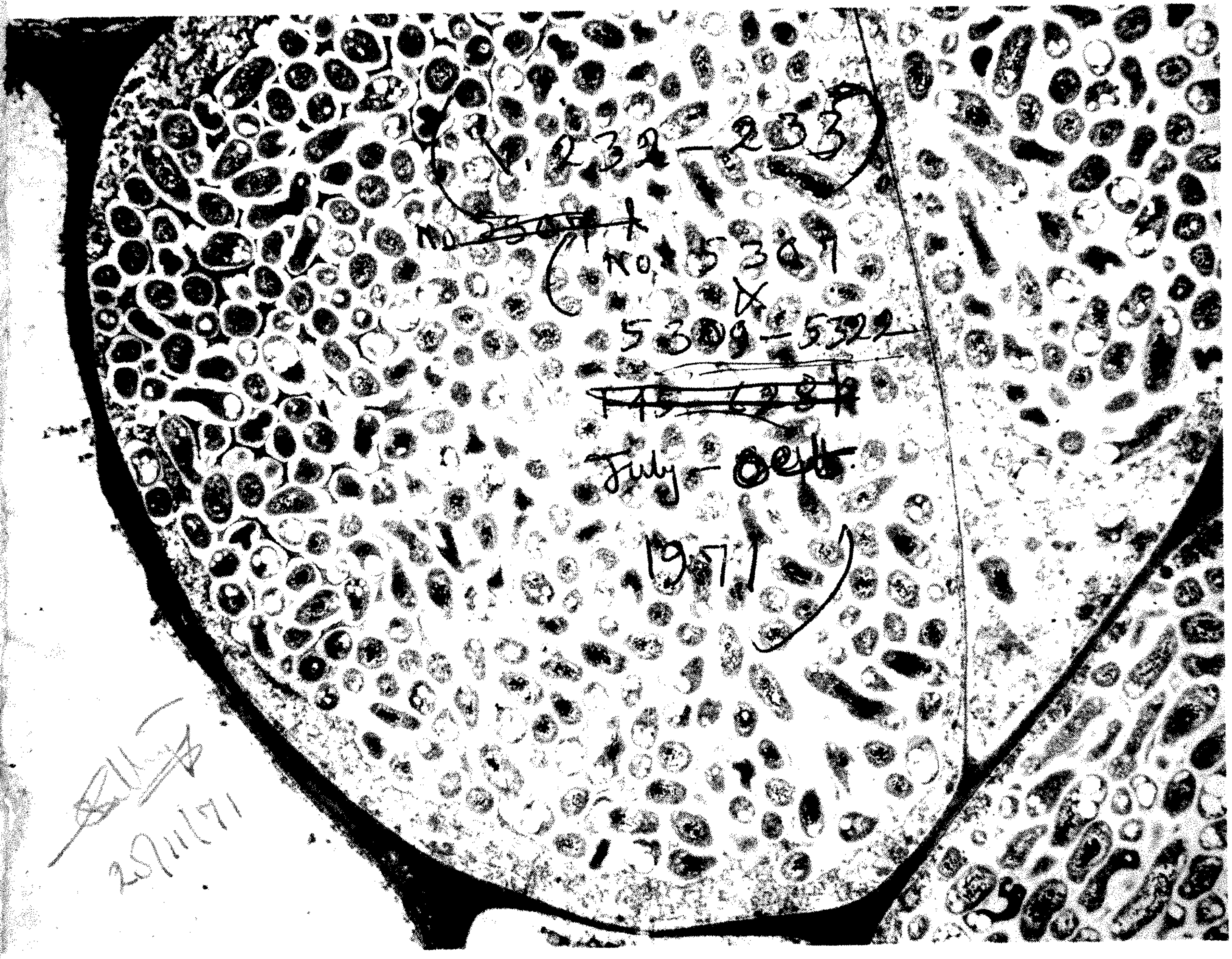
NATURE

Vol. 232 No 5307 July 16 1971 FRIDAY

26 NOV 1971
Pure Chemistry Library
SCIENCE COLLEGE
92 Upper Circular Road
CALCUTTA



V. 232–233
~~No 2307~~
No. 5307
&
5309–5322
~~145–628~~
July–~~Oct~~
1971

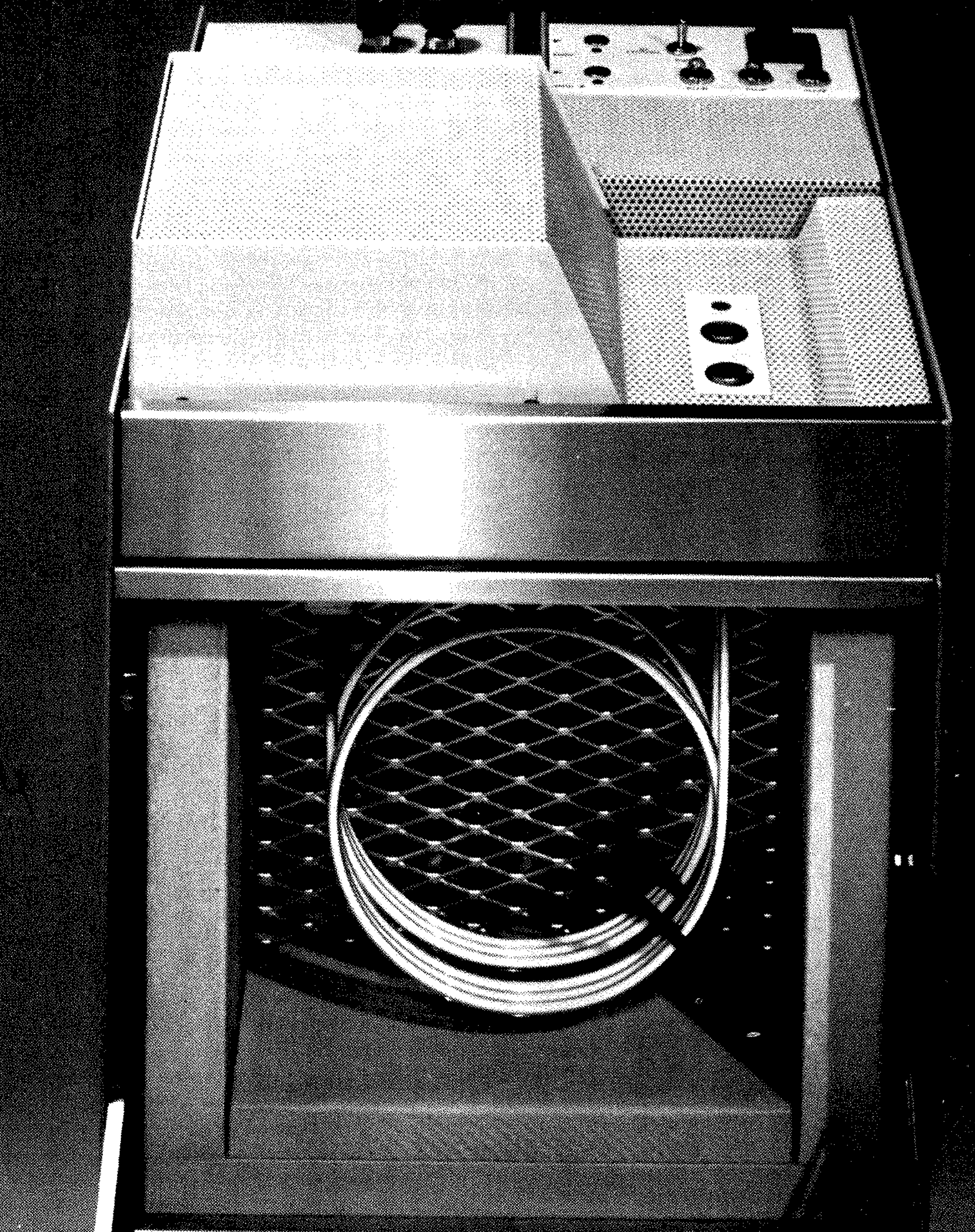
29/11/71

Rhizobium and plant cells symbiotic *in vitro*

234

Mercury in the brain . . . Carbon-14 in people
LSD and DNA . . . Circular polarization from planets
Rifting in the N-E Atlantic

25p UK $1.00 USA



The basic oven

Flexible.
Get-attable.
The basis for 7 different chromatograph oven systems.
To go with 4 detectors—ECD, FID, Thermionic or Katharometer.
To make 10 detector systems.
To give you precisely what you want.
Sensitivity. Versatility.
Add the automatic solid injection system or the Curie Point Pyrolyser or any one of a wide range of accessories and you have one of the world's really great chromatographs. For routine, research or computer controlled analyses. The Pye Series 104.
Write or telephone for details today.

Pye Unicam Ltd
York Street, Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

CUK-H06834-234-RP364

NATURE

MACMILLAN JOURNALS LTD

No. 5307 FRIDAY JULY 16 1971 Vol. 232

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page
Whose Rate for the Job? 145
What Future for Euratom? 146
No Jobs for the Boys 147

OLD WORLD

Scientists' Pay/Mineral Resources/Stars in Rock . . 148

NEW WORLD

Cancer Compromise Almost Unites Senate/Unemployment 149

NEWS AND VIEWS

Circular Polarization and Astronomy/Nuclear Fusion/RNA/Bacteriophages/Continental Drift . . . 151

Transport in the Phloem—SPANNER (London) . . 157

The Space Scars of Earth—MILLMAN (National Research Council of Canada) 161

Planets—Circular polarization of Jupiter and other planets—KEMP, WOLSTENCROFT (Hawaii) and SWEDLUND (Oregon) 165

North-East Atlantic—Evolution deduced from magnetic data—WILLIAMS and McKENZIE (Cambridge) . 168

Symbiosis—Establishment between *Rhizobium* and plant cells *in vitro*—HOLSTEN, BURNS, HARDY and HEBERT (E.I. du Pont de Nemours and Company) . 173

LETTERS TO *NATURE*

Physical Sciences

Radio pulses—Search for pulses from the galactic centre—CHARMAN, FRUIN, JELLEY (AERE, Harwell), HAYNES, HODGSON, SCOTT, SHAKESHAFT (Cambridge), BAIRD, DELANEY, LAWLESS (University College, Dublin), DREVER and MEIKLE (Glasgow) 177

Variable cosmic sources—Rapid optical variability of the radio source PKS 1514-24—BIRAUD (US National Radio Astronomy Observatory) 178

Page
Lyman-α radiation—Discussion of the Vela 4 results—FEHLAU, CHAMBERS, FULLER and KUNZ (California) 179

Palaeogeography—The expanding Earth theory reappraised—HALLAM (Oxford) 180

Lidar and infrared radiation—Enhancement near small cumulus clouds—PLATT (CSIRO, Aspendale) and GAMBLING (Adelaide) 182

Rock failure—A servocontrolled testing machine—HUDSON (Minnesota) 185

Ball lightning—Optical and acoustic detection—WAGNER (Pennsylvania) 187

—Observation in. Italy—GIBBS-SMITH (Victoria and Albert Museum) 187

Biological Sciences

Visual chromophores—Crystal structures of 11-*cis* and all-*trans* retinal—GILARDI, KARLE, KARLE (US Naval Research Laboratory) and SPERLING (Ohio State) 187

LSD—Circular dichroism fails to detect intercalation in DNA—BRADY, BRADY and BOUCEK (Miami) . 189

—Failure of LSD-25 to intercalate in DNA—SMIT and BORST (Amsterdam) 191

Myeloma protein—Induced optical activity when specifically bound to 2,4-dinitrophenyl-lysine in mouse—ROCKEY, DORRINGTON (MRC, Cambridge) and MONTGOMERY (MRC, London) . . . 192

Membrane immunochemistry—Localization of A antigen sites on human erythrocyte ghosts—PINTO DA SILVA, BRANTON (California) and DOUGLAS (Mount Sinai School of Medicine, NY) . . . 194

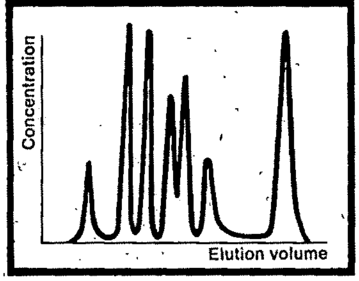
Murine leukaemia—Treatment with heterologous antiserum or cyclophosphamide—HILL (Colorado) . 196

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 152 to 156.

(Continued overleaf)

Sephadex®

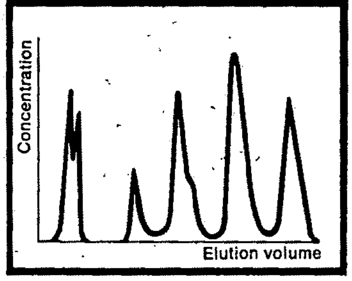
Partition chromatography



Separation of esterified catecholamine metabolites by partition chromatography in 1,2-dichloroethane: methanol (7:3).

(Anggard, E., et al., J. Chromatog. 50 (1970) 251-259)

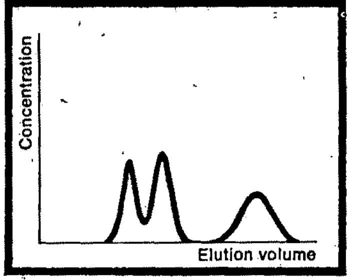
Gel filtration



Gel filtration of triglycerides and fatty acids in chloroform.

(Adapted from Downey, W.K., et al., J. Chromatog. 46 (1970) 120—124. Reproduced by kind permission of the Authors and the Publisher.)

Adsorption chromatography



Separation of oestrogens by adsorption chromatography in distilled water.

(Van Baelen, H., et al., J. Chromatog. 30 (1967) 226—227. Reproduced by kind permission of the Authors and the Publisher.)

Sephadex® LH-20

in organic solvents and aqueous solutions

Sephadex LH-20 is a lipophilic derivative prepared from Sephadex G-25 by hydroxypropylation. It is supplied in bead form (dry particle size 25—100 μ) as a dry, free-running powder which swells in water and polar organic solvents. The degree of swelling decreases with decreasing solvent polarity. Although introduced as a medium for gel filtration in organic solvents Sephadex LH-20 has rapidly become established as a medium for other types of chromatography which depend on partition and adsorption effects.

Sephadex LH-20 is used in polymer fractionation, lipid and steroid separations, aliphatic and aromatic hydrocarbon separation as well as in a host of diverse applications.

Special solvent resistant columns are available.

Pharmacia (Great Britain) Ltd.
Paramount House
75 Uxbridge Road
LONDON W5
Telephone 01-579 0102/7

Pharmacia

[illegible] CALCUTTA

Page

Leukaemia—Induction of auto-immune response to leukaemic cells by chemically tagged white blood cells —SAHASRABUDHE, MADYASTHA, PREMA (Cancer Research Institute, Bombay), LELE (Grant Medical College, Bombay), GOLLERKERI (Tata Memorial Hospital, Bombay) and RAO (Haffkine Institute, Bombay) 197

—Preparation of anti-leukaemic serum by exploitation of differences in antibody surface charge —SAHASRABUDHE, PREMA, MADYASTHA (Cancer Research Institute, Bombay), GOLLERKERI (Tata Memorial Hospital, Bombay) and RAO (Haffkine Institute, Bombay). 198

Cancer—N-nitrosopiperidine formed from piperidine and sodium nitrite in the stomach and isolated intestinal loop of the rat—ALAM, SAPOROSCHETZ and EPSTEIN (Harvard) 199

Mercury—Distribution and concentration in human brain—GLOMSKI, BRODY and PILLAY (State University of New York) 200

—Elimination from animals accelerated by absorption with reduced human hair—TAKAHASHI and HIRAYAMA (Kumamoto) 201

Page

Learning—Two-stage recovery of a response in cuttlefish —MESSENGER (Sheffield) 202

—Retention performance during long term memory formation in octopus—SANDERS and BARLOW (London and Naples) 203

BOOK REVIEWS

by P. G. Werskey, Benjamin Lewin, Alan T. Bull, A. W. Johnson, J. M. Creeth, T. Mulvey, B. C. Vickery . 205

CORRESPONDENCE

Aid for Bengal (P. J. S. Brooks) 209

Obituaries—Professor I. Vincke/Academician V. V. Parin 209

Announcements—University News/Miscellaneous/International Meetings. 210

Next Monday's issue will include

Cosmic rays—Clusters of neutron stars may provide injection mechanism for Fermi process—BOCCALETTI, DE SABBATA, FORTINI and GUALDI (Bologna)

Solar microwave bursts—Homologous events from different active centres—SCALISE, BASU, PAES DE BARROS and KAUFMANN (Mackenzie)

Liquids—Use of models to study instantaneous structure—WOODCOCK (Southampton)

Crystallography—Structure of low chalcocite—EVANS (US Geological Survey)

X-ray photoelectron spectroscopy—Spectra of DPPH and diphenylpicrylhydrazine—DRUMMOND (AEI Scientific Apparatus Limited) and HARKER (Newcastle upon Tyne)

Next Wednesday's issue will include

Gene linkage—Analysis in human chromosomes using man-mouse hybrid cells—RUDDLE, CHAPMAN, RICCIUTI, MURNANE (Yale), KLEBE (Salk Institute) and KHAN (Leiden)

Cytotoxins—When released from stimulated lymphocytes they mediate target cell lysis—PETER and DAWKINS (California)

Lymphocytes—Cytotoxic lymphocytes from rats depleted of thymus processed cells—HARDING, PUDIFIN, GOTCH and MacLENNAN (Radcliffe Infirmary, Oxford)

Seminal proteases—Influence on vascular permeability in humans—SUOMINEN and NIEMI (Turku)

Calcitonin—Explanation for the unusual potency of the hormone in salmon—HABENER, SINGER, DEFTOS, NEER and POTTS (Harvard)

—Effects on strontium retention in the dog—ANDERSON, BALK, CRACKEL, AUSTIN, BOLLMEIER and SLUSHER (Illinois)

Animal behaviour—Effect of arousal induced by noise on reinforcement of a response by light—WILLIAMS, WELLS and LOWE (Hull)

Interference of Electromagnetic Waves

A. H. COOK

This fundamental account of the aspects of interference that are of the greatest practical importance in spectroscopy, optics, mechanical engineering, and radio astronomy is written for research workers, teachers, and post-graduate and honours-year undergraduate students. The treatment is based mainly on the idea of coherence of electromagnetic fields and an attempt has been made to give a unified account of both optical and radio interferometers. Laser and radio-astronomical sources have led to much simpler accounts of interference methods. These developments and the use of interferometers for precise measurements in engineering and optical spectroscopy are emphasized. 90 text figures £5 *International Series of Monographs on Physics*

DOMINVS ILLVMINATIO MEA

OXFORD UNIVERSITY PRESS

ADVANCES IN BIOPHYSICS

Edited by Masao Kotani

The new annual series that concentrates on the theoretical aspects of the latest developments in biophysics. Published under the scientific direction of the Biophysical Society of Japan, each volume presents the year's most important research results by leading biophysicists from Japan and other countries. As reviewed in *Science*: "In all cases the papers are amply illustrated and clearly presented . . . As many of the authors chosen for this and forthcoming volumes are important contributors to their respective fields, the series promises to be an interesting addition to the biophysics literature."

Volume 2

Crystal Structure of Tropomyosin. Electrogenic Sodium Pump. One-Electron and Two-Electron Transfer Processes in Enzymic Oxidation and Reduction. Phase Transition in Membranes with Reference to Nerve Excitation. Stochastic Theory of Reaction Kinetics.

230 pages. Illus. July 1971. $12.50

Volume 1

The Lateral-Line Organ of Shark as Chemoreceptor. Ribonuclease T_1 — Structure and Function. Polymerization of Flagellin and Polymorphism of Flagella. Spin Changes in Hemoproteins.

193 pages. Illus. 1970. $11.50

Your continuation order for the series ensures automatic shipment of each new volume immediately upon publication.

Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS
IN SCIENCE AND MEDICINE

ATOMIC AND MOLECULAR RADIATION PHYSICS

by **L. G. Christophorou**, *Oak Ridge National Laboratory and the University of Tennessee.*

A coherent picture of many apparently different areas of research in atomic and molecular photophysics and atomic and molecular collision processes, this work discusses the relevance and importance of these fundamental studies, at atomic and molecular level, to radiation science and biology. It covers at a fairly fundamental level the recent research on atoms and molecules (small and complex), obtained both by photophysical and particle collision processes, and discusses the importance of such studies to the understanding of the interaction between radiation and matter and biological action of molecules.

Due July 1971 682 pages *£10.50*

**Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex**

The Social Impact of Modern Biology

EDITED BY WATSON FULLER

In this book, based on a conference organized by the British Society for Social Responsibility in Science, leading world biologists discuss modern biological discoveries and their implications. 'Up-to-date and cheap, it is a remarkable publication which deserves the widest reading and discussion, especially in universities and schools.'–*New Society*, cloth £1, paper 50p

ROUTLEDGE & KEGAN PAUL
68 Carter Lane, London EC4V 5EL

NATURE

MACMILLAN JOURNALS LTD

No. 5309 FRIDAY JULY 30 1971 Vol. 232

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Responsibility without Government 287

OLD WORLD

Scientists' Pay/Biology / Brackish Water / Astronomer Royal 288

NEW WORLD

AEC Bruised but not Cut/FDA 290

NEWS AND VIEWS

***X*-Chromosome Inactivation in Mules and Hinnies/New Class of Varying Stars/Confusion over Fossil Man/** Transformation / Photochemistry / Antibodies / High Energy Physics/Narcotics/Bacteriology . . . 293

Why Hydrology?—RODDA (Institute of Hydrology) . 301

Prospects for Carbon Fibres—JEFFRIES (Cotton, Silk and Man-made Fibres Research Association) . . 304

Hominidae—New cranium of *Homo* from Bed I, Olduvai Gorge—LEAKEY, CLARKE and LEAKEY (Centre for Prehistory and Palaeontology, Nairobi) . . 308

***X* chromosome inactivation**—Non-random *X* chromosome expression in female mules and hinnies—HAMERTON, RICHARDSON, GEE (Children's Hospital, Winnipeg and Manitoba), ALLEN and SHORT (Cambridge) 312

***X* chromosomes**—Non-random late replication in mules and hinnies—GIANNELLI (London) and HAMERTON (Children's Hospital, Winnipeg, and Manitoba) 315

LETTERS TO *NATURE*

Physical Sciences

Microtektites—Fission track ages and geomagnetic reversals—DURRANI and KHAN (Birmingham) . 320

Gegenschein—Measured from space—ROUY, CARROLL (Rutgers), ALLER (California), ROACH and SMITH (Battelle Northwest Laboratories) . 323

Mars—Explanation of blue haze clearings—WELLS and HALE (Marshall Space Flight Center) . . 324

Rock permeabilities—High values for Precambrian Onverwacht cherts—SANYAL (Stanford), KVENVOLDEN (NASA) and MARSDEN (Stanford) . 325

Recent sediments—Conversion of oleic acid to saturated fatty acids in the Severn Estuary—RHEAD, EGLINTON, DRAFFAN and ENGLAND (Bristol) . . 328

Carbon dating—Stratigraphy of Lake Keilambete, Victoria—BOWLER (Australian National) and HAMADA (Institute of Physical and Chemical Research, Yamato-Machi Saitami Prefecture) . . . 330

Asbestos fibres—In beverages and drinking water—CUNNINGHAM and PONTEFRACT (Canada Department of Health and Welfare) . . . 332

Biological Sciences

Nucleosides—Molecular conformation of deoxyuridine—RAHMAN and WILSON (Dundee) . . . 333

Bone structure—Pole figures of the orientation of apatite—NIGHTINGALE and LEWIS (Surrey) . . 334

Cardiac function—Assessment by oesophageal ultrasonic Doppler shift blood velocity probe—SIDE and GOSLING (London) 335

Lithium—Attenuation of drug-induced hyperactivity in rats—COX, HARRISON-READ, STEINBERG and TOMKIEWICZ (London) 336

Procoagulant synthesis—Triggering by cell adhesion in cultured fibroblasts—ZACHARSKI (Veterans Administration Center, Vermont) and McINTYRE (Dartmouth Medical School) 338

Fungal pathogenesis—Action of pollen in breaking resistance of *Vicia faba* to *Botrytis cinerea*—MANSFIELD and DEVERALL (London) . . . 339

Neurosecretory terminals—Coated microvesicles in posterior pituitary shed their coats—DOUGLAS, NAGASAWA and SCHULZ (Yale) 340

—Micropinocytotic origin of coated and smooth microvesicles of posterior pituitary gland—NAGASAWA, DOUGLAS and SCHULZ (Yale) 341

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 295 to 300.

Pure Chemistry Library
SCIENCE COLLEGE

(*Continued overleaf*)

Aspects of Terpenoid Chemistry and Biochemistry

Proceedings of the Phytochemical Society Symposium, April 1970

edited by T. W. Goodwin

June 1971, xiv+442 pp., £8.50.

This, the proceedings of the second Phytochemical Society symposium on terpenoids, reveals the extensive progress which terpene chemistry, biochemistry and biology have made in the past few years and complements the first symposium. The recent developments in the biosynthesis, metabolism and function of plant terpenes are presented by international experts.

As with previous volumes in the series, this is intended for research workers and graduate level students in the field.

Neurotransmitter–Receptor Interactions

D. J. Triggle

June 1971, x+610 pp., £9.00

This text discusses the molecular basis of neurotransmitter-receptor interactions from a multidisciplinary standpoint, for an effective understanding of the actions of neurotransmitters will require collaboration between disciplines. Emphasis has been placed on two broad areas, into which the author believes knowledge of neurotransmitter-receptor interactions must be incorporated; these areas are the regulatory control of macromolecules and membrane structure and function.

As a result of the emphasis on interdisciplinary cooperation, the book should not only be of value to workers researching drug-receptor interactions, but also as a guide to scientists from other disciplines who wish to enter this field.

Biochemical Society Symposium Number 32

The Biochemistry of Steroid Hormone Action

edited by R. M. S. Smellie

June 1971, x+174 pp., £3.50.

Contents: Estrogen action: a study of the influence of steroid hormones on genetic expression. Interaction of steroid hormones and histones. Steroid hormones, ribonucleic acid synthesis and transport, and the regulation of cytoplasmic translation. Steroid hormones and enzyme induction. The effect of steroids on the transport of electrolytes through membranes. Androgen stimulation of prostatic cation-dependent ATPase. Hormone-receptor interaction as a guide to biochemical mechanism. Author index. Subject index.

Ecology of Leaf Surface Micro-organisms

Proceedings of an International Symposium held at the Universty of Newcastle-upon-Tyne, September 1970

edited by T. F. Preece and C. H. Dickinson

June 1971, xviii+640 pp., £9.00

These proceedings are a unique presentation of the current state of knowledge on various aspects of leaf surface phenomena, especially their microbiological, chemical and physical characteristics.

As well as contributions from a large number of international experts, this volume contains recorded discussions, many of which will be as valuable as the papers in stimulating further work and in revealing gaps in our knowledge. Research workers and graduates in botany, agriculture, horticulture, microbiology and general biology at any level will find the papers and discussions of value and interest.

Gustation and Olfaction

An International Symposium, Geneva, June 1970

edited by G. Ohloff and A. F. Thomas

June 1971, x+276 pp., £4.00

This symposium was attended by chemists, biochemists, physiologists and those working in the perfume and flavour industries. These leading specialists were able to present original ideas and conclusions, which will greatly assist all researchers in this field. A significant coverage is given of the relationship between behaviour and taste or smell in both insects and mammals (including man). Several aspects of molecular structure in relation to odour and taste and particularly of stereochemistry are also investigated. A stimulating discussion followed during which varied opinions on present-day and future progress were expressed, a summary of which is reproduced in this book.

Academic Press
London and New York
Berkeley Square House, Berkeley Square, London WIX 6BA, England
111 Fifth Avenue, New York, NY 10003, USA

Page

Muscle—Changes in ATP and creatine phosphate storage in muscle of rats exercised at high altitude—GALE (Sonoma State College) and NAGLE (Wisconsin) 342

Follicular cells—Fertilization of denuded rabbit eggs *in vitro* by sperm recovered from the uterus or vagina—CHANG, HANADA and HUNT (Worcester Foundation for Experimental Biology) 343

Toad hormones—Role of skin and neurohypophyseal hormones in the adaptation of *Bufo viridis* to high salinities—KATZ and WEISBERG (Hebrew University of Jerusalem) 344

Mosquitoes—Sterilization of *Aedes aegypti* (L.) by chronic exposure to low doses of beta radiation—GUTHRIE (Atomic Energy of Canada Limited) and BRUST (Manitoba) 345

Cocoa pod rot—Transmission by invertebrates—EVANS (Cocoa Research Institute, Ghana) . . 346

Page

Polymorphism—Relationship to metabolic function—JOHNSON (Stanford) 347

X **chromosomes**—Phenotypic evidence for selective differences between cells with an active horse *X* and cells with an active donkey *X* in the female mule—HOOK and BRUSTMAN (New York State Department of Health) 349

Australopithecines—Determination of height—BURNS (Case Western Reserve) 350

BOOK REVIEWS

by Kenneth Dewhurst, John Phillipson, Michael A. Simpson, Alexander De Grassie, J. Leggett Bailey, S. Cohen, J. A. Creighton J. Mason, R. J. W. Reynolds 351

CORRESPONDENCE

Smoking and Cancer (John Higginson), Defoliation by Gypsy Moth (Thomas H. Jukes) 355

Next Monday's issue will include

Petrology—Synthesis of grunerite—FORBES (Illinois)

NP 0532—Pulsed gamma rays above 50 MeV—BROWNING, RAMSDEN and WRIGHT (Southampton)

Hydrogen bonds—Position of atom in *A*-type bonds—KROON, KANTERS, PEERDEMAN (Utrecht) and VOS (Groningen)

Optical rotation—Symmetry rules—STILES (Cambridge)

Interstellar dust—Role of soft galactic X-rays in alignment of dust—WICKRAMASINGHE (Cambridge)

—Reply—MACK (Houston)

Next Wednesday's issue will include

Myoglobin—Primary structure—HERRERA and LEHMANN (MRC, Cambridge)

Ribosomes—Silver causes dissociation—HARDY and TURNOCK (Leicester)

Endocytosis—Role of microfilaments in macrophages—ALLISON, DAVIES and DE PETRIS (Clinical Research Centre, Harrow)

Liver—Activation of protein synthesis in cell sap by amino-acids—CLEMENS and KORNER (Sussex)

Pacemaker neurones—Recording from completely isolated neurones of *Aplysia californica*—CHEN, VON BAUMGARTEN and TAKEDA (Michigan)

HIGH QUALITY
OPTICS by
OS
Optical Surfaces
LIMITED
GODSTONE ROAD, KENLEY, SURREY
CR2 5AA
Telephone: 01-668 2843

MANUFACTURERS OF
ASTRONOMICAL, SCIENTIFIC
and INDUSTRIAL EQUIPMENT

If you work with instruments where a high degree of accuracy is vital, an optical component that falls short of the specified performance costs time and causes frustration. Our business and pleasure is to produce components of the highest optical quality, or lower qualities if desired.

Foremost in the first category are our Fabry-Perot etalon plates; diameters up to 150 mm, with matching accuracies to better than $\lambda/200$. (We can supply standard 60 mm diameter plates from stock.) Solid etalons, plane-parallel plates, resonant reflectors, and super-flats to $\lambda/200$ are other examples of our more sophisticated production range.

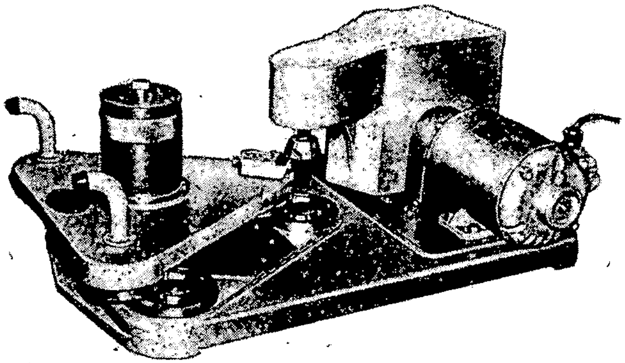
We make $\lambda/20$ optics for astronomical purposes, including all the conic sections—both on-axis and off-axis—and objective prisms, Schmidt plates ($\lambda/10$) and plane parallel windows. Outside the normal astronomical range, we will make almost any on-axis or off-axis conic (including grazing-incidence surfaces) to accuracies which constitute the present limits of optical skill. Our handwork department offers a specialized service in the production of prisms and small optics of all types to the most demanding specifications. Our craftsmen will produce almost any optical component you may specify in glass, silica or glass ceramic, and certain crystals.

It is our desire to provide the type of service and collaboration essential to so many research projects, and we invite your enquiries which will be given our prompt and careful attention.

CUTROCK

CROFT PARALLEL GRINDING MACHINE (MOTOR DRIVEN)

For precision grinding of thin sections and fossils



The travelling carriage of the machine moves in a circular track over the grinding plate controlled by steel balls running in precision machined tracks. Mounted in the centre of the carriage is a metric micrometer which houses the specimen holder. When the carriage is in the grinding position as illustrated, the micrometer can be adjusted until the specimen is in contact with the grinding plate, at this point the reading should be taken and then the amount to be ground off should be set on the micrometer. The specimen holder will lift by this amount and as the machine grinds the holder will return to its setting position, which once reached will stop any further grinding of the specimen even if the machine continues to run.

Grinding plates can be supplied in zinc, copper, cast iron, glass or lead.

CUTROCK ENGINEERING CO., LTD.

Telephone: 01-346-5978/9 35 BALLARDS LANE LONDON N3

NATURE

MACMILLAN JOURNALS LTD

No. 5310 FRIDAY AUGUST 6 1971 Vol. 232

LONDON
4 Little Essex Street, London WC2R 3LF., Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Black Arrow Aborts 357

Sheep and Goats 358

OLD WORLD

Gas Centrifuge/Scientists' Pay/Computers/Earthquake Research/Astronomy/Freshwater Biology/Space Policy 360

NEW WORLD

Davis Committee Exposes the Problems/Pesticides in the Open/Atomic Energy/Uranium Enrichment . . 364

NEWS AND VIEWS

New Baskets for Old/Filling the Dating Gap/History of Biology/Galaxies/Seafloor Spreading/Haemoglobin/Stratigraphy/Olfaction/Nutrition/Reverse Transcriptase/Conservation 367

Value of Historical Records of Earthquakes—AMBRASEYS (London) 375

Homo erectus—Postcranial remains and associated artefacts from Olduvai Gorge—LEAKEY (Centre for Prehistory and Palaeontology, Nairobi) 380

—Left femur shaft and hip bone from Olduvai Gorge—DAY (London) 383

Microtubule assembly—Localization of subunits in the cytoplasm during morphogenesis—TUCKER (St. Andrews) 387

LETTERS TO *NATURE*

Physical Sciences

Atmosphere—Total ozone increase in 1960s—KOMHYR, BARRETT, SLOCUM and WEICKMANN (US Air Resources Laboratory) . . . 390

Neutron stars—Do they have an ocean surface?—CARTER and QUINTANA (Cambridge) . . 391

Coma cluster—X-ray limits do not imply that virial theorem is inapplicable—NOERDLINGER (New Mexico Institute of Mining and Technology) . . 393

Pollution—Mercury in the tidal Thames—SMITH, NICHOLSON and MOORE (Institute of Geological Sciences) 393

Oxfordshire coalfield—Seams below Steeple Aston—POOLE (Institute of Geological Sciences) . . . 394

Dioxin—Formation through thermal decomposition of sodium 2,4,5-trichlorophenate — MILNES (Coalite and Chemical Products Ltd) 395

Page

Biological Sciences

Sex pheromone—Identification in rhesus monkeys and properties of synthetic attractants—CURTIS, BALLANTINE (University College of Swansea), KEVERNE, BONSALL and MICHAEL (Bethlem Royal Hospital) 396

Sexual response—*p*-chlorophenylalanine and copulatory behaviour in the male rat—MALMNÅS and MEYERSON (Uppsala) 398

—*p*-chlorophenylalanine facilitates copulatory behaviour in the male rat—SALIS and DEWSBURY (Florida) 400

Sexual attraction—Mate-seeking by female ticks on bovines—GLADNEY (US Department of Agriculture) 401

Insect deterrent—Protection of beans by natural insect repellants—GILL and LEWIS (London) . . . 402

Trophoblast—Identification of HL-A antigens—LOKE, JOYSEY and BORLAND (Cambridge . . . 403

Leukaemia—Transplantation of radiation-induced canine myelomonocytic leukaemia—SHIFRINE, BULGIN, DOLLARHIDE, WOLF, TAYLOR, WILSON, DUNGWORTH and ZEE (California) . 405

Reproduction—Persistence of corpora lutea in mice in a cold environment—BARNETT and MUNRO (Glasgow) 406

Haemoglobin—Stereochemical interpretation of abnormal oxygen affinities—MORIMOTO, LEHMANN and PERUTZ (MRC, Cambridge) 408

Ramapithecus—Ecology of the Nagri environment, North India—PRASAD (Geological Survey of India) 413

Sequential choice—Evaluation of compound probabilities—COHEN, CHESNICK and HARAN (Manchester) 414

Similarity coefficient—Similarity measure for mixed attribute types—ANDERSON (MRC, Edinburgh) . 416

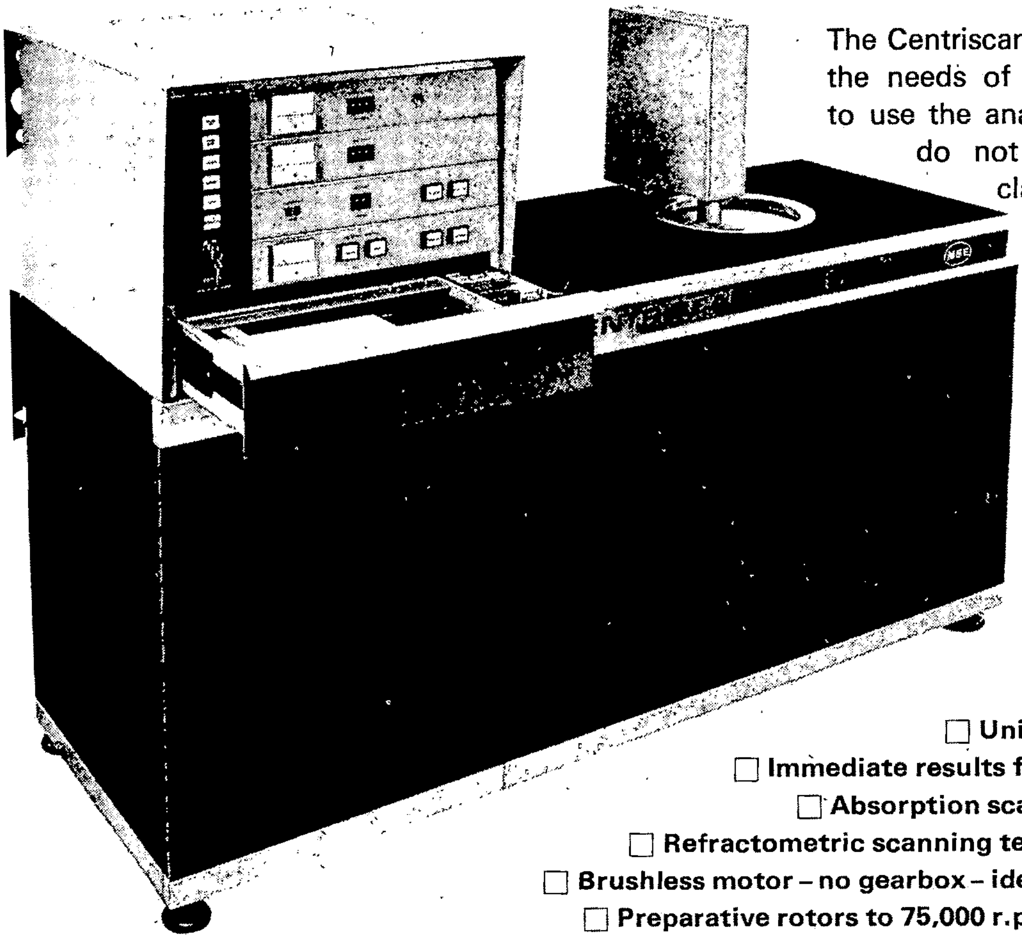
Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 368 to 374.

(Continued overleaf)

an ANALYTICAL ULTRACENTRIFUGE
with INTEGRAL SCANNER and RECORDER
plus PREPARATIVE FACILITIES

MSE CENTRISCAN 75

(and all at a price you can afford)



The Centriscan 75 meets, at relatively low cost, the needs of the many scientists who wish to use the analytical centrifugal technique but do not require the full facilities of a classical Analytical Ultracentrifuge. However, with recording of the absorbance of up to four samples at a time, the Centriscan 75 is capable of work of the highest precision, even on submicrogram quantities – work that was previously possible only with much more expensive equipment. In addition, the 75,000 r.p.m. preparative capability gives unique versatility.

- ☐ **Unique optical system**
- ☐ **Immediate results from direct traces – no cameras**
- ☐ **Absorption scanning down to 254nm**
- ☐ **Refractometric scanning technique giving Schlieren traces**
- ☐ **Brushless motor – no gearbox – ideal for equilibrium runs**
- ☐ **Preparative rotors to 75,000 r.p.m. – 500,000 x 'g'**

3345

MSE

Measuring and Scientific Equipment Ltd.,
Manor Royal, Crawley, Sussex. *Telephone: Crawley (STD 0293) 31100.*
Cables: Emesetool, Crawley Telex: 87119.

Page

Nitrogen fixation—Occurrence and significance in a tropical lake—HORNE (California) and VINER (Royal Society African Freshwater Biological Team) . 417

Radioactive fallout—Bomb^{14}C in the human population—NYDAL, LÖVSETH and SYRSTAD (Norwegian Institute of Technology) 418

Cosmic ray flashes—Explanation for astronauts' observations—McAULAY (Trinity College, Dublin) . 421

Cholinergic receptors—Reply to Chothia and Pauling—BEERS and REICH (Rockefeller) 422

Bacterial coats—Presence and function of disclinations in surface coats of unicellular organisms—NABARRO (Paris) and HARRIS (Witwatersrand) 423

Page

Daucic acid—Possibility of general occurrence in plants isolated from carrots —KEYS (ARC, London), LEAVER, BARTON, BROWN and WIDDOWSON (London) 423

BOOK REVIEWS

by Eric Ashby, C. C. Booth, John Patterson, Garth Underwood, A. G. Mackie, D. H. Sadler, Bernard Pagel, H. M. Frey 425

CORRESPONDENCE

Science and Development (K. C. Tripathi) . . . 429

Obituary—Sir W. Le Gros Clark 429

Next Monday's issue will include

Hexahelicene—Absolute configuration—LIGHTNER, HEFELFINGER, FRANK, POWERS and TRUEBLOOD (University of California, Los Angeles)

Calcium sulphate hemihydrate—Infrared studies of the possibility that two forms exist—CLIFTON (National Bureau of Standards)

NP 0532—Observations at 30–100 KeV—SMATHERS, CHUBB (NRC, Washington) and SADEH (Tel-Aviv)

Deferred luminescence—Electron tunnelling responsible at 4.2 K in an organic glass—KIEFFER, MAGAT, MEYER and RIGAUT (Orsay)

Anomalous water—Reply to Pethica Thompson and Pike—DERJAGUIN and CHURAEV (USSR Academy of Sciences)

Next Wednesday's issue will include

Polar mutants—Mechanism for polarity based on mRNA degradation—MORSE and GUERTIN (Harvard)

RNA—Aminoacetylation of bisulphite-modified yeast tyrosine transfer RNA—KUCAN, FREUDE, KUCAN and CHAMBERS (New York)

Actinomycin D—Effects on infection of tumour cells by fowl plague virus—MALLUCCI (London) and SKEHEL (MRC, London)

L-**Asparaginase**—Antiviral activity demonstrated—MARAL and WERNER (Société des Usines Chimiques Rhône-Poulenc)

DILLON'S UNIVERSITY BOOKSHOP

All current technical and scientific textbooks

Lists of new books issued—state subject

1 MALET ST., LONDON WC1
01-636 1577

BACK VOLUMES OF SCIENTIFIC PERIODICALS

We are always interested in buying your collections of duplicates of Scientific, Technical and Medical Journals at the highest prevailing prices. Please send us your lists of surplus journals, which will receive our immediate attention

We are Distributors of Back Issues of Journals including Pergamon Press Journals and many other Publishers. We invite you to write in for our regularly issued Bulletins and Catalogues and should be pleased to be of assistance in your acquisitions.

ROBERT MAXWELL & CO. LTD.
Back Issues Department
Midland Road, OLNEY, Bucks, England. Tel. Olney 529

"*Must reading* for any lab director contemplating computerization . . ."

CLINICAL LABORATORY COMPUTERIZATION

By Arthur F. Krieg, Thomas J. Johnson, Jr., Clement McDonald, and Ernest Cotlove

The most highly acclaimed text yet published on computerization for clinical laboratories:

". . . must reading for any lab director contemplating computerization, and should be the first book he picks up on the subject . . . The reading is fast, the approach direct, and computer jargon kept at a useful minimum. Probably worth the price of the book alone is the chapter listing 73 questions the lab director should ask a prospective vendor in requesting a proposal . . . a guideline for minimizing cost overruns while maximizing the chances for selecting the best system for a given type of laboratory operation.

"This is one book that fulfills its purpose and provides the laboratory director with an effective road map through the maze of computer claims and counterclaims."

—*Laboratory Management*

". . . offers all possible factual information to help pathologists, laboratory directors, technologists, and hospital administrators choose an efficient, economic, and reliable computer system."

—*Lab World*

"The authors even give lists of example installations so that the prospective user can get some honest opinions. Easily readable and full of useful information."

—*Computer Decisions*

132 pages. Illus. 1971. $8.50

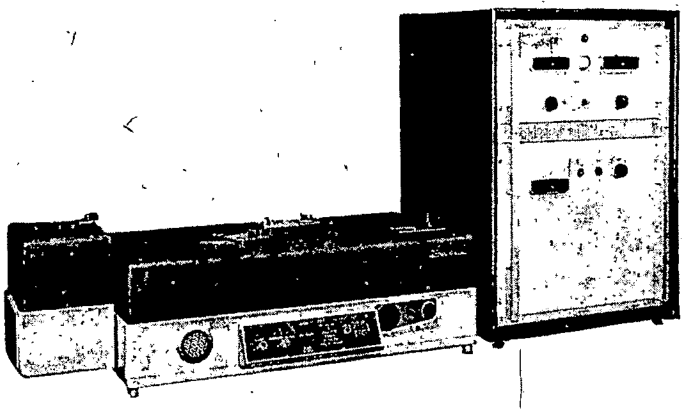
Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS IN SCIENCE AND MEDICINE

B&S Precision Instruments

The POLARMATIC 62 Automatic Spectropolarimeter



SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

NOW O.R.D + C.D

We are pleased to announce that a Circular Dichroism attachment has been designed by the National Physical Laboratory and will shortly be available for this outstanding spectropolarimeter. Like the Polarmatic itself, this new device will once again open up new thresholds in biological research.

A great deal of engineering development has enabled us to offer this attachment to both new and present users of the O.R.D. instrument. For full details write to Dept. N.

We now have capacity for aluminising certain types of mirrors, at short notice.

BELLINGHAM & STANLEY LIMITED B&S
61, MARKFIELD RD. LONDON N15. TEL. 01-808 2675

Pure Chemistry Library
SCIENCE COLLEGE.
[illegible], Upper Circular Road
CALCUTTA

NATURE
MACMILLAN JOURNALS LTD

No. 5311 FRIDAY AUGUST 13 1971 Vol. 232

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page
Have We Been Here Before ? 431
Throwing in the Sponge 432
Howzat ! 432

OLD WORLD

Nuclear Weapons/Scientists' Pay/Enzyme Research/Metrication/Building Research/Electricity Supply/Meteorology/Eastern Science/European Research/Hovercraft 434

NEW WORLD

Metric Standards Round the Corner 439

NEWS AND VIEWS

The Universe Considered as Hole/Significance of Sex Linked Antigens/DNA Replication/Antiquities/Behaviour/Proteins/The Earth/Pesticides/Aircraft Dynamics/Seafloor Soils/Microearthquakes 440
Statistical Mechanics and Quantum Mechanics— UHLENBECK (Rockefeller) 449
Research Environments and Productivity—GUPTA and SHARMA (CSIR, New Delhi) 450
Upper Teesdale—Survival of relict arctic–alpine flora related to local geology—JOHNSON, ROBINSON (Durham) and HORNUNG (Nature Conservancy Research Station) 453
Cognition—Ability of children to make transitive inferences—BRYANT (Oxford) and TRABASSO (Princeton) 456
Periodicals—Method of finding the optimum selection for libraries—BROOKES (London) 458

LETTERS TO *NATURE*

Physical Sciences

Solar activity—Apollo samples suggest constant proton flux over 10^6 years—LAVRUKHINA and USTINOVA (USSR Academy of Sciences) 462
Quasars—Faraday rotation consistent with local distances—ARP (California Institute of Technology) . 463
Black holes—Possible occurrence in binary systems—GIBBONS and HAWKING (Cambridge) . . . 465
Palaeotemperatures—In the Arctic Ocean—HERMAN (Washington State), GRAZZINI (Paris) and HOOPER (Washington State) 466
Cariaco Trench—Palaeoclimatic significance of its stagnation—ADAM (Arizona) 469
Semiconductors—Monolithic light entering diode arrays using gallium phosphide—CARTER, MOTTRAM, PEAKER, SUDLOW and WHITE (Ferranti Ltd) . 469
Drag reduction—Value of adhesive tapes—BARNARD and PRITCHARD (Essex) 470

Biological Sciences

Thermal death—Quantitative evidence that protein denaturation is the cause—ROSENBERG, KEMENY, SWITZER and HAMILTON (Michigan State) . . 472
Arylsulphatase — Preparation and characterization of an arylsulphatase insolubilized on porous glass—WEETALL (Corning Glass Works) 473
Cyclic AMP—Possible role in short term regulation of adrenal steroidogenesis—BERGER (Children's Hospital, Rondebosch) 474
Hepatotoxic alkaloids—Effects of pyrrolizidine alkaloids mimicked by synthetic compounds—MATTOCKS (MRC, Carshalton) 476
Drug addiction—Time course and reversibility of physical dependence on opioid narcotics in mice—CHENEY and GOLDSTEIN (Stanford) 477
Sperm antigens—Serological demonstration of H-Y (male) antigen on more than 50% of mouse sperm—GOLDBERG, BOYSE, BENNETT, SCHEID and CARSWELL (Cornell and Sloan-Kettering Institute) 478
Steroids—Effect on the level of zinc in the blood—BRIGGS, BRIGGS (Zambia) and AUSTIN (College of Technology, Belfast) 480
Meiosis—Studies of the seminal fluid of humans with normal sperm count and oligospermia—SPERLING and KADEN (Free University, Berlin) 481
Glandular secretion—Material from flour moth larvae contains a pheromone and elicits oviposition in a parasite—CORBET (London) 481
Sex chromosomes—Presumptive X monosomy in black rats from Malaysia—HOI-SEN (Malaya) . . . 484

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 442 to 447.

(*Continued overleaf*)

For protein synthesis

A choice of three carbon-14 labelled amino-acid mixtures

Equimolar mixture for t-RNA
Specially provided for those studying acceptor-activity of t-RNA. An equimolar mixture of amino-acids-C14(U), each amino-acid having a specific activity accurately adjusted to 10mCi/mmol.
Code No CFB : 152

High specific activity mixture
A mixture of individually purified amino-acids uniformly labelled with carbon-14. The amino-acids are mixed in approximately the same proportions as in typical algal protein hydrolysate. The current batch has an isotopic abundance of carbon-14 of 87% (54 mCi/matom of carbon).
Code No CFB : 104

Purified Protein Hydrolysate – for economy
Protein hydrolysate-C14(U)—obtained by the acid hydrolysis of algal protein. The products of hydrolysis are extensively purified by ion exchange and paper chromatography to give an amino-acid content of not less than 95%. This is still the most economical protein labelling mixture available.
Code No CFB : 25

Sole supplier

The Radiochemical Centre is the only radiochemicals supplier able to offer you a readily available choice of products in this field. If you are interested in protein synthesis (or any other application of radiochemical products) please write for further information.

The Radiochemical Centre Amersham England

0514/5/71

Page

Mosquito—Frequency of blood feeding in *Aedes aegypti* — McCLELLAND (California) and CONWAY (London) 485

Pest control—Effects of mimics of juvenile hormone on grain insects—PALLOS, MENN, LETCHWORTH and MIAULLIS (Stauffer Chemical Company) . . 486

Proteins—Evolution and natural selection—CLARKE (Nottingham) 487

Cephalopod—Deepest photographic evidence of an abyssal species—JAHN (US Naval Oceanographic Office) . 487

Fossil elk—Late Glacial skeleton associated with barbed points — BARNES, EDWARDS, HALLAM and STUART (Lancashire Record Office and Manchester) 488

Primates—Relationships of *Amphipithecus* and *Oligopithecus*—SIMONS (Yale) 489

Identification keys—Selection of tests allowing for equivocal or unknown test results—GOWER (Rothamsted Experimental Station) and BARNETT (Food Research Institute) 491

Cell walls—Electron microscope and optical diffraction studies on *Chlamydomonas*—HORNE, DAVIES, NORTON and GURNEY-SMITH (John Innes Institute) 493

Protein source—*Wolffia arrhiza* as a possible source of cheap protein—BHANTHUMNAVIN (Mahidol) and McGARRY (Asian Institute of Technology) . . 495

Page

Hypogonadism—Effect of purified luteinizing hormone releasing factor on normal and hypogonadotrophic anosmic men—NAFTOLIN, HARRIS and BOBROW (MRC, Oxford) 496

Ewe fertility—Haemoglobin type of marino ewes grazing oestrogenic pastures—OBST, SEAMARK (Adelaide) and McGOWAN (Australian Department of Agriculture) 497

Tetrahydrocannabinol—Effects on behavioural tasks in rats—WEBSTER (Addiction Research Foundation, Toronto), WILLINSKY (Toronto), HERRING (Addiction Research Foundation, Toronto) and WALTERS (Toronto) 498

Sponge—Mechanism of stratification of siliceous spicules —SCHWAB (Owens-Illinois Technical Center) and SHORE (Toledo) 501

BOOK REVIEWS

by Lancelot Law White, Mark S. Bretscher, G. Ainsworth Harrison, Anthony Robertson, S. Zeki, M. J. Tucker, W. J. Megaw and R. F. Carswell . . 503

CORRESPONDENCE

Scientists' Salaries (Anon)/Baffling Brevity (Tom Correyback)/Micrometeoroid Muddle (Friedrich Horz) . 507

Announcements/University News/Appointments/Miscellaneous/International Meetings 508

Next Monday's issue will include

Pulsar glitches — Mechanism for Crab Nebula pulsar — SCARGLE (California) and PACINI (Frascati)

Radio sources—Precise positions of twenty-eight sources—SMITH (Cambridge)

Solar corona—Comments on radioactive decay of neutrons as an energy source—CHUPP (New Hampshire)

X-ray sources—Rotating magnetic white dwarfs can be X-ray emitters—APPARAO (Tata Institute)

Catalysis—Effect on an alumina support on a $CuCl_2$ catalyst—BLANCO, BLANCO, CORTES and SORIA (Instituto Rocasolano, Madrid)

Next Wednesday's issue will include

Radiation damage—Repair by mammalian daughter cells after potentially lethal treatment—BELLI (Harvard)

Immunoglobulins—Detection of alpha, kappa and lambda chains in mammalian immunoglobulins using fowl antisera to human IgA—ORLANS (Cambridge) and FEINSTEIN (ARC, Cambridge)

Vacuoles — Lysosomal activities in damaged and recovering plant cell vacuoles—VILLIERS (Natal)

Yeasts—Selection of tests for identification—BARNETT (Food Research Institute)

Bone—Piezoelectric effect at p-n junction—WILLIAMS and PERLETZ (Illinois)

Tweeddale Court
14 High Street
Edinburgh EH1 IYL

Oliver & Boyd

SALMON AND TROUT

A Resource, its Ecology, Conservation and Management

Derek H. Mills

Exploitation and pollution; hydro-electric development and erosion; just a few of the factors which affect the salmon and trout fisheries. So what of the future, when without the continued and enlightened efforts of man, the exploiter and polluter, there will be none.

Salmon and Trout is *the* book for anyone associated or interested in the present state and future development of the salmonid industry. Containing all the essential knowledge for the study, conservation and management of game fish, including a summary of the development of the high seas salmon fishery and methods for regulating stocks in home waters—it is a must for the scientist and layman alike.

372 pages lavishly illustrated £4.00

PL-1 PROGRAMMING IN TECHNOLOGICAL APPLICATIONS

by **Gabriel F. Groner,** The Rand Corporation, California

Describes how to write Programming Language One computer programmes for solving technical problems. Motivates the engineer, scientist, or student by introducing him to programming early in the book, then expanding his skills through numerous relevant examples and exercises at the end of each chapter.

June 1971 442 pages £4.75 (cloth)
£3.50 (paper)

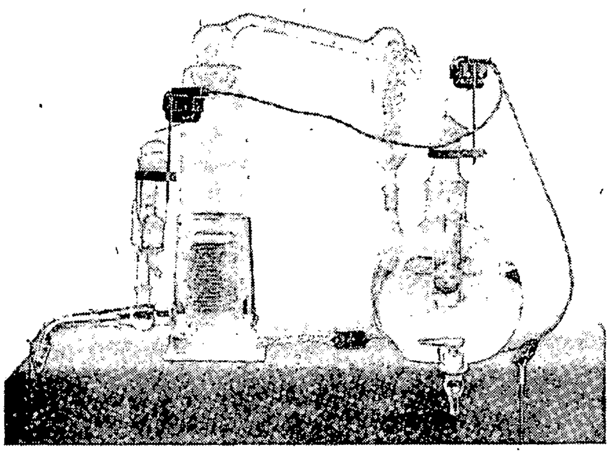
Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex

Display Advertising for

Nature

USA
NATURE SCIENTIFIC PUBLICATIONS INC.
711 National Press Building
Washington DC 20004
202 737-2355

Elsewhere
T. G. SCOTT AND SON LIMITED
1 Clement's Inn, London WC2A 2ED
01-242 6264



This unique water still is in use in a number of research establishments and hospitals where water of the highest purity is essential.

PRICE, complete with heater and all electrical equipment 230 volt (a.c. only)—**£45.00**

Obtainable only from

L. V. D. SCORAH, M.Sc.
(Manufacturer of the "SCORAH" Blowpipe)
44 NORTHFIELD ROAD, KINGS NORTON,
BIRMINGHAM, 30 021-458 1885

The SCORAH Automatic Electric all glass WATER STILL (patented)

Automatic Can be left on without attention. Heater current cuts off if water supply is interrupted, or inadequate or forgotten. When receiver (5 litres capacity) is full, current cuts off. When distilled water is drawn from receiver, the still resumes operation until receiver is refilled.

Purity Electrostatically charged condenser droplets can pick up fumes, dust and air-borne organisms from the laboratory atmosphere. The design of the receiver vessel ensures absence of such contamination.

Efficiency Heater of Scorah design and manufacture, consumption 1,700 watts, output 2.4 litres per hour (the full theoretical yield). Cost 3½d. per gallon at 1d. per unit.

Safety The heating element is out of contact with the feed water, thus eliminating danger from "live" water supply or electrolytic effects or "burn out".

Assembly No retort stands or clamps are required. The 2½-in. diameter ball joint gives a strainless set-up. Outlet cap on boiler for quick periodical drain-off of sediment. The still may be installed on a shelf or platform about 1 ft. above bench level near a sink and enclosed in a transparent plastic cover to keep out dust.
Phoenix heat-resisting glass. Interchangeable ground glass joints.

Pure Che :stry Librar
SCIENCE COLLEGE
92, Upper Circular Road
CALCUTTA

NATURE

MACMILLAN JOURNALS LTD

No. 5312 FRIDAY AUGUST 20 1971 Vol. 232

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page
Waiting for Lord Rothschild 509
Ducks and Drakes 511

OLD WORLD

Census Returns/Scientists' Pay/Box Girders/Atomic Energy/Foundations/Conservation/United Nations/Archaeology/Industrial Archaeology 512

NEW WORLD

NSF and NASA get their Money/Higher Education . 517

NEWS AND VIEWS

New Ways with Volcanoes?/Earthquakes/Visna Virus/Jupiter/Barn Owl/Bacteria/Ichthyology/Toxins/Sea-floor Spreading/Biological Materials 519
NRDC and the Environment for Innovation—HAIGH PEARSON, WATKINS (Manchester Business School) and GIBBONS (Manchester) 527
Lithium in Psychiatry—PEARSON (Sheffield) and JENNER (MRC, Sheffield) 532
Perspectives in Organometallic Chemistry—STONE (Bristol) 534
Lightning—Satellite data from OSO-5—SPARROW and NEY (Minnesota) 540
Neural connexions—Functional reorganization after partial deafferentation in the rat—WALL and EGGER (London) 542
Adiabatic decompression—Effect on temperature changes in geological processes—WALDBAUM (Princeton) 545

LETTERS TO *NATURE*

Physical Sciences

Ehrenfest's Paradox—The rotating disk—WEINSTEIN (Houston) 548
Earth—Geomagnetic dynamos in a stable core—BULLARD and GUBBINS (Cambridge) 548
—Correlation between variations in magnetic intensity and climatic changes—WOLLIN, ERICSON and RYAN (Columbia) 549
Peculiar A star HR 465—Spontaneous fission of super-heavy elements as an explanation of the observed promethium abundance—KUCHOWICZ (Warsaw) . 551
Page
Fission products—Tungsten-181 and other products in ground level air in Northern Europe—KOLB (Brunswick) 552
Lead pollution—Composition of airborne particles—TER HAAR (Ethyl Corporation) and BAYARD (Walter McCrone Associates Inc.) 553

Biological Sciences

Atherosclerosis—Effect of inclusion of cellulose in an "atherogenic" diet on the blood lipids of children—SHURPALEKAR, DORAISWAMY, SUNDARAVALLI and NARAYANA RAO (Central Food Technological Research Institute, Mysore) . . 554
Sex chromosomes—Cytological evidence for the association of the short arms at *X* and *Y*—CHEN and FALEK (Emory) 555
***Y* chromosome**—Para-nucleolar position in interphase nuclei—BOBROW, PEARSON and COLLACOTT (MRC, Oxford) 556
Leukaemia—Evidence for a genetic factor in pigs—McTAGGART, HEAD and LAING (Edinburgh) . 557
Cell division—Comparison of lines transformed by different agents in medium lacking serum growth factor—SMITH and SCHER (US National Institutes of Health) 558
Mycoplasma—Incidence of infection in guinea-pigs—HILL (MRC, Carshalton) 560
Interferon—Transplacental transmission in mice inoculated with influenza or Newcastle disease virus—KORSANTIYA and SMORODINTSEV (All-Union Research Institute of Influenza, Leningrad) . . 560
Malaria parasites—Investigation of genetic recombination in new strains—WALLIKER, CARTER and MORGAN (Edinburgh) 561
Thirst—Frequency analysis of control—OATLEY and TOATES (Sussex) 562
Ammonoids—One, two or three links connecting the Triassic and Jurassic faunas?—TOZER (Geological Survey of Canada) 565
Plesiadapis—Reply to Szalay—GINGERICH (Yale). 566
—CARTMILL (Duke) . 566

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 520 to 526.

Pure Che :stry Librar
SCIENCE COLLEGE

(Continued overleaf)

THE PHYSICS OF CLOUDS

R. B. MASON

This book has been rewritten and expanded to take account of research done up to the end of 1969, both experimental and theoretical, on the microphysical processes of nucleation, condensation, droplet growth, initiation and growth of snow crystals, and the mechanisms of precipitation release. Rain-making experiments are appraised and there are chapters dealing with radar studies of precipitating clouds and the electrification of clouds. It is both a textbook for the student and a reference book for the research worker, primarily for the atmospheric physicist, although it contains much of interest for those working on nucleation phenomena, crystal growth, and aerosol physics.
Second edition 40 plates 143 text figures £12

**OXFORD
UNIVERSITY
PRESS**

READINGS IN MOLECULAR BIOLOGY

Selected by Dr. W. B. Gratzer

A topical review which puts molecular biology research under the microscope

The book is a selection of over 60 articles describing the significant experiments and advances in molecular and cell biology, virology and biochemistry during the past year. The reports first appeared in *Nature* during 1969 and 1970 as weekly contributions by the Molecular Biology, Cell Biology and other specialist correspondents to the journal. They take the form of short and lively reviews of topics in a broad area of biological research and are the first contributions of their kind to the scientific literature in which scientists write critical appraisals of their colleagues' work.

The wide-ranging subjects they discuss are necessarily diverse and include

nucleic acids
membranes
replication
enzymes
cells
techniques

The book will be of value to the research worker as a record of progress in his field, and to the third year undergraduate as a comprehensive introduction to the changing pattern of molecular biology.

SPECIAL OFFER

By special arrangement with the publishers, Macmillan and Co. Ltd., *Nature* is able to offer its readers copies of READINGS IN MOLECULAR BIOLOGY at the special rate of £1 (inc. p. & p.). If you wish to place an order for the book, please complete the form below and return it with your remittance.

(American distributor M.I.T. Press)

Please send me................copy/copies of READINGS IN MOLECULAR BIOLOGY at £1 net each.
I enclose a remittance for £...........
NAME ....
ADDRESS ....
....
....

Please return this order form to:
Nature, Macmillan Journals Ltd., Little Essex Street, LONDON WC2R 3LF, England.

176 pages Published by Macmillan and Co. Ltd.

Page

Hominines—Is Vertesszöllös II an occipital of *Homo erectus*?— WOLPOFF (Case Western Reserve) . 567

Seeds—New evidence that *Canna* seed from Argentina was 600 years old when germinated—LERMAN (Bern) and CIGLIANO (La Plata). 568

Isozymes—Variation under selection in *Zea mays*—BROWN (York) 570

Shrubs—Ecotypes in the salinity gradient of a salt desert—GOODMAN (Welsh Plant Breeding Station) and CALDWELL (Utah) 571

Blood flow—Arrest by ultrasound—DYSON, WOODWARD and POND (London) 572

Animal behaviour—Defence and territorial behaviour dissociated by hypothalamic lesions in the rat—ADAMS (Wesleyan) 573

Cosmic ray muons—Production of visual sensations—CHARMAN and ROWLANDS (Manchester) . 574

Anticholinergic drugs—Absolute configuration and crystal structure of dexbenzetimide—SPEK, PEERDEMAN (Utrecht), VAN WIJNGAARDEN and SOUDIJN (Janssen Pharmaceutica) 575

Bone fracture—Effects of compression at temperatures between −200° C and +200° C—ARMSTRONG, ARKAYIN and HADDAD (Maryland) . . . 576

Page

Prebiotic synthesis—Cystine formed in simulated primitive conditions—KHARE and SAGAN (Cornell) . . 577

Hashish—Two new constituents, cannabivarin and tetrahydrocannabivarin—MERKUS (RC Hospital, Sittard) 579

Narcotics—Acid monoamine metabolites in cerebrospinal fluid during methadone maintenance—BOWERS, KLEBER and DAVIS (Yale) 581

Cnidaria—Life cycle of *Tripedalia cystophora* Conant (Cubomedusae)—WERNER (Biologische Anstalt Helgoland), CUTRESS and STUDEBAKER (Puerto Rico) 582

BOOK REVIEWS

by J. G. Crowther, S. B. Barnes, Bryan Clarke, V. M. Craddock, W. H. R. Lumsden, A. S. V. Burgen, R. Mason 584

CORRESPONDENCE

Metric Chains (S. D. Steel)/Telescope Administration (A. Hunter), (John Lyons)/ Orbital Planes of Planets (Ernest H. Wells, Daniel Payne Hale) 588

Obituary—Lord Boyd Orr 589

Announcements—International Meetings . . . 590

Next Monday's issue will include

Crab Pulsar—Smith's model criticized—MANCHESTER and TADE MARU (NRAO)

Lasers—Inhibition of gold deposition by intense optical standing waves—LITTLE, KEY, WILTSHER and ROWLEY (London)

Matrix Raman spectroscopy—Depolarization measurements and isotope splitting—HUBER, OZIN and VANDER VOET (Toronto)

Ideal liquids—Empirical relation for temperature dependence of compressibility—GROSSE (Germantown Laboratories Inc)

Ivory Coast microtektites—Corrected values of uranium contents—DURRANI and KHAN (Birmingham)

Next Wednesday's issue will include

Possible Mechanisms of *X* chromosome Inactivation—LYON (Cambridge)

Mutants—1 : 1 distribution between mutants induced by ultraviolet and phenocopies—FJELD (Oslo)

Mycoplasmas—Modification of amino-acid concentrations induced in cell culture medium—STANBRIDGE, HAYFLICK (Stanford) and PERKINS (MRC, London)

Blood coagulation—Role of leucocytes in the Schwartzman reaction—NIEMETZ (VA Hospital, Bronx) and FANI (New York)

Carrier effect—Cooperation between carrier-reactive and hapten-sensitive cells *in vitro*—CHEERS, BREITNER, LITTLE and MILLER (Royal Melbourne Hospital)

Proceedings of the International Conference on

ALTERNATIVE INSECTICIDES FOR VECTOR CONTROL

Atlanta, Georgia

15–19 February, 1971

Sponsored by Emory University, the Center for Disease Control, and the World Health Organization

published in

BULLETIN OF THE WORLD HEALTH ORGANIZATION

Volume 44, Nos. 1–3

Sw. fr. 30.— £10.00 $3

This special issue brings together the most recent information on the design, mode of action, and toxicity of insecticides, with particular attention to anticholinesterase insecticides that might serve as alternatives to DDT. Pyrethroids, DDT analogues and insect hormones are also covered.

Orders may be addressed to:

Her Majesty's Stationery Office, P.O. Box 569, London S.E.1.

or

World Health Organization
Distribution and Sales Service
1211 Geneva 27, Switzerland

'MICHROME'

STAINS & DYES

for use in Biology, Medicine, Chemistry and as tools of Research, Production and Quality Control in a variety of Industries.

Over 2,000 Stains, Dyes, etc. kept in stock including:

Acridine orange, Azocarmine, Biebrich scarlet R, Brilliant green, Aurantia, Celestine blue, Crystal violet, Cyanine, Aurine tricarboxylic acid, Dicyanine, DPX, p-Ethoxychrysoidine, Fast ponceau L salt, Fast Bordeaux O, Giemsa, Fluormount, Fluoroil, Cresyl fast violet, Lacmoid, Leishman, Janus green, Fluorescein, Nile blue, Pinacryptol green, Methyl red, Matamount, Leishman, Papanicolaou, Optoil, Pyronin Y (G), Rhodanile blue, Rosolic Acid, Shorr, Thionine, Quinacrine mustard dihydrochloride, etc.

Catalogue available on request

INDUSTRIAL RESEARCH ESTABLISHMENTS & COMMERCIAL ORGANIZATIONS

are invited to make use of our professional services as consultants on the selection and use of dyes as research and production tools.

EDWARD GURR LTD.

42, Upper Richmond Rd. West, London, SW14
Cables: Micromlabs, London SW14. Telephone: 01-876-8228

QUALITY — RELIABILITY — SERVICE
PROMPT DELIVERY

COMPLEX VARIABLE METHODS IN ELASTICITY

by **A. H. England,** *Department of Theoretical Mechanics, University of Nottingham*

Deals with the plane strain and generalized plane stress boundary value problems of linear elasticity; these problems are formulated and solved using complex variable theory. In this book the basic representation of the displacement field in terms of functions of a complex variable is derived directly from the equations of elasticity. Also the three basic methods relying on series representation, Cauchy integral representation, and the solution via continuation, are all described in detail. Worked examples and sets of problems are included.

July 1971 192 pages £5.25

Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex

BACK VOLUMES OF SCIENTIFIC PERIODICALS

We are always interested in buying your collections of duplicates of Scientific, Technical and Medical Journals at the highest prevailing prices. Please send us your lists of surplus journals, which will receive our immediate attention

We are Distributors of Back Issues of Journals including Pergamon Press Journals and many other Publishers. We invite you to write in for our regularly issued Bulletins and Catalogues and should be pleased to be of assistance in your acquisitions.

ROBERT MAXWELL & CO. LTD.
Back Issues Department
Midland Road, OLNEY, Bucks, England. Tel. Olney 529

Pure Chemistry Library
SCIENCE COLLEGE
92, Upper Circular Road
CALCUTTA

NATURE
MACMILLAN JOURNALS LTD

No. 5313 FRIDAY AUGUST 27 1971 Vol. 232

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737–2355

Page
From Intelsat to Aerosat 591
What Market for Computers? 592
Compromise Without Solution 593

OLD WORLD

Scientists' Pay/Electronics/Engineering/Science Museums/Conservation 594

NEW WORLD

Drug Office Wins Friends, Disarms Enemies/Ailing Health Services/DDT on Mock Trial 596

NEWS AND VIEWS

Rutherford and Geiger Re-evaluated/Earth Sciences Converge in Moscow/Adenoviruses/Photomorphogenesis/Rhipidistia/Continental Drift/Triassic Glider/Sensory Physiology/The Earth 599
Supermassive Objects in Astrophysics — TRIMBLE (Cambridge) 607
North Atlantic—New interpretation of South Labrador Sea in terms of evolution—LAUGHTON (National Institute of Oceanography) 612
Tumour virus—Genome expression of C-type in wild house mice—GARDNER, OFFICER, RONGEY (Southern California), ESTES, TURNER and HUEBNER (Bethesda) 617

LETTERS TO *NATURE*

Physical Sciences

Chandler wobble—Calculations of the effect of earthquakes—SMYLIE (British Columbia) and MANSINHA (London, Ontario) 621
Quasars—Optical variability of bright source B2 1215+30—LUCCHETTI and USHER (Pennsylvania State University) 622
Lasers—Applications in analysing geological material —SCOTT, JACKSON and STRASHEIM (CSIR, Pretoria) 623
Soils — Transformation of hematite to goethite — SCHWERTMANN (Munich) 624
Ball lightning—Support for a physical explanation—WITTMANN (Göttingen) 625
Particle detector—Proposal for a new high precision instrument—RICE-EVANS (Bedford College). . 625

Biological Sciences

Page
Prostaglandin—Myometrial activity inhibited by progesterone—PORTER and BEHRMAN (Harvard) . 627
Semen—Possible nucleic acid and protein synthesis—SHEID and WILSON (New York) . . . 628
Prostaglandins—Possible role for $F_{2\alpha}$ in parturition in sheep—LIGGINS and GRIEVES (Auckland) . 629
Synthetic peptides—Extension of Vane's warning—BANGHAM, CALAM, PARSONS and ROBINSON (National Institute for Medical Research, London) 631
Ovary—Reply to Flint and Denton on the role of reduced TPN in the response to interstitial cell stimulating hormone—HALL (Victoria) . . . 631
Cervical cancer—Relation of chromosome number to malignant potential—KIRKLAND and STANLEY (Woodville, South Australia) 632
Kidney transplants—Transformation of lymph cells *in vivo*—HAMBURGER, DIMITRIU, BANKIR, DEBRAY-SACHS and AUVERT (Hôpital Necker, Paris) 633
Thalidomide—Effect on human embryonic tissues *in vitro*—LASH and SAXÉN (Helsinki) . . . 634
Blastocoele—Perfusion technique for rabbit—CROSS (Pennsylvania) 635
Cell cooperation—Limited synergism between rat thymus and mouse bone marrow—MORGADO and FOLCH (Chile) 637
Graft versus host—Cytotoxic antibody for epithelial cells — MERRITT, MANN and ROGENTINE (Bethesda) 638
Insect immunology—Teratocytes as a means of resistance to cellular defence reactions—SALT (Cambridge) . 639
Immunologic memory—Cellular basis discussed—GERSHON, KRÜGER, NAYSMITH and WAKSMAN (Yale) 639
Polychlorinated biphenyls—Photolysis of 2, 4, 6, 2′, 4′, 6′-hexachlorobiphenyl—SAFE and HUTZINGER (National Research Council of Canada) . . . 641

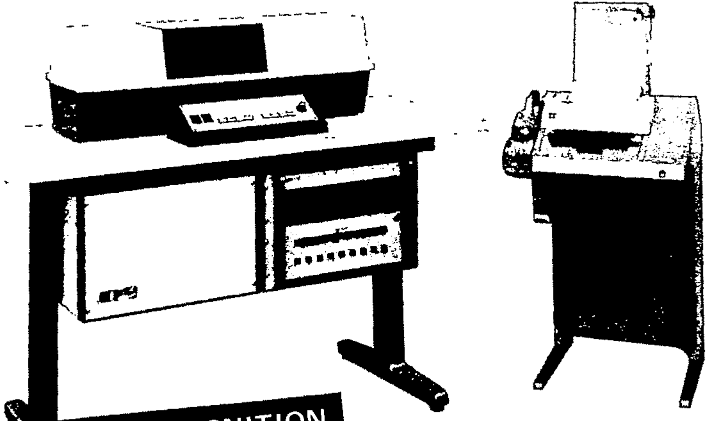
Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 601 to 606.

(Continued overleaf)

the *VERY BEST* high-quality image processing system for your dollars

- RASTERS AND APERTURES TO 12.5 MICRONS
- ORTHOGONAL SAMPLING GRID
- PHOTOGRAPHIC DENSITY RANGE 0–3D
- DATA RATES TO 60 KC
- FORMATS TO 10" x 10" FLEXIBLE FILM
- GRAY LEVEL RESOLUTION: READ MODE – 256 PARTS
  WRITE MODE – 64 PARTS

REMOTE SENSING



PATTERN RECOGNITION

Photomation Mark II

An electro-mechanical high-speed, high-accuracy photographic digitizer and plotting system for image processing and/or generation. Operates on-line and can be computer-controlled, or interfaced directly to magnetic tape.

CONTOUR PLOTTING

Photoscan

An electro-optical drum scanner with high-accuracy, bidimensional position in micron range for rapidly digitizing photographic data on film negatives or positives. Typical scan time for 100 micron raster of 5" x 5" format is 5 minutes.

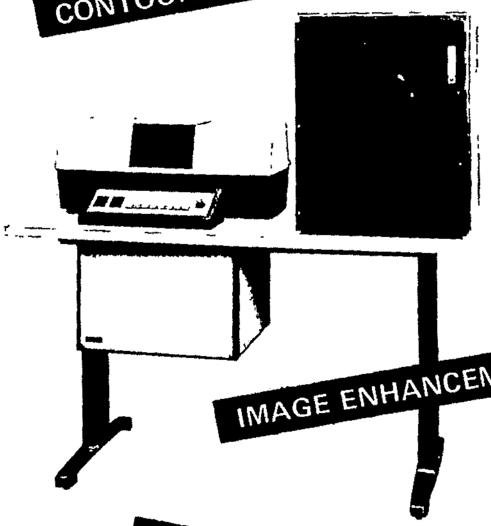
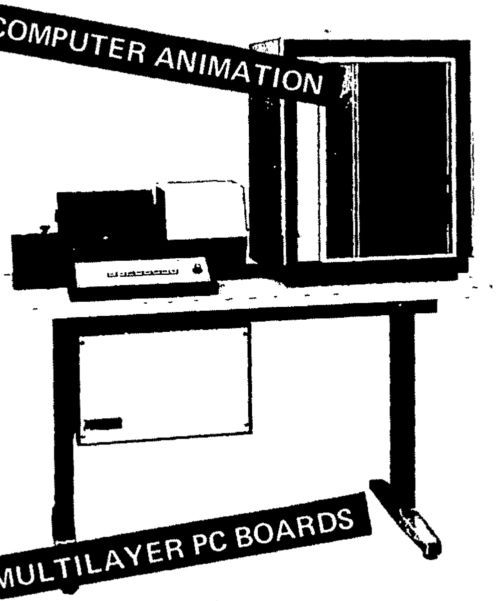


IMAGE ENHANCEMENT

COMPUTER ANIMATION

MAP MAKING



X-RAY CRYSTALLOGRAPHY

Photowrite

An electro-optical high-speed digital film writing system. Digital information from magnetic tape or directly from a computer modulates the beam from a light source and exposes the film producing up to 64 repeatable gray levels. Typical reconstruction time for a 5" x 5" format at 100 micron raster is 2 minutes.

MULTILAYER PC BOARDS

OCEANOGRAPHY

The Leader in Computer-Oriented Image Processing Instrumentation

---

OPTRONICS INTERNATIONAL, INC.

7 STUART ROAD, CHELMSFORD, MASS. 01824 USA (617) 256-4511 TLX 94-7443

Offices and Reps

Page

Insecticides—Resistance to carbamates and organophosphates in *Anopheles albimanus*—ARIARATNAM and GEORGHIOU (California) . . . 642

Heartworms—Acceptability of daily *l*-tetramisole by pound dogs infected with *Dirofilaria immitis*—TULLOCH and ANDERSON (Texas) . . . 644

Insecticides—Steric model used to design degradable insecticides—HOLAN (Melbourne) 644

Plant growth—Photomimetic effect of acetylcholine—GRESSEL, STRAUSBAUCH and GALUN (Rehovot) 648

Oxygen uptake—Two distinct effects of blue light in *Chlorella*—SARGENT and TAYLOR (Ontario) . 649

Ancient America—Possible ball court at La Venta, Mexico—WYSHAK, BERGER, GRAHAM and HEIZER (California) 650

Heart beat—Regulation of strength by sodium and potassium concentrations—GADSBY, NIEDERGERKE and PAGE (London) 651

Host antigens—Appearance on *Schistosoma mansoni* cultivated with human cells *in vitro*—CLEGG, SMITHERS (National Institute for Medical Research) and TERRY (Brunel) 653

Page

Alcohol adaptation—A reversible biphasic response by goldfish—GOODWIN, DOWD and GUZE (Missouri) 654

Acanthaster planci—Larval development and metamorphosis—HENDERSON (Brisbane) and LUCAS (Townsville). 655

Culex pipiens—A new hypothesis for the cause of cytoplasmic incompatibility—YEN and BARR (Los Angeles) 657

BOOK REVIEWS

by R. D. Martin, C. R. B. Joyce, B. E. Juniper, W. H. Rees, J. R. Cann, J. Sutton, D. F. Brewer, J. Farradane 659

CORRESPONDENCE

Chemical Societies (J. C. Williams) / Smoking and Cancer (C. B. Goodhurst 663

Announcements—University News/Miscellaneous. . 664

Next Monday's issue will include

Lake District—Relationship between the Skiddaw and Borrowdale Volcanic groups—HELM (Goldsmiths' College) and ROBERTS (Birkbeck)

Rayleigh scattering—Caused by obliquely oriented uniform needles—SHAH (TIFR, Bombay)

Polyhalite—Recent polyhalite from Sebkha el Melah—PERTHUISOT (Paris)

Particles—Absolute determination of size and shape—CHENG and SUTTON (Department of Trade and Industry)

Norwegian sea—New crustal model—HINZ (Hannover) and MOE (Bergen)

Next Wednesday's issue will include

Myeloma proteins—Structural differences in hinge region of subclasses of γA myeloma proteins—ABEL and GREY (Denver)

Myofibrillar ATPase—Neural control in rat skeletal muscle—BULLER (Bristol), MOMMAERTS and SERAYDARIAN (Los Angeles)

Bacteriochlorophylls—Found in gliding filamentous prokaryotes from hot springs—PIERSON and CASTENHOLZ (Oregon)

Hemiplegia—Dysfunction of lower motor neurone associated with atrophy—McCOMAS, SICA, UPTON, AQUILENE and CURRIE (Buenos Aires)

Membranes—Evidence against hydrogen-calcium competition model for activation of electrically excitable membranes—EHRENSTEIN (Bethesda) and FISHMAN (Woods Hole)

Advances in Biomedical Engineering Volume 1

edited by R. M. Kenedi

July 1971, x+252 pp., £5.00

This new *Advances* series aims to present significant pioneer work of a wide range, from research to clinical and technological applications in this new multidisciplinary field. It will also provide a review of the existing state of the subject as well as presenting further additions to present knowledge.
The series will be of particular interest to clinicians, practitioners, teachers, researchers and students at all levels of medicine, biology and engineering. It will provide an invaluable source of reference and it is hoped that the material included will help to further applications and stimulate interest and research in this field.

Scientists and the Sea 1650-1900

A Study of Marine Science

Margaret Deacon

June 1971, xvi+446 pp., £5.50

During the past 30 years the oceans have become one of the principal areas of scientific exploration. However, little has been heard of the origins of marine science. This book traces its growth from remote beginnings in the ancient world, through the scientific revolution of the seventeenth century to the point reached late in the nineteenth century when it at last achieved recognition as an independent scientific discipline. 'Scientists and the Sea' takes a new look at the men who took part in the development of this science, including Robert Boyle, Robert Hooke, Sir Isaac Newton, Sir Joseph Banks, William Whewell and T. H. Huxley.

East African Mammals Volume 1

An Atlas of Evolution in Africa

Jonathan Kingdon

July 1971, x+446 pp., £12.00

This is the first of a three volume work. It has an introduction to the East African environment but deals mainly with the primates. Mammalian evolution is the central theme; but the work aims to be a comprehensive inventory, textbook and atlas as well. This volume relates existing knowledge of geography, vegetation and anatomy to mammology and to the evolution of the species. *East African Mammals* presents a broad background for the student of mammology and much new information about species. This contribution to the understanding of evolution on the African continent will be of interest to zoologists, naturalists, veterinary and museum workers, primatologists and paleontologists.

Methods in Microbiology Volume 5B

edited by J. R. Norris and D. W. Ribbons

July 1971, xiv+696 pp., £9.50

Contents
The disintegration of micro-organisms. Centrifugal techniques for the isolation and characterization of sub-cellular components from bacteria. Chemical analysis of microbial cells. Chemical extraction methods of microbial cells. Biphasic separation of microbial particles. Separation and purification of proteins. Zone electrophoresis of the separation of microbial cell components. Free-flow electrophoresis. Disc electrophoresis. Isoelectric focusing and separation of proteins. Reflectance spectrophotometry. Base composition of nucleic acids. Author index. Subject index.

Symposia Mathematica Volume 6

Published by The Italian National Institute of Higher Mathematics

July 1971, vi+396 pp., £7.30

Internationally known scholars have presented original, unpublished works on diverse scientific and mathematical questions. Functional analysis, evolutionary problems in the solar system, number theory, homological algebra, categorical algebra and algebraic topology are some of the broad fields covered in recent volumes. Mathematicians working directly with the theories discussed in these papers will find them valuable and interesting; physicists, astrophysicists and engineers will also find them useful for further scientific studies.

Academic Press London and New York

Berkeley Square House, Berkeley Square
London WIX 6BA, England

111 Fifth Avenue
New York, NY 10003, USA

NATURE

MACMILLAN JOURNALS LTD

No. 5314 FRIDAY SEPTEMBER 3 1971 Vol. 233

LONDON

4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON

711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Pure Chemistry Library
SCIENCE COLLEGE
92, Upper Circular Road
CALCUTTA

Page

Making Water Run Uphill 1

The Next Step with Nuclear Explosions 2

Making Voters of Universities 3

OLD WORLD

Pollution/Employment/Industrial Relations/River Flooding 4

NEW WORLD

Patent Policies Aim to Provide Incentives to Industry/Dermatology/Alaska/Short Notes 6

NEWS AND VIEWS

Detecting Underground Nuclear Explosions at Last?/Ocean Floor/Astronomy/X-ray Astronomy/Minicells/Haemoglobin/Upper Mantle/Central Nervous System/Chemosystematics 8

Glaciation and the Stones of Stonehenge—KELLAWAY (Institute of Geological Sciences) 30

Was there really a Big Bang?—BURBIDGE (San Diego) 36

Anomalous redshifts—A new interpretation and ideas on the nature of mass—HOYLE and NARLIKAR (Cambridge) 41

Plate tectonics—Origin of the Alps and the western Mediterranean—HSÜ (Zurich) 44

Allostery—Molecular mechanisms of chromosome pairing, folding and function—COMINGS and RIGGS (Duarte, California) 48

LETTERS TO *NATURE*

Physical Sciences

Earthquakes—Discrimination of earthquakes and explosions using short period seismic array data—ANGLIN (Ottawa) 51

Black holes—Difficulty in retaining detectable magnetic fields—WALD (Princeton) 52

Infrared astronomy—Map of galactic nucleus at 10 μm—RIEKE and LOW (Arizona) 53

Biological Sciences

Page

Sex ratio—Adaptive significance of biased sex ratio in *Orchestia*—WILDISH (New Brunswick) 54

Vision—Stereopsis in the cat—FOX and BLAKE (Tennessee) 55

Prostaglandin E_2—Relation to adrenaline reversal in rat uterus—TOTHILL, RATHBONE and WILLMAN (London) 56

Lepidoptera—Limitations in use of electroantennograms for bioassay of male pheromones—BIRCH (Berkeley, California) 57

Monosodium glutamate—Confirmation of neuronal degeneration in hypothalamic nucleus—BURDE, SCHAINKER and KAYES (Missouri) 58

"Neuromythology"—Comments on neuronal death with ageing—TOMASCH (Shiraz). 60

Polychlorinated biphenyls—Sensitivity of fish ATPases—YAP, DESAIAH, CUTKOMP and KOCH (Minnesota) 61

Pregnancy—Effect on isoantibody response in rabbits—WOODROW, ELSON and DONOHOE (Liverpool) . 62

Sleep—Brain catecholamines and human sleep—WYATT, CHASE, KUPFER, SCOTT and SNYDER (National Institute of Mental Health), SJOERDSMA and ENGELMAN (National Heart Institute) . . 63

Cell differentiation—Induction of mesodermal and endodermal structures and primordial germ cells in *Triturus* ectoderm by vegetalizing factor from chick embryos—KOCHER-BECKER (Heiligenberg) and TIEDEMANN (Berlin) 65

Nitrogen budget—Importance of denitrification and nitrate reduction in sediments of lakes—KEENEY, CHEN and GRAETZ (Wisconsin) 66

Dispersion patterns—Uniform distribution of objects in a homogeneous field—GLASS (Chicago) and TOBLER (Michigan) 67

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 10 to 14.

Pye Unicam has a new automatic enzyme analyser. This is what makes it tick.

It is the new Unicam AC61 Programme Delay Unit, which holds the reacting mixture in a spectrophotometer flow cell from 5 seconds to 5 minutes while the change in absorbance is measured.

The whole system – comprised of an automatic chemical processing unit, a spectrophotometer and a recorder – has been designed for versatility and precision at low cost.

The system is simple to operate. Programming takes only a few minutes. Sample and reagent volumes are controlled by preformed templates. No initial sample preparation is required.

Enzyme reaction rates can be measured and recorded completely automatically at speeds ranging from 12 – 60 an hour, while other chemical analyses can be completed at up to 120 samples an hour.

Above all, the system is backed by methods proved in the Pye Unicam Applications Laboratory and over 20 method sheets are currently available including LDH, CPK, SGOT and SGPT in serum.

Find out more about the Unicam Automatic Analytical System by telephoning or writing to Pye Unicam today.

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

BOOK REVIEWS

Page

by William Cooper, Jack Simmons, D. M. Knight, R. W. Clark, W. A. Coates, A. J. Duxbury, Clive A. Edwards, Christopher Perrins 68

CORRESPONDENCE

Page

Freedom of Scepticism (T. D. H. Sterling)/Weaned from Confusion (F. R. Spratling)/ Alluvial Gold (R. M. McMullen) 73

Obituary—Sir Lawrence Bragg 74

Next Monday's issue will include

X-ray scattering—Compton and Rayleigh scattering from suspensions and slurries—BRAMWELL, CARR-BRION and STUART (Warren Spring Laboratory)

NGC 4151—Photometric measurements at $\lambda = 11$ μm—STEIN (Minnesota) and GILLETT (San Diego)

Ion-exchange resins—Study of ion diffusion by an autoradiographic method—MADI and VARRO (Hungary)

Hydrogen atoms—Produced in carbohydrate photolysis—BOS and BUCHANAN (Victoria)

Cygnus X-1—Optical identification—MURDIN and WEBSTER (Royal Greenwich Observatory)

Next Wednesday's issue will include

Homopolymers—Relationship of biological activity of poly I.poly C and molecular weight—NIBLACK and McCREARY (Connecticut)

Immunoglobulins—Detection of alpha, kappa and lambda chains in mammalian immunoglobulins using fowl antisera to human IgA—ORLANS and FEINSTEIN (Babraham)

Radiation damage—Repair by mammalian daughter cells after potentially lethal treatment—BELLI (Massachusetts)

Muscular dystrophy—Alterations in ganglioside composition of mouse skeletal muscle—MAX and BRADY (National Institutes of Health)

Bone—Piezoelectric effect at p-n junctions—WILLIAMS and PERLETZ (Illinois)

Pure Chemistry Library
SCIENCE COLLEGE.
[illegible] Upper Circular Road
CALCUTTA

MEASURING SOLAR RADIATION ?

Then our range of instruments will interest you.

S.R.I.3 Solarimeter.
S.R.I.3A Actinometer.
S.R.I.3Ph Photo-synthesis Solarimeter.
S.R.I.4 Nett Radiometer.
S.R.I.4U Underwater Nett Radiometer.
S.R.I.5 Solari-Albedometer.
S.R.I.6 Reversing Albedometer.
S.R.I.7 Miniature Nett Radiometer.
S.R.I.8 Linear Nett Radiometer.
S.R.I.9 Soil Heat Flux Plate.
S.R.I.10 Stiletto Heat Flux Plate.

Pre-calibrated elements, and sensors available. Maintenance Service by air mail parts exchange.

We invite your enquiries.

SOLAR RADIATION INSTRUMENTS
21 ROSE STREET, ALTONA, VICTORIA
AUSTRALIA . . . 3018

CURRENTLY FROM GRIFFIN

Prof. F. N. David's
First Course in Statistics

New, enlarged version (regression, correlation and contingency tables) of the Author's tried and popular *Primer*: minimum maths, plenty of examples, mainly from biology. Now No. 31 in Griffin's flexiback series. *Sept 6* **£1.90**

Statistical Papers of
George Udny Yule

Prof. ALAN STUART and Dr. M. G. KENDALL select 12 major papers by the Cambridge pioneer (mainly relevant to social and medical research) for a centenary volume. 460 pp. 11 × 7 in. *Aug. 31* **£6.90**

Distribution Management

Prof. S. EILON and Imperial College colleagues **C. D. T. WATSON-GANDY** and **Dr. N. CHRISTOFIDES** study mathematical modelling and analysis. 248 pp. 68 diags. **£4.50**

Astronomy for Surveyors

Prof. J. B. MACKIE's probably unique textbook: revised **Seventh Edition.** 269 pp. 91 illus. **£3.50**

GRIFFIN

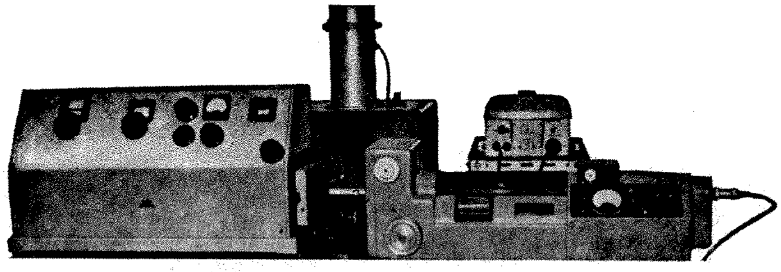
1820

Fully descriptive folders from Chas Griffin & Co. Ltd., 42 Drury Lane, London WC2

BS Precision Instruments

The Pepol 60

O.R.D.



The B-S 'PEPOL 60', a photo-electric polarimeter for Optical Rotatory Dispersion measurement. Specifically designed for O.R.D. work in the visible and u.v. regions, this instrument yields results accurate to ±0.002 angular degrees over the majority of the range 2,200–6,000 Å.

THIS FINE INSTRUMENT CAN NOW BE FOUND IN MANY LEADING RESEARCH AND EDUCATIONAL ESTABLISHMENTS. MAY WE SEND YOU DETAILS?

We now have capacity for aluminising certain types of mirrors, at short notice.

We are the sole distributors in the U.K. of Schott Monochromatic Interference Filters

Please write to Dept. N

BELLINGHAM & STANLEY LIMITED BS

NATURE

MACMILLAN JOURNALS LTD

No. 5315 FRIDAY SEPTEMBER 10 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Prosperity and Science as Chicken and Egg . . . 77

Where Real Trouble Lies 79

OLD WORLD

British Association/Radio Telescopes/Pollution/Nuclear Reactors/Space Science/Environment/Biomedical Research/Graduate Employment 80

NEW WORLD

AEC Causes Anger and Delight/Employment . . . 85

NEWS AND VIEWS

Of Mice and Men/Cancers/Proteins/Permo-Triassic/Reaction Mechanisms/Hepatitis/Aphids/Oceanography 88

Mechanisms of Sound Localization — WHITFIELD (Birmingham) 95

Earthquakes—Their effects on deep-sea sediments—FRANCIS (UKAEA, Blacknest) 98

Leukaemia virus—Group specific antigen in human cell line containing C-type virus—GILDEN, PARKS, HUEBNER and TODARO (Maryland) . . . 102

Hodgkin's disease — Virus-like agents from patients—EISINGER, FOX, HARVEN, BIEDLER and KINGSLEY SANDERS (Sloan-Kettering) . . . 104

LETTERS TO *NATURE*

Physical Sciences

Cygnus XR-1—Eclipsing binary model—DOLAN (Ohio) 109

—Optical identification—NURDIN and WEBSTER (Royal Greenwich Observatory) . . 110

Collapsed objects—Possible black hole in β Lyrae—DEVINNEY (South Florida). 110

Radio sources—New observations of Sgr A—DOWNES and MARTIN (Cambridge) 112

Dinitrogen—Reduction in aqueous systems—HILL and RICHARDS (ARC Unit, Sussex) 114

Atlantic Ocean—Occurrence of biogenic siliceous sediments—RAMSAY (East Anglia and Illinois) . . 115

Page

Fossils—First diagnostic carboniferous fossils from New Zealand—JENKINS (Christchurch) and JENKINS (Sydney) 117

Biological Sciences

Molecular evolution—Production of functional organization of genetic material—KIMURA and OHTA (Mishima) 118

Insecticides—Toxicity of photoisomers of cyclodiene insecticides to freshwater animals—GEORGACAKIS and KHAN (Illinois) 120

Phenotypic masking—Occurrence in drug dependent strains of *E. coli*—QUESNEL, YORK and SKINNER (Manchester) 121

Mitosis—Cell division after laser microirradiation of mitotic chromosomes—BERNS and CHENG (Michigan) 122

Heterosis—Appearance in a single gene after inbreeding in *Drosophila*—WILLS and NICHOLS (Connecticut) 123

Mouse embryos—Survival after freezing and thawing—WHITTINGHAM (Cambridge) 125

Behaviour transfer—Failure to transfer morphine tolerance of passive avoidance by brain extracts—GOLDSTEIN, SHEEHAN and GOLDSTEIN (Stamford) . 126

Cerebrospinal fluid—Formation in spinal subarachnoid space—SATO, ASAI, AMANO, HARA, TSUGANE and YAGI (Nagoya) 129

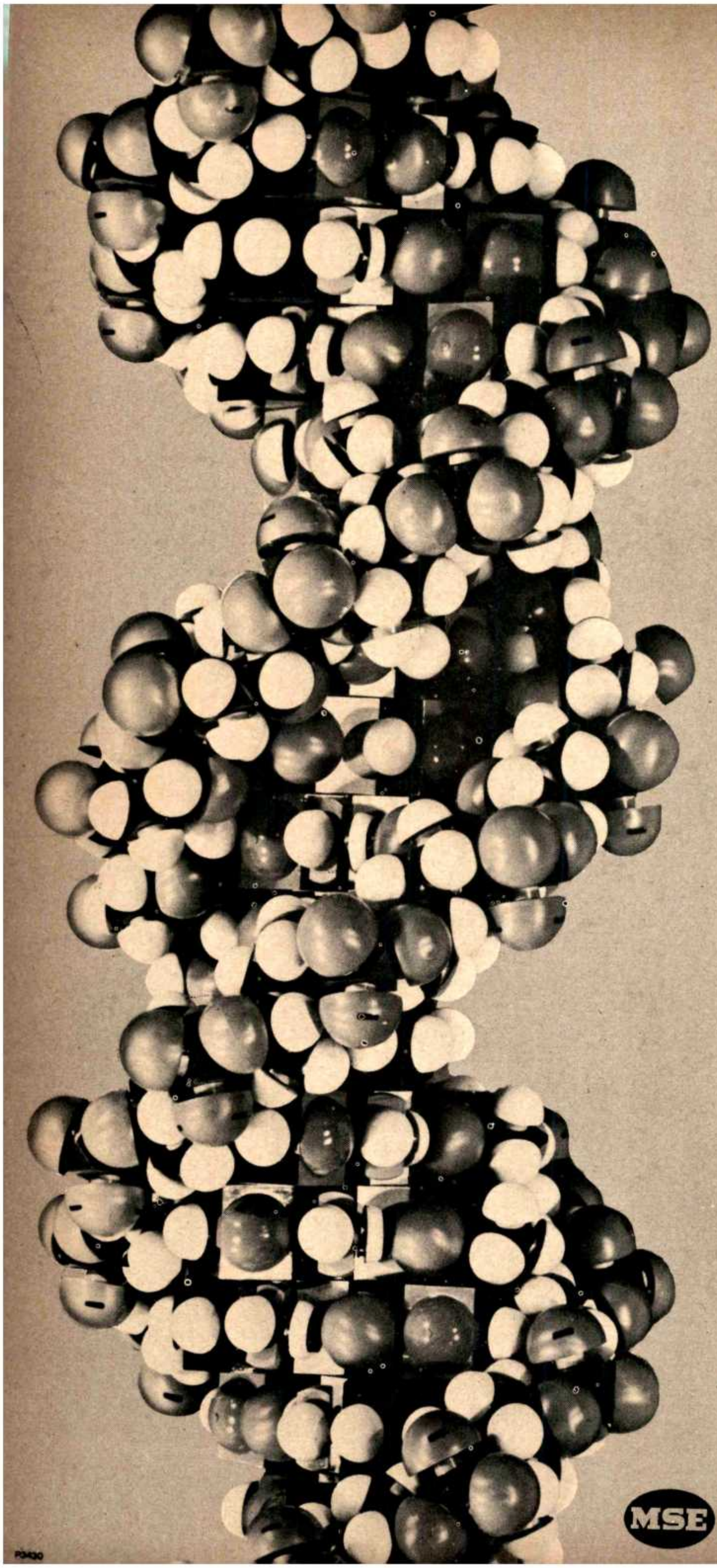
Tapetum—Reflecting spheres in weakfish (Sciaenidae) eyes—ARNOTT (Austin), NICOL (Texas) and QUERFIELD (Colorado) 130

Mycorrhiza—Possible role in litter decomposition—GADGIL and GADGIL (New Zealand) . . . 133

Tumours—Immunological suppression of spontaneous tumours in C3H/He mice—IRIE and IRIE (Tokyo) . 133

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 89 to 94.

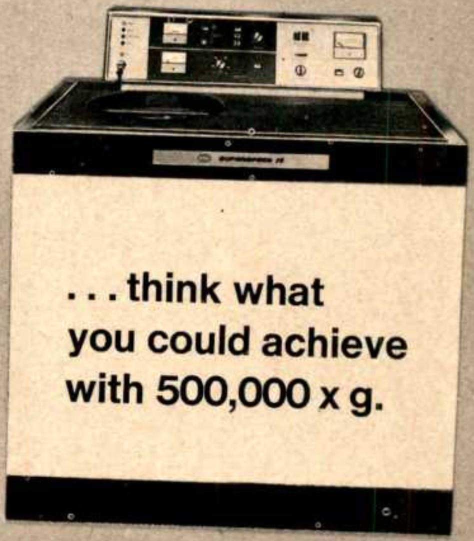
(Continued overleaf)



World health looks to the life sciences . . .

Here is one of our contributions:

Is this the golden age of the life sciences? Recent and continuing research into the molecular anatomy of cells is piercing the mists that have shrouded the chemical basis and control of all living things for centuries. But scientists must have the tools, hence the MSE Super Speed 75 preparative ultracentrifuge. It meets every possible centrifugation requirement of the research scientist—whether it be for the highest g value (511,000 x g at 75,000 rpm with the 8 x 14ml rotor) the best possible combination of capacity and speed (e.g. 75,000 x g at 21,000 rpm with the 6 x 250ml rotor) or for the zonal technique for which the Super Speed 75 will accept all "B" type zonal rotors. Fully automatic, the Super Speed 75 incorporates all those features that scientists themselves, most exacting of people, have asked for.



MSE

Measuring & Scientific Equipment Ltd.

Manor Royal, Crawley, Sussex, England
Telephone: Crawley (STD 0293) 31100

P3430

Page

Chromosome polymorphism—Negro and white populations differ—LUBS (Colorado) and RUDDLE (Connecticut) 134

Ferredoxins—Their role in the origin of life and in evolution—HALL, CAMMACK and RAO (King's) . 136

α-Keratin—Structure by X-ray diffraction—FRASER and MACRAE (Victoria) 138

Myosin—Dimer geometry by analysis of concentration-viscosity dependence in the monomer-dimer equilibrium—BURKE and HARRINGTON (Baltimore) . 140

Sleep—Extended sleep and performance—TAUB, GLOBUS, PHOEBUS and DRURY (California) . 142

Algae—Mutants lacking cell walls are a potential source of protein—DAVIES (Norwich) 143

BOOK REVIEWS

by P. J. Farago, Grenville Turner, J. N. Murrell, Samuel S. Epstein, W. Ashworth, E. W. Elcock, David Pye, Marie Jahoda, G. G. Hall 145

CORRESPONDENCE

Tolerable Pollution (R. E. Eckardt) 149

Obituary—Wendell Meredith Stanley 149

Next Monday's issue will include

Japanese geology—Rifting in the Japanese late Palaeozoic geosyncline—SUGISAKI, MIZUTANI, ADACHI (Nagoya) HATTORI and TANAKA (Kawasaki)

Type 1 Supernovae—Wavelength shifts of intensity minima—BRANCH and PATCHETT (Royal Greenwich Observatory)

Solar wind—Shock free decelaration?—WALLIS (Stockholm)

Next Wednesday's issue will include

Prostaglandins—Model building used to deduce stable conformation—RABINOWITZ, RAMWELL and DAVISON (California)

Immunological tolerance—Induction of "low dose" tolerance to a bacterial somatic antigen in neonatal mice—ALLEN and FRIEDMAN (Pennsylvania)

Immunoglobulin—Interaction of macrophages and lymphocytes with surface immunoglobulin—SCHMIDTKE (California) and UNANUE (Massachusetts)

A revolutionary concept in anatomical illustration . . .

PHOTOGRAPHIC ANATOMY OF THE HUMAN BODY

By Professor Chihiro Yokochi

- *VIVID* . . . completely realistic full-color and black and white photographs of actual human specimens skillfully prepared to show every major system and organ of the human body.
- *CLEAR* . . . descriptive text and imaginative analogs ensure a lasting comprehension of anatomical structure and function.
- *RECOMMENDED* . . . for undergraduate medical students, and for students of nursing, dentistry, technology, and the allied health professions. Written and illustrated to the highest standards of medical publishing.

105 pages in large format (8½" x 12"). Full-color photographs. (Slides available.) 1971. $12.50

Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS IN SCIENCE AND MEDICINE

POLARIZATION INTERFEROMETERS

Applications in Microscopy and Macroscopy

by **M. Françon** *and* **S. Mallick**, *both of the Laboratoire D'Optique, Faculté des Sciences, Université de Paris*

This monograph aims to present in simple language the fundamentals of polarization interferometers, their important applications and the latest developments in the field. As many non-physicists use these instruments a minimum of mathematics is used, and the emphasis is on the physical principles involved. Some technical details and numerical values are given to facilitate the setting up of the instrument in the laboratory. (*Wiley Series in Pure and Applied Optics.*)

July 1971 172 pages £4.50

Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex

RUSSIAN

JOURNALS REPORT ON IMPORTANT SCIENTIFIC & TECHNICAL RESEARCH

Publications in your field include:

BIOCHEMISTRY Ref. 70054.	6 issues yearly £12.50 p.a.
MOLECULAR BIOLOGY Ref. 70562	6 issues yearly £7.50 p.a.
BIOPHYSICS Ref. 70053	6 issues yearly £10.90 p.a.

Usually in Russian but many with English summaries.
Send for 1972 list.

Collet's
Denington Estate,
WELLINGBOROUGH, U.K.

DOMI NVS ILLV MINA TIO MEA

Affinity and Matter

Elements of Chemical Philosophy 1800-1865

T. H. LEVERE

Affinity served as a unifying concept both within chemical theory and between chemistry and physics. It also played a crucial role in the development of the concept of matter, significant for the history of ideas. This book stresses these two aspects of affinity, relating them to each other and to their nineteenth-century intellectual background in science, philosophy, and religion. The development of the concept of affinity is introduced through Newton's legacy to the eighteenth century, and is then studied mainly through the contributions of major figures such as Davy, Faraday, Berzelius, Oersted, and Berthelot. 14 plates 7 figures £4.50

OXFORD
UNIVERSITY
PRESS

NATURE

MACMILLAN JOURNALS LTD

No. 5316 FRIDAY SEPTEMBER 17 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Reform by Inches or Half-inches 151

Opportunities for Collaboration in Europe 152

From Prayer-meeting to Trade Fair 153

OLD WORLD

Brain Drain / Molecular Biology / Communications / Nuclear Physics/Technology 154

Clearing the Air from Geneva 157

NEW WORLD

NRC Committee Knocks Anti-knock/Technology Assessment/Marine Pollution/Space Collaboration . . 158

NEWS AND VIEWS

Haemoglobin Synthesized in *Xenopus* Eggs/Gerontology/Enzymes/Mitochondrial DNA/Stomata/Bioengineering 161

Reminiscences of Rutherford 167

Has Science Never Had It So Good? 169

Science and the Common Market—FLOWERS (Science Research Council) 173

RNA—Frog eggs and oocytes for study of messenger RNA and its translation—GURDON, LANE, WOODLAND (Oxford) and MARBAIX (Brussels) 177

Antibiotics—Formation as secondary metabolites in response to toxic initiators—DHAR and KHAN (Central Drug Research Institute, Lucknow) . . 182

LETTERS TO *NATURE*

Physical Sciences

Lunar glass spheres—Similarity to glass shot formed during manufacture of mineral wool—CROSS (Northwich) 185

Solar rotation—Influence of eddy viscosity—STARR (MIT) 186

Carbon monoxide—Natural atmospheric sources—McCONNELL, McELROY and WOFSY (Harvard) . 187

Meteorites—Precambrian impact structure in West Greenland—JENSEN (Institut for Almen Geologi, Copenhagen) 188

Page

Biological Sciences

Chromosomes—Quinacrine fluorescence in evolutionary studies on *Drosophila*—BARR and ELLISON (Wisconsin) 190

Oocytes—Chromatid disjunction after ageing in the absence of fertilization—RODMAN (Cornell) . . 191

Blood clotting—Sequential activation of factors—JANSSEN and VAN LEEUWEN (Netherlands Red Cross) 193

Oncogenesis—Interruption by human foetal antigen—AMBROSE, ANDERSON and COGGIN (Oak Ridge National Laboratory and Tennessee) . . 194

Humoral antibody—Correlation with regression of tumours induced by feline sarcoma virus—ESSEX, KLEIN (Karolinska Institute), SNYDER and HARROLD (California) 195

Antimalarial substances—Their inhibiting effect on glucose-6-phosphate dehydrogenase—COTTON and SUTORIUM (Nijmegen) 197

Allergic neuritis—Effects of trypanosomes on development in rabbits—ALLT, EVANS, EVANS and TARGETT (London) 197

Nuclear magnetic resonance—^{13}C NMR spectra of lecithins vesicles and erythrocyte membranes—METCALFE, BIRDSALL, FEENEY, LEE, LEVINE and PARTINGTON (MRC, Cambridge) 199

Calcitonin—Release from thyroid granules—GREENBERG, MARTIN and MELICK (Melbourne) . . 201

Lipid monolayers—Significance of surface potential measurements during action of phospholipase A on lecithins—COLACICCO (Albert Einstein College of Medicine) 202

Vision—Perceptual fading of a stabilized cortical image—BLAKEMORE, MUNCEY and RIDLEY (Cambridge) 204

Iridescent corneas—An underwater study of fish corneas—LITHGOE (MRC, Sussex) 205

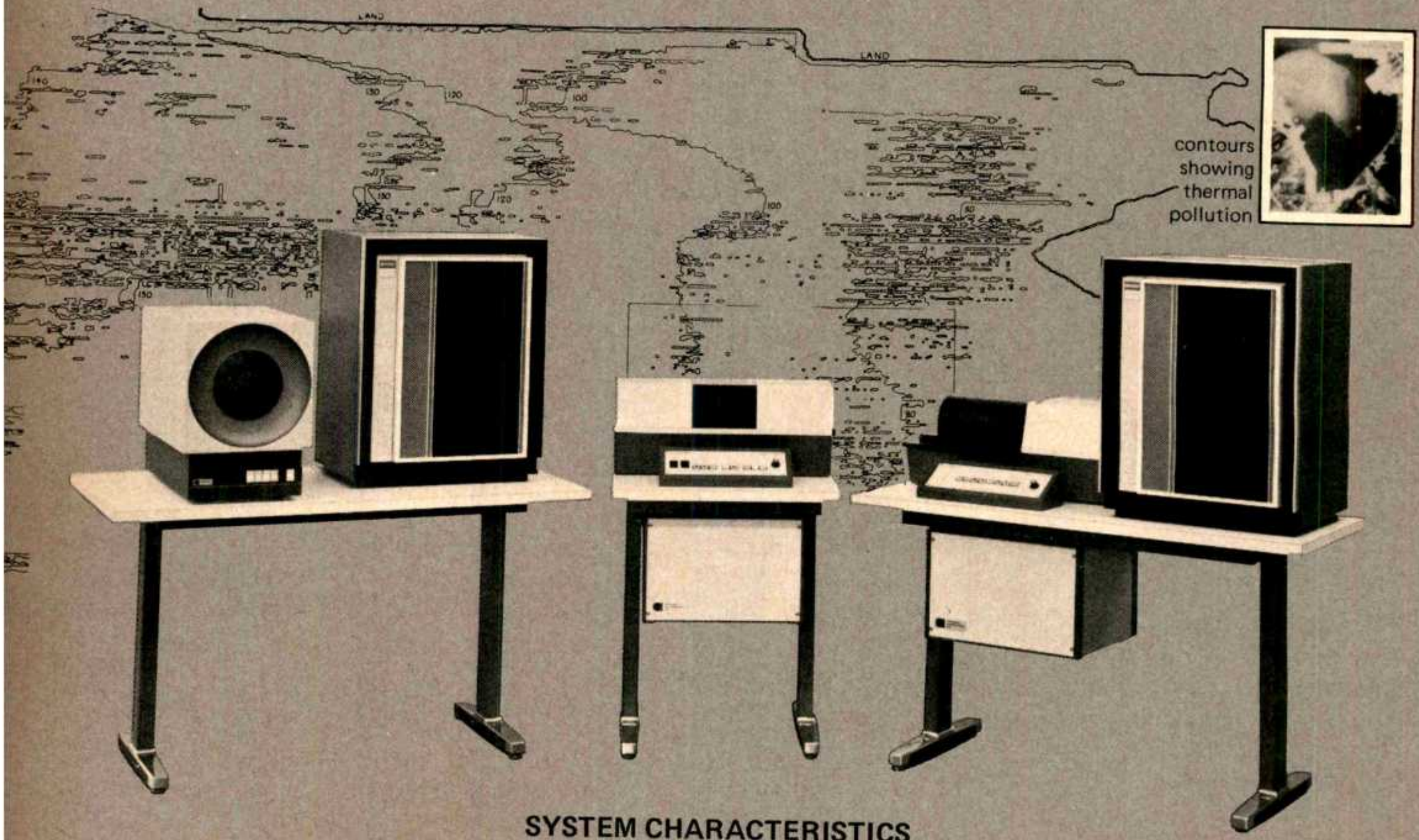
Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 162 to 166.

(Continued overleaf)

Photomation Mark I

The high-speed image processing system that extracts all your photographic data for storage and viewing

The P-1600 is fully integrated and can be computer-controlled, with a scanning microdensitometer on the input side and high-speed film writing unit on the output side, all supplemented by a tape transport and high-resolution CRT storage display



contours showing thermal pollution

SYSTEM CHARACTERISTICS

- Rasters and apertures to 12.5 microns
- Data rate to 25,000 picture elements per second
- Photographic display range from 0 to 3D
- Gray level resolution — 256 parts in read mode 64 parts in write mode
- Formats to 10" by 10" flexible film
- Color separation and reconstruction

Use the Mark-I with a small or large computer for:

- Remote Sensing
- Pattern Processing & Recognition
- Contour Plotting
- Industrial & Medical Radiology
- Electron Microscopy
- X-Ray Crystallography
- Particle Photography
- Image Enhancement
- Computer Animation
- Map Making

Input and output units of the Photomation Mark I system are obtainable separately and software is available. Contact our application engineers for further information today.

The Leader in Computer-Oriented Image Processing Instrumentation

OPTRONICS INTERNATIONAL INC.
7 STUART ROAD, CHELMSFORD, MASS. 01824 USA (617) 256-4511 TLX 94-7443

Page

Smoking—The effects of oral nicotine intake on smoking in monkeys—GLICK, ZIMMERBERG and JARVIK (Albert Einstein College of Medicine) 207

Morphine antagonism—Effects of cyprenorphine hydrochloride on sensory reinforcement in the rat—LOWE and WILLIAMS (Hull) 208

Steroidal saponins—Characterization in starfish—TURNER, SMITH (Aberdeen) and MACKIE (NERC Fisheries Biochemical Research Unit) 209

Lead pollution—Accumulation by wild oats (*Avena fatua*) in a contaminated area—RAINS (California) . . 210

RNA—Effect on acquisition of a one-way avoidance response in rats—MALIN, GOLUB and McCONNELL (Michigan) 211

Page

BOOK REVIEWS

by Dorothy S. Zinberg, Norman E. Zinberg, Ashley Montagu, J. M. Barnes, A. S. V. Burgen, J. S. Rowlinson, M. Gumbrell, A. L. Cullen, O. S. Heavens . . 213

CORRESPONDENCE

Six Years for a PhD (Jay M. Pasachoff)/Bird Navigation (Eric Hardy)/Black Hole to the North? (William F. Dryden)/Unfair to Scientists (Cyril Cooper)/History of "Plasmas" (Harold M. Mott-Smith) 217

Announcements—British Diary 219

Next Monday's issue will include

Solar X-ray spectrum—Observation of weak satellite lines—PARKINSON (Leicester)

Crab Nebula—Measurement of 10 µm flux—AITKEN and POLDEN (London)

NP 0532—No evidence of pulsed gamma ray emission above 50 MeV—VASSEUR, PAUL, PARLIER, LERAY, FORICHON and AGRINIER (Saday), BOELLA, MARASCHI and TREVES (Milan) and BUCCHERI, CUCCIA and SCARSI (Palermo)

Underground explosions—An explanation of the complexity of P signals—DOUGLAS, MARSHALL and CORBISHLEY (UKAEA, Blacknest)

Isomeric liquids—Dipolar interaction and flow birefringence—CHAMPION, MEETEN and TENCH (City of London Polytechnic)

Next Wednesday's issue will include

Salicylate—Inhibition of human embryonic cell culture growth—PAINE (Cambridge) and NAGINGTON (Public Health Laboratory Service)

Leukaemia—Cytidine deaminase and the development of resistance to arabinosyl cytosine—STEUART and BURKE (Johns Hopkins)

Haematophagy—Role of blood platelets—GALUN (Israel Institute for Biological Research) and RICE (Makerere)

tRNA—Sequence relationship of three valine acceptors from *E. coli*—YANIV (Paris and Stanford) and BARRELL (MRC, Cambridge)

Membranes—Conformation of glycerylphosphoylethanolamine in the crystal structure of its monohydrate—DETITTA and CRAVEN (Pittsburgh)

POLYMER

international journal on the science and technology of polymers and biopolymers

Polymer *is the international medium for the publication of original papers on the chemistry, physics and application of polymer research and on other disciplines which contribute to the development of polymer science.*

Polymer is published monthly to ensure the swift appearance in print of papers accepted for publication. All papers are scrupulously refereed and the journal is is guided by an editorial board of distinguished scientists whose activities cover all aspects of polymer research. Short communications, which deal with work that merits publicity before a full paper can be prepared, are published to keep readers abreast of current polymer research.

Annual subscription £15.00 ($37.50). From January 1st, 1972: £18.50 ($46.25). Pre-paid orders received before the end of 1971 will be accepted at the current rate of £15.

IPC Science and Technology Press Ltd,
IPC House, 32a High Street,
Guildford, Surrey, England.

TRANSFERABLE DRUG RESISTANCE FACTOR R

Edited by Susumu Mitsuhashi. Foreword by Bernard D. Davis

An up-to-date, critical study of resistance-transfer factors by one of the main contributors to their discovery and analysis — an achievement considered perhaps the most significant development in microbiology in the past decade.

In addition to presenting a complete review of the papers and reports of the many scientists engaged in R factor research, the book includes a large amount of previously unpublished data from the highly original investigations conducted by Dr. Mitsuhashi and his associates, with far-reaching implications in chemotherapy and antibody theory, as well as in questions of gene evolution and the urgent problems of lysogeny and latency in viral infection, including oncogenic viruses. *Contents:* Epidemiology of Bacterial Drug-Resistance; Epidemiology of R Factors; Genetic Properties of R Factors; Physiology of R Factors; Biochemical Mechanisms of R Factor Resistance; The Origin of R Factors. Name index. Subject index.

210 pages. Illus. 1971. $14.50

DRUG ACTION AND DRUG RESISTANCE IN BACTERIA

Edited by Susumu Mitsuhashi

Describes the action of macrolide antibiotics and lincomycin, the mechanism of bacterial resistance to these drugs, and the genetic and biochemical aspects of inducible resistance to macrolide antibiotics. The contributors to this volume include J. W. Corcoran, Bernard Weisblum, Hamao Umezawa, and other pre-eminent investigators in this field.

About 300 pages. Illus. August 1971. About $14.50

Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS
IN SCIENCE AND MEDICINE

BACK VOLUMES OF SCIENTIFIC PERIODICALS

We are always interested in buying your collections of duplicates of Scientific, Technical and Medical Journals at the highest prevailing prices. Please send us your lists of surplus journals, which will receive our immediate attention

We are Distributors of Back Issues of Journals including Pergamon Press Journals and many other Publishers. We invite you to write in for our regularly issued Bulletins and Catalogues and should be pleased to be of assistance in your acquisitions.

ROBERT MAXWELL & CO. LTD.
Back Issues Department
Midland Road, OLNEY, Bucks, England. Tel. Olney 529

RUSSIAN

JOURNALS REPORT ON IMPORTANT SCIENTIFIC & TECHNICAL RESEARCH

Publications in your field include:

CYTOLOGY & GENETICS Ref. 74565	6 issues yearly £2.50 p.a.
GENETICS Ref. 70211	12 issues yearly £18.40 p.a.
BIOLOGICAL SCIENCES Ref. 70089	12 issues yearly £12.10 p.a.

Usually in Russian but many with English summaries.
Send for 1972 list.

Collet's
Denington Estate,
WELLINGBOROUGH, U.K.

NATURE

MACMILLAN JOURNALS LTD

No. 5317 FRIDAY SEPTEMBER 24 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Why Not to Test Cannikin . . . 221

Creating a Professional Labour Market . . . 221

Pugwash Joins the Establishment . . . 223

OLD WORLD

Environment/Biological Manpower/Mathematics/Research and Development/Museums/Education/Airports . . . 224

NEW WORLD

Yet Another Prescription for Cancer/Detergents/AEC . 228

NEWS AND VIEWS

Does Molecular Shape Determine Odour Quality?/Limits on Seyfert Galaxies/Developmental Biology/RNA Synthesis/Reefs/Fluid Mechanics/History of Science/Viral Infections/Nucleic Acids/Acoustics . . . 231

Combustion Temperatures: The Future?—WEINBERG (London) . . . 239

Obsidian—Sources identified by fission track analysis—DURRANI, KHAN, TAJ (Birmingham) and RENFREW (Sheffield) . . . 242

Continental drift—Formation of the Scotia Arc—DALZIEL and ELLIOT (Columbia and Ohio State) 246

Andes—History of the Andean orogeny—RUTLAND Adelaide) . . . 252

LETTERS TO *NATURE*

Physical Sciences

Seyfert galaxies—Evidence for infrared variability of NGC 1068—LOW and RIEKE (Arizona) . . 256

North-East Atlantic—Comments on evolution—LE PICHON and SIBUET (Centre Océanologique de Bretagne) . . . 257

Superheavy elements—Decay lifetime implied from meteorite studies—SCHRAMM (California Institute of Technology) . . . 258

Xenon—Isotopic composition from a Greenland anorthosite—JEFFERY (Western Australia) . . . 260

Stereochemistry—Steranes from oil shale—BALOGH, WILSON and BURLINGAME (California) . . 261

Page

Biological Sciences

Multiple sclerosis—Cellular lipid inclusions in white matter—BAYLISS and ADAMS (London) . . 264

Tourniquets—Nerve lesions caused by pressure—OCHOA, DANTA, FOWLER and GILLIATT (London) . . . 265

Fibroblasts—Local origin deduced from sarcomas induced in chimaeras by implants of pliable disks—BARNES, EVANS and LOUTIT (MRC, Harwell) 267

Fertilization—Induction of zona reaction in golden hamster eggs by cortical granule material—BARROS and YANIGIMACHI (Hawaii) . . . 268

Malnutrition—Brain cholesterol in newborn rats—IM, BARNES and LEVITSKY (Cornell) . . . 269

Odour—Stereochemical and vibrational theories—AMOORE (US Department of Agriculture) . . 270

Serotonin—Evidence for inhibition of mouse killing by rats—DI CHIARA, CAMBA and SPANO (Cagliari) . . . 272

Neuronal growth—Expansion and retraction of dendritic trees as a function of neural activity *in vivo*—SUMNER and WATSON (Edinburgh) . . . 273

Fluid balance—Of a Toureg nomad and an Englishman in the Sahara—BEIGHTON (London) . . . 275

Mimicry—Experimental study involving the salamanders, *Notophthalmus viridescens viridescens* and *Pseudotriton ruber schencki*—HOWARD and BRODIE (Clemson) . . . 277

Knee joint—Reply to Roberts on kinematics—McLEISH (Manchester) . . . 278

—Reply to McLeish—ROBERTS (Glasgow) 278

Mycorrhizas—Mechanism of absorption of phosphate from soil by *Endogone* species—SANDERS and TINKER (Oxford) . . . 278

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 233 to 238.

(Continued overleaf)

Announcing a new important Pergamon International Research Journal

Computers and Structures

Editor-in-Chief: Harold Liebowitz
School of Engineering and Applied Science
George Washington University
Washington DC 20006 USA

To be published bi-monthly - first issue September 1971

Size 247mm x 187 mm

Subscription rates:

one year	**£24.00** **$60.00**
two years (saving 10%)	**£ 43.20** **$108.00**

The objective of this new international journal is to provide a medium for the communication of information concerning the applications of computers (digital, analog and hybrid) and computer programs to the solution of scientific and engineering problems related to hydrospace, aerospace and terrestrial structures. The journal is intended to be of interest and utility to the researcher and practitioner in the academic, governmental and industrial communities. It is planned to publish technical papers and computer program papers in such relevant technical areas as structural mechanics, fluid mechanics and oceanography as utilized to establish structural loading, soil mechanics and foundations engineering, geology and geophysics and materials science and engineering including fatigue, creep, relaxation, fracture, stress corrosion, etc. Particular attention will be devoted to the practical engineering aspects of structural analysis, design and optimization.

Although the journal will publish authoritative papers on theoretical and experimental research and advanced applications of computer programs, it may also feature, when appropriate:

a *tutorial survey-types of papers reviewing some field or fields of computers and/or structures*
b *educational type of papers showing what computers are and how they could be used to assist the engineer in designing structures*
c *computer program described in sufficient detail as to be usable with minimum effort*
d *a special section entitled "Design Data and Methods" for contributions concerning computer-aided design of structures and structural elements and*
e *several pages devoted to reviews of books and papers published elsewhere, meetings, schedules and other information.*

return to:-
R. C. Dean
Marketing Department
Journals Division
Pergamon Press
Headington Hill Hall
Oxford OX3 0BW
England

ORDER FORM

Please enter my/our subscription to **COMPUTERS & STRUCTURES**

☐ 1 year ☐ 2 years ☐ Payment attached ☐ send invoice

Name ______________________________

Address ______________________________

____

____

____

☐ Please send details of related journals in this field

Nature September 24th

Page

Mimosa—Rapid closure of leaves in response to light—APPLEWHITE and GARDNER (Yale) . . 279

Neanderthals—Child's skull from southern Italy—ASCENZI (Rome) and SEGRE (Messina) . . 280

Nocturnal moths—Attraction to submillimetre radiation source—ELDUMIATI and LEVENGOOD (Sensors Inc.) 283

Lymphocytes—Effects of serotonin and related compounds on sensitization to basic protein of brain in malignant neoplasia — FIELD, CASPARY and CARNEGIE (MRC, Newcastle upon Tyne) . . 284

Page

BOOK REVIEWS

by E. Gaskell, H. McIlwain, R. G. Edwards, Alun M. Roberts, D. C. Smith, R. F. Phillips, N. A. Hampson, Hugh Firth, B. V. Smith 287

CORRESPONDENCE

Elitism in Astronomy (Neville J. Woolf)/Conquest of Cancer (Solomon Garb)/Research and Development (Duncan Burn) 291

Announcements—British Diary 292

Next Monday's issue will include

AP Lib—Spectrophotometric similarity to BL Lac—RODGERS (Australian National)

Pollution—Identification by flame photometric sulphur detector—ADLARD and MATTHEWS (Shell Research Ltd)

Atomic spectroscopy—Mechanisms of atom formation in flames—COKER, OTTAWAY and PRADHAN (Strathclyde)

Interstellar silicates—Spectrum of VI Cyg No. 12 searched at 2.2 to 11.0 μm—STEIN (San Diego and Minnesota) and GILLETT (San Diego)

Astrophysics—Should the spallation cross-sections for ^{10}Be production from oxygen be modified?—RAISBECK and YIOU (CNRS)

Next Wednesday's issue will include

Neuroblastoma—Morphological differentiation induced *in vitro* by dibutyryl 3′5′ cyclic monophosphate—PRASAD and HSIE (Colorado)

Tumour viruses—Hormone-activated expression of the C-type RNA tumour virus genome—HELLMAN and FOWLER (US National Institutes of Health)

Cancer detection—Increased polyamine levels in urine of cancer patients—RUSSELL (Baltimore Cancer Research Center)

Membranes—Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*—OESTERHELT and STOECKENIUS (California)

—Structure of purple membrane of *Halobacterium halobium*—BLAUROCK and STOECKENIUS (California)

MYOCARDIOLOGY

Recent Advances in Studies on Cardiac Structure and Metabolism

Edited by Eors Bajusz and George Rona

Selected papers from The Seminar on Preventive Myocardiology of the American College of Cardiology (June 1970) and the Third Annual (First American) Meeting of the International Study Group for Research on Cardiac Metabolism. A detailed, authoritative study of the most recent advances in investigative cardiology, ranging from functional-metabolic-structural interrelationships of heart in health and disease to the practical application of new research findings by preventive medicine.

Two volumes. About 900 pages. Illus. September 1971. About $45.00 the set

EXERCISE AND CARDIAC DEATH

Edited by Ernst Jokl and J. T. McClellan

A detailed clinical study of the phenomenon of sudden heart failure in seemingly healthy individuals. Presents extensive evidence showing that it is now possible to differentiate between the natural history of heart disease in patients with recognizable symptoms and those in whom the first overt symptom is terminal collapse. More than 30 eminent specialists document their clinical and laboratory experience with athletes, pilots, and others in sedentary and non-sedentary occupations, as well as with cases involving identical twins and successive generations in families. An authoritative source of basic clinical data and laboratory experimentation for cardiologists, pathologists, and internists. For every physician whose practice includes sports, aviation, military, insurance, or industrial medicine this volume provides information of critical importance to their diagnosis and treatment of cases of hidden heart disease.

183 pages. Illus. 1971. $11.50

Available in Europe from S. Karger

Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS IN SCIENCE AND MEDICINE

H. K. LEWIS ESTABLISHED 1844

for all books

ON PURE AND APPLIED SCIENCE

including

CHEMISTRY · BIOLOGY · PHYSICS
MATHEMATICS, ENGINEERING AND
AGRICULTURE, etc.

Catalogues on application, please state interests

H. K. LEWIS & Co. Ltd., 136 Gower Street
Telephone] **LONDON, WC1E 6BS** *[01-387 4282*

REACTIONS OF MOLECULES AT ELECTRODES

Edited by **Noel S. Hush,** *University of Bristol*

This book is concerned with the study of processes occurring at metal, semiconductor or insulator electrodes, an important topic which frequently overlaps with other often apparently unrelated fields. Specialists in other areas of chemistry are often unaware of the possible relevance of developments in the theory of electrode processes or of kinetic and thermodynamic data for such processes to their own fields, as the value of electrochemical methods in preparative chemistry has not yet been fully realized. The topics covered in this book are of importance not only to electrochemists but to research workers in many other branches of chemistry.

September 1971 514 pages £9.50

Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex

CUTROCK

MODEL VRS 150 LARGE SPECIMEN CUTTING MACHINE

Maximum cutting area 18″ × 9″

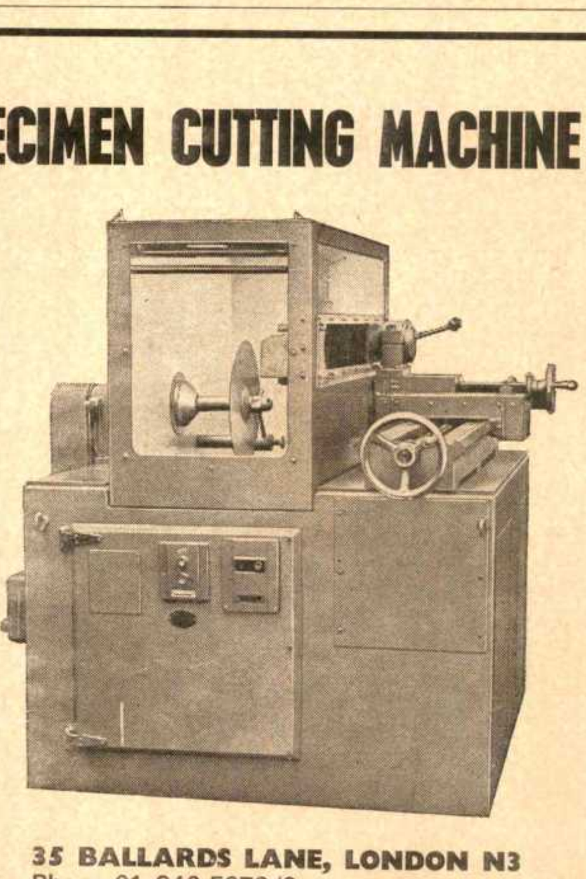
The machine is designed to cut specimens of rock, refractories, concrete and any hard materials requiring a diamond wheel as a cutting agent.

Using a 14″ diameter cutting disc the 9″ depth of cut is achieved by its reversing vice unit. The specimen is cut 4½″ deep with the first pass, is then turned through 180° and cut again, the second cut meeting the first, giving 9″ depth of cut. Specimens of 4½″ and under can be cut by one pass.

The machine is totally enclosed to contain all splash thus making it ideal for laboratory use. The illustration shows the machine fitted with the standard rock vice. A core vice is also available to cut both longitudinal and lateral cuts on cores up to 6″ dia × 18″ long.

Power feeds can be fitted if required.

Dimensions 54″ long 53″ wide 63″ high wt. 9 cwts.



CUTROCK ENGINEERING CO. LTD.

35 BALLARDS LANE, LONDON N3
Phone 01-346 5978/9

Pure Chemistry Library
SCIENCE COLLEGE
Upper Circular Road
CALCUTTA

NATURE

MACMILLAN JOURNALS LTD

No. 5318 FRIDAY OCTOBER 1 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Pure Chemistry Library
SCIENCE COLLEGE
Upper Circular Road
CALCUTTA

Page

Public Money for University Research 293

Publish and be Damned a Second Time 294

OLD WORLD

European Laboratory/Detergents/Intelligence/Human Ecology/Patents/Computers 295

Gesture of Independence 298

NEW WORLD

DDT Nailed Again/Manpower 299

NEWS AND VIEWS

Cancer and Immunity/Nucleic Acids/Cosmic Rays/Mineral Resources/Entomology/Pacific Science/Cancer Cells/Plant Roots/Stereology/Botany/Mycology . 303

Immunoglobulin E (Reagin) and Allergy—STANWORTH (Birmingham) 310

Blood groups—Cyclic expression of determinants in murine cells and their relation to growth control—THOMAS (London) 317

Tumours—Cytostatic antibody and SV40 tumour immunity in hamsters—AMBROSE, ANDERSON and COGGIN (Oak Ridge National Laboratory and Tennessee) 321

LETTERS TO *NATURE*

Physical Sciences

Upper atmosphere rotation—Rotation rate decreases above 350 km—KING-HELE (Royal Aircraft Establishment) 325

Aerosol pollution—Use of astronomical telescopes to monitor aerosol pollution in atmosphere—PORCH, MANNERY, CHARLSON and HODGE (Washington, Seattle) 326

Sulphur dioxide—Behaviour is similar to water vapour when crossing natural air-water interfaces—LISS (East Anglia) 327

Page

Density of seawater—Comment on Kremling's method—CREASE (National Institute of Oceanography) . 329

Fibrous carbon—Spatial relationship between graphite planes and catalyst particles—BAIRD, FRYER and GRANT (Glasgow) 329

Biological Sciences

Lymphocytes—T cells needed for potentiation by adjuvants of antibody formation—ALLISON (Clinical Research Centre, Harrow) and DAVIES (Chester Beatty Research Institute) 330

HL-A antigens—Evolution of expression during long term culture of fibroblasts—SASPORTES, DEHAY and FELLOUS (Institut de Recherches sur les Maladies du Sang, Paris) 332

***Y* chromosome**—Fluorescent screening of newborns in hospital—LEWIN and CONEN (Hospital for Sick Children, Toronto) 334

Scrapie—Disease in immunologically deficient mice—McFARLIN, RAFF, SIMPSON and NEHLSEN (MRC, London) 336

Prostaglandins—Method for extraction from human blood—UNGER, STAMFORD and BENNETT (London) 336

Porous biomaterials—Made by replication of echinoderm skeletal microstructures—WEBER, WHITE and LEBIEDZIK (Pennsylvania State) 337

Biological rhythms—Model for spontaneous oscillations in blood pressure and thermoregulatory systems—HYNDMAN, KITNEY and SAYERS (London) . 339

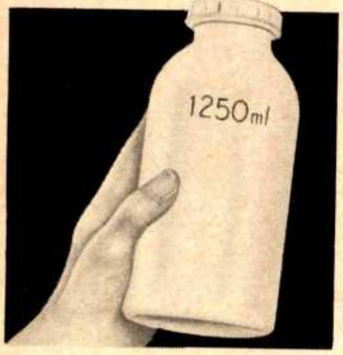
Feeding behaviour—Inhibition by injection of amino-acids into the hypothalamus—PANKSEPP and BOOTH (Sussex) 341

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 303 to 308.

(Continued overleaf)

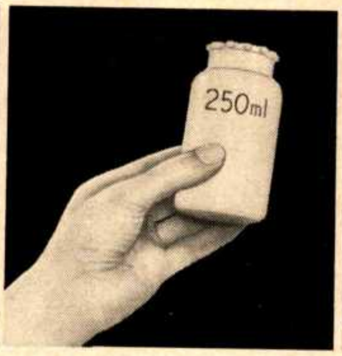
The MSE MISTRAL 6L Refrigerator Centrifuge really can take it...

4 of these

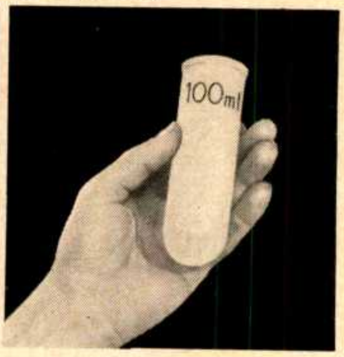


6 of these

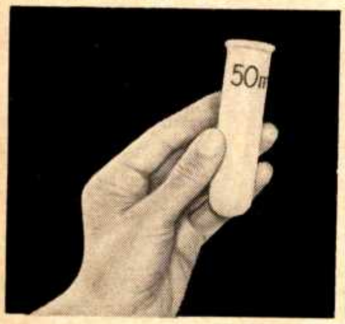
6 of these



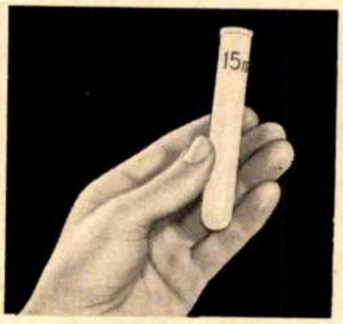
12 of these



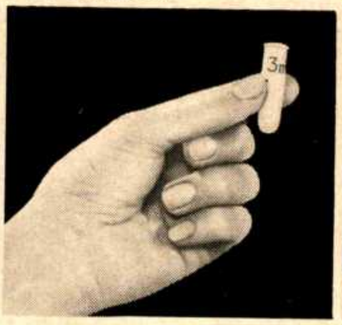
24 of these



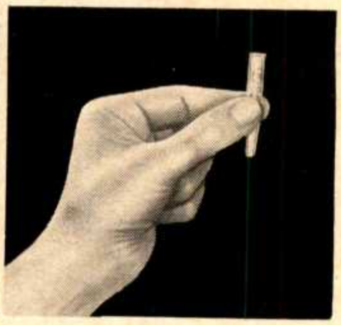
96 of these



144 of these



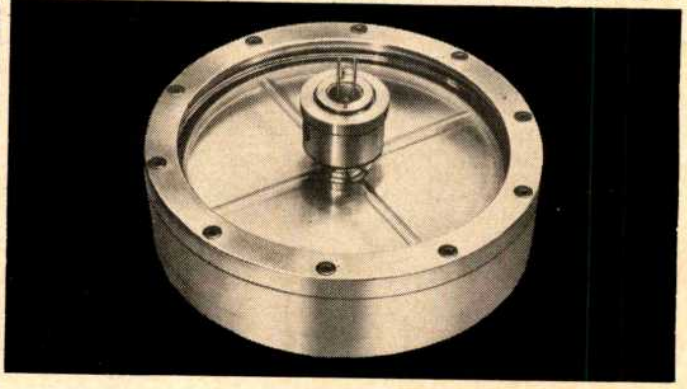
288 of these



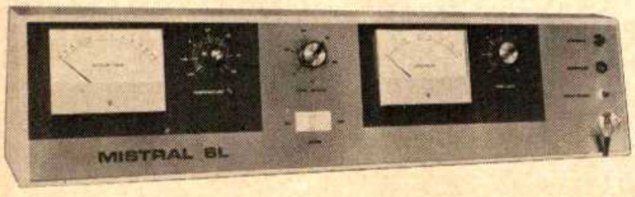
6 of these

or 4 of these

.. and of course the 'A' TYPE ZONAL ROTOR



— and now we've added a smart new control panel and even more precise temperature control. Perfection!



The versatile carrying capacity of the MSE Mistral '6L', like its dependability, is proverbial in the world of temperature controlled centrifuging. But now the temperature system has been improved to give more accurate and rapid temperature readings of the actual contents of the centrifuge tubes. The modern ergonomically designed control panel is simple to use and gives at-a-glance information on centrifuging progress. Send today for Publication No. 476/B covering the Mistral range.

Measuring and Scientific Equipment Ltd.,
Manor Royal, Crawley, Sussex, England.
Telephone: Crawley (STD 0293) 31100

Page

Teratology—Effects of methadone HCl in rats and rabbits—MARKHAM, EMMERSON and OWEN (Eli Lilly and Company) 342

Marihuana—Impairment of recent memory in Rhesus monkeys—ZIMMERBERG, GLICK and JARVIK (Albert Einstein College of Medicine) 343

Prey selection—Oddity and "specific searching image" more important than conspicuousness—MUELLER (North Carolina) 345

Chloroplasts—CO_2 dependent oxygen evolution by envelope-free chloroplasts—WALKER, McCORMICK (Sheffield) and STOKES (Melbourne) . . . 346

Geotropism—Elimination in lettuce seedlings—BERRIE (Glasgow) 347

Pa

Mitosis—Duration of mitotic cycle in dicotyledons and monocotyledons—EVANS and REES (University College of Wales) 3

Plant breeding—Importance of synchrony of ear emergence—PARODA (University College of Wales) . 351

Bicuculline—Evaluation as an antagonist of GABA—STRAUGHAN, NEAL, SIMMONDS, COLLINS and HILL (London) 352

BOOK REVIEWS

by F. Greenaway, Allan Maccoll, Norman Clark, H. Carter, W. H. Brock, D. M. Hirst, J. H. E. Griffiths . 355

Next Monday's issue will include

3C273B—Confirmation of coincidence with associated QSO—HAZARD, SUTTON, ARGUE, KENWORTHY (Cambridge), MORRISON and MURRAY (Royal Greenwich Observatory)

Planetary atmospheres—Simulation of absorption spectra in cloudy atmospheres—McCLEESE, MARGOLIS and HUNT (California Institute of Technology)

Earth's core—Upper limit on viscosity—HADE (Meteorological Office)

Next Wednesday's issue will include

Sodium pump—Role of ATPase in an energized state—CHARNOCK, COOP and OPIT (Alberta and Birmingham)

Lactation—Intermittent release of oxytocin during suckling in the rat—WAKERLEY and LINCOLN (Bristol)

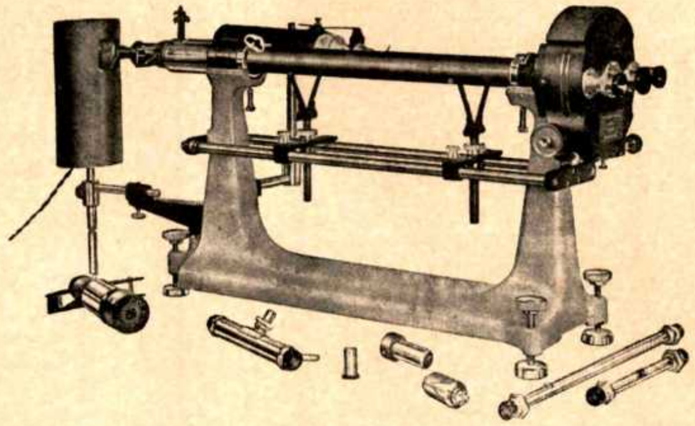
Instrumental learning—Performance of neodecorticate rabbits—OAKLEY (MRC, London)

Many authors will have experienced delay in the receipt of reprints of articles published in *Nature* during recent months. Everything possible is being done to rectify the situation and the forbearance of those concerned is greatly appreciated.

Editor, *Nature*

BS **Precision Instruments**

Polarimeter Model A



This instrument gives the highest degree of accuracy with divided circle on glass reading to 0.01 degree.

Circle centre on ball bearings · Easily removable trough · Divided drum replacing verniers · Stationary observation telescope · Circle reader enlarging image of scale · Slow motion adjustment always in action · Suitable, with additions, for measurement of rotatory dispersion.

This polarimeter has been supplied in large numbers both in this country and abroad, and has always been acclaimed as being of advanced design and workmanship. Please ask for particulars "N".

We now have capacity for aluminising certain types of mirrors, at short notice.

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

BELLINGHAM & STANLEY LIMITED BS
61, MARKFIELD RD. LONDON N.15. TEL. 01-808 2675

'MICHROME'
STAINS & DYES

for use in Biology, Medicine, Chemistry and as tools of Research, Production and Quality Control in a variety of Industries.

Over 2,000 Stains, Dyes, etc. kept in stock including:

Acridine orange, Azocarmine, Biebrich scarlet R, Brilliant green, Aurantia, Celestine blue, Crystal violet, Cyanine, Aurine tricarboxylic acid, Dicyanine, DPX, p-Ethoxychrysoidine, Fast ponceau L salt, Fast Bordeaux O, Giemsa, Fluormount, Fluoroil, Cresyl fast violet, Lacmoid, Leishman, Janus green, Fluorescein, Nile blue, Pinacryptol green, Methyl red, Matamount, Leishman, Papanicolaou, Optoil, Pyronin Y (G), Rhodanile blue, Rosolic Acid, Shorr, Thionine, Quinacrine mustard dihydrochloride, etc.

Catalogue available on request

INDUSTRIAL RESEARCH ESTABLISHMENTS & COMMERCIAL ORGANIZATIONS

are invited to make use of our professional services as consultants on the selection and use of dyes as research and production tools.

EDWARD GURR LTD.

42, Upper Richmond Rd. West, London, SW14
Cables: Micromlabs, London SW14. Telephone: 01-876-8228

QUALITY — RELIABILITY — SERVICE
PROMPT DELIVERY

CORONARY HEART DISEASE AND PHYSICAL FITNESS

Edited by O. Andrée Larsen and R. O. Malmborg

Fully covers the physiological and metabolic adaptations to exercise and training in normal man and in patients with coronary heart disease. Particular attention is given to the effect on the central and peripheral circulation, methods for measurements of physical fitness, training programs, and clinical studies evaluating the relation between physical inactivity and the risk of coronary heart disease.

About 284 pages. Illus. July 1971. $17.50

ELECTRICAL STIMULATION OF THE HEART IN THE STUDY AND TREATMENT OF TACHYCARDIAS

By H. J. J. Wellens

A detailed description of the techniques and procedures perfected for the evaluation and treatment of tachycardias in clinical trials at the widely respected cardiology clinic of the University of Amsterdam, directed by Dr. D. Durrer. Covers classification and diagnosis of tachycardias, emphasizing differential diagnosis between supraventricular tachycardias with aberrant conduction and ventricular tachycardias, theoretical considerations on tachycardias and methods used in this study, and detailed reports on studies in patients with atrial flutter, A-V junctional tachycardias, and tachycardias related to the pre-excitation syndrome.

160 pages. Illus. 1971. $10.00

Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS IN SCIENCE AND MEDICINE

NATURE

MACMILLAN JOURNALS LTD

No. 5319 FRIDAY OCTOBER 8 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Research Councils on the Brink 361

Phosphates are Free Again 362

OLD WORLD

Astronomy/Atomic Energy/Research Councils/Civil Service / Transport / Research Councils / Concorde ?/ Education 364

NEW WORLD

Safeguard Survives Another Attack/Manpower . . 369

NEWS AND VIEWS

Transduction of Human Cells by Lambda/Anomalous Redshifts Explained?/Haemoglobin/Adenovirus/Parasitology / Magnetic Resonance / Prostheses / Bacteriophages/Nuclear Physics 370

Egg Transfer in Domestic Animals — ROWSON (Cambridge). 379

Global Atmospheric Research Programme—MASON (Meteorological Office) 382

Cainozoic—Vertebrate succession in Kenya—BISHOP, CHAPMAN, HILL (London) and MILLER (Cambridge) 389

Development—Antigens in the development of *Drosophila melanogaster*—ROBERTS (Oxford) . . . 394

Gene expression—Bacterial gene transcribed and translated by human cells *in vivo*—MERRIL, GEIER and PETRICCIANI (US National Institutes of Health) 398

LETTERS TO *NATURE*

Physical Sciences

PKS 1514-24—Radio and optical appearance—HUNSTEAD (Sydney) 401

Radio galaxies—Cosmological evolution—MACKAY (Cambridge) . . . .

—Reply to Mackay—ROWAN-ROBINSON (London) 403

Page

Electromagnetic radiation—Interplanetary time delay measurements need not contradict general relativity —FERENCZ (Committee on Space Research of Hungary) and TARCSAI (Eötvös) 404

Lavas—Age determinations using measurements of weathering changes—ATKINSON (DSIR, Lower Hutt) and SWINDALE (Hawaii) 406

River morphology—Experimental study of channel patterns—SCHUMM and KHAN (Colorado State) . 407

Acoustics—Aerodynamic noise produced by gliding owl —GRUSCHKA, BORCHERS and COBLE (Tennessee) 409

Biological Sciences

Fish pheromones—Determinants of local orientation in anadromous species—NORDENG (Oslo) . . . 411

Neuroblastoma—Expression of differentiated functions mediated by dibutyryl cyclic AMP in mouse—FURMANSKI, SILVERMAN and LUBIN (Dartmouth Medical School) 413

Leukaemia virus—Suppression of *in vitro* leucocyte migration—FRIEDMAN and CEGLOWSKI (Albert Einstein Medical Center) 415

Hybrid cells—Chromosomes of faster growing cell of hamster-mouse hybrid preferentially eliminated—HANDMAKER (Oxford) 416

ATPase—Proposed reaction mechanism for the (Na^+ $+K^+$)-dependent adenine triphosphatase—ROBINSON (New York) 419

Lysozyme—Role of distortion in lysozyme-catalysed hydrolysis of glucosides—SYKES and DOLPHIN (Harvard) 421

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 372 to 377.

(Continued overleaf)

NMR

Basic Principles and Progress
Grundlagen und Fortschritte

Editors: P. Diehl, E. Fluck, R. Kosfeld

Vol. 1: P. Diehl, C. L. Kethrapal: NMR Studies of Molecules Oriented in the Nematic Phase of Liquid Crystals
R. G. Jones: The Use of Symmetry in Nuclear Magnetic Resonance

53 fig. V, 174 pp. 1969. Cloth DM 39,–; US $ 11.30

Vol. 2: H. J. Keller: NMR-Untersuchungen an Komplexverbindungen

22 Abb. III, 88 S. 1970. Geb. DM 32,–; US $ 9.20

Vol. 3: O. Kanert, M. Mehring: Static Quadrupole Effects in Disordered Cubic Solids
F. Noack: Nuclear Magnetic Relaxation Spectroscopy

73 fig. v, 144 pp. 1971. Cloth DM 48,–; US $ 13.90

Vol. 4: Natural and Synthetic High Polymers

Lectures presented at the Seventh Colloquium on NMR Spectroscopy. Held in the Institut für Physikalische Chemie, April 13-17, 1970, Technische Hochschule Aachen. Editors: P. Diehl, E. Fluck, R. Kosfeld
202 fig. X, 309 pp. 1971. Cloth DM 64,–; US $ 18.50

Vol. 5: R. A. Hoffman, S. Forsén, B. Gestblom: Analysis of NMR Spectra

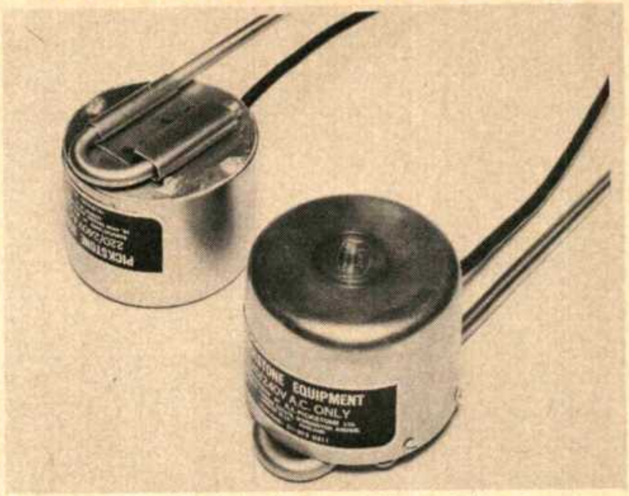
A Guide for Chemists
63 fig. III, 165 pp. 1971. Cloth DM 64,–; US $ 18.50

Vol. 6: P. Diehl, H. Kellerhals, E. Lustig: Computer Assistance in the Analysis of High-Resolution NMR Spectra

Approx. 11 fig. Approx. 100 pp. Due October 1971
Cloth DM 48,–; US $ 13.90

Springer-Verlag
Berlin · Heidelberg · New York

PICKSTONE MAGNETIC STIRRER



Suitable for use with stirring bars up to 37 mm in length (optimum 26 mm) with low viscosity solutions in volume of up to 1 litre. 600 r.p.m. Stainless steel case.

Clip-on fixing for mounting on a retort stand and the stirrer can stand on the bench with or without the fixing rod.

Magnetic Stirrer £5.00

Stirring Bars 26 mm 40p

Carriage extra on all orders under £10

R. E. PICKSTONE LTD.
FARADAY PLACE, THETFORD, NORFOLK.
Telephone: THETFORD 2238 (STD 0842–2238)

MULTIVARIATE ANALYSIS:

Techniques for Educational and Psychological Research

by **Maurice M. Tatsuoka,** *University of Illinois*

Discusses multivariate statistical techniques useful in education and psychological research, including multivariate significance testing, multivariate analysis of variance, principal components analysis, discriminant analysis, and canonical correlation analysis. Develops the necessary matrix algebra in the context of statistical applications.

September 1971 336 pages £4.85

Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex

Display Advertising for

Nature

USA

NATURE SCIENTIFIC PUBLICATIONS INC.
711 National Press Building
Washington DC 20004
202 737-2355

Elsewhere

T. G. SCOTT AND SON LIMITED
1 Clement's Inn, London WC2A 2ED
01-242 6264

Page

Blastocysts—Rabbit ova cultured from single-cell stage—OGAWA, SATOH and HASHIMOTO (Nihon) . 422

Arousal—Effects of amphetamine potentiated by starvation—CAMPBELL (Princeton) and FIBIGER (British Columbia) 424

Catecholamines—Effects of 6-hydroxydopamine on sleep in the rat—HARTMANN, CHUNG (Tufts), DRASKOCZY and SCHILDKRAUT (Harvard) . . 425

Plankton blooms—Trace metal variations in seawater caused by bloom of *Phaeocystis*—MORRIS (Marine Science Laboratories, Anglesey) 427

Page

BOOK REVIEWS

by B. W. Cunliffe, G. N. Cantor, D. B. Scott, D. ter Haar, J. Dyson, G. Y. Craig, D. C. Blackley, E. G. Richards, Bryan Clarke 429

CORRESPONDENCE

Doomsday Syndrome (Wayland Kennet)/Working Europeans (C. H. Waddington)/Research Associations (S. V. Vaeck)/Bradford's Law (Melvin Weinstock)/Ageing Ova (W. A. F. Watson)/Suckling Etymology (J. C. Rigg)/Retino-rectal Connexions? (Anthony Robertson) 433

Announcements—University News/Appointments/Miscellaneous/International Meetings 435

Next Monday's issue will include

Extensive air showers—Mechanism for 22.25 MHz radio emission from showers with $\sim 10^6$ particles is geomagnetic charge separation—PRESCOTT, HOUGH and PIDCOCK (Calgary)

Solar corona—Neutron theory of coronal heating requires a high gamma ray flux at the Earth—CHENG (NASA)

Iron meteorites—Detection of chromium nitride—BUCHWALD (Technical University, Lyngby) and SCOTT (Cambridge)

Cape Verde Islands—Evidence for a Mesozoic oceanic ridge—KLERKX (Liège) and DE PAEPE (Ghent)

Acrylic fibres—Small angle X-ray scattering studies—FILLERY and GOODHEW (Surrey)

Next Wednesday's issue will include

Mitochondria—Products of mitochondrial protein synthesis in yeast—THOMAS and WILLIAMSON (MRC, London)

Chromatin—Tissue differences in antigenic properties of non-histone protein-DNA complexes—CHYTIL and SPILSBERG (Vanderbilt)

Membranes—Structural changes in membranes of synchronized cells demonstrated by freeze-cleavage—SCOTT, CARTER and KIDWELL (US National Institutes of Health)

Drosophila melanogaster—Simultaneous transmission of three stable genotypes—CORWIN, HANRATTY and WOLAK (Pittsburgh)

Photoreceptors — "Dark" current generated passively—ZUCKERMAN (Rochester)

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA.

Practical, time-saving reference books for biochemists in teaching and research . . .

SELECTED PAPERS IN BIOCHEMISTRY

Series Editor: Denichi Mizuno

The twelve volumes in this series present a selection of the most significant papers published in biochemistry throughout the world, including early basic research as well as recent developments and discoveries. Each volume contains the full text, including original illustrations, of the seminal papers in a specific field of biochemistry of importance to modern study and research. An innovative concept in scientific publishing, designed specifically for advanced students, research workers, and permanent reference collections in schools and libraries.

Publication of the series begins July 1971 for completion by Spring 1972. Each volume will contain 250-300 pages. Price per volume: $12.50

Vol. 1: BACTERIAL GENETICS AND TEMPERATE PHAGE
Vol. 2: VIRULENT PHAGE
Vol. 3: CODING
Vol. 4: RADIATION BIOLOGY OF MICROORGANISMS
Vol. 5: RNA SYNTHESIS
Vol. 6: TRANSFER RNA
Vol. 7: PROTEIN SYNTHESIS
Vol. 8: ALLOSTERIC REGULATION
Vol. 9: ACTIVE TRANSPORT
Vol. 10: MITOCHONDRIA
Vol. 11: ENZYME MECHANISM
Vol. 12: PHOTOSYNTHESIS

Your continuation order for the series ensures automatic shipment of each new volume immediately upon publication.

Order from your bookseller or write directly to:

UNIVERSITY PARK PRESS
Chamber of Commerce Building
Baltimore, Maryland 21202

INTERNATIONAL PUBLISHERS
IN SCIENCE AND MEDICINE

Recently published

COMPLEX PERMITTIVITY

Contributors: **B. K. P. Scaife, W. G. S. Scaife, R. G. Bennett** and **J. H. Calderwood.**

This book contains up-to-date reviews on three important aspects of dielectrics. The first is concerned with the molecular behaviour underlying the observed variation in the complex permittivity of dielectrics with frequency.

The second section is concerned with the work done in the high pressure field. Such work is capable of yielding important information concerning molecular behaviour, and is one in which research activity is increasing.

The third review is concerned with the various methods that have been devised to measure complex permittivity at frequencies below those requiring the use of wave guides.

£3.45 net 0 340 11536 X

Available through your bookshop

THE ENGLISH UNIVERSITIES PRESS

St. Paul's House, Warwick Lane, London EC4P 4AH

Whither the Universities?

the Universities?

In the first issue of THES out on 15 October Sir Eric Ashby takes a long hard look at the present state and future of the universities, and warns of the possibility that increasing numbers of university and college students will be there who don't want to be there – Sir Eric terms them social conscripts – and flatly concludes that the academic establishment has no solution to offer.

Also in the first issue The Reverend Sir Herbert Andrew; and a feature on multi-disciplinary courses for scientists. Later, an interview with Sir Brian Flowers, and articles on: teaching applied mathematics – what the professors say; immunology – origins and advances; and the future of the Research Councils.

From 15 October – the THES: Friday's paper. 8p.

THE TIMES
Higher Education
SUPPLEMENT

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA

NATURE

MACMILLAN JOURNALS LTD

No. 5320 FRIDAY OCTOBER 15 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page
DDT may be Good for People 437
Where are the Bounds of Decent Practice? 438
One Way to Europe 439

OLD WORLD

Trade Unions/European Laboratory/Insecticides/Coal . 440

NEW WORLD

Fighting the Great ABM Battle Again/Cancer/DDT/Drug Addiction 442

NEWS AND VIEWS

Seafloor Spreading and Mountain Building/Putting the Cerebellum to Sleep?/Haemoglobin/Mitochondrial DNA/Electron Microscopy/Hepatitis/Acetylcholine/Behaviour / Palaeontology / Schizophrenia / Reactor Technology 445
Politics of Nuclear Power in the United States—WILSON (Harvard) 453
The Oxford Botanic Gardens: 1621–1971—DARLINGTON (Oxford) 455
Muscle—A general model for myosin-containing filaments—SQUIRE (Aarhus) 457
Plate tectonics—Correlation between data from Western North America and the North Atlantic—CONEY (Middlebury) 462

LETTERS TO *NATURE*

Physical Sciences

Lib γ-1—New γ-ray source possibly associated with PKS 1514-24—FRYE, ALBATS, ZYCH (Case Western Reserve), STAIB (Madison College), HOPPER, RAWLINSON and THOMAS (RAAF, Melbourne) 466
Cygnus XI—Short term variability of pulsation in X-ray flux—CRUISE, NEWTON and CHAPMAN (University College, London) 468
Galactic spurs—Soft X-ray emission—ILOVAISKY and BOWYER (Berkeley) 469
Environmental Mercury—Contribution from Hawaiian volcanoes—ESHELMAN, SIEGEL and SIEGEL (Hawaii) 471
Page
Isotope separation—A new method—STANGEBY and ALLEN (Oxford) 472
Solar System—Properties of Jupiter not affected by evolution of solar system—KUMAR (Virginia) . . 473
Atmospheric dust—Transportation by the Atlantic north-east and south-east trade winds—CHESTER (Liverpool), ELDERFIELD (Leeds), and GRIFFIN (California) 474
Experimental Mohole—Upper Miocene Neogene Zone 18 recognized—BANDY (California) 476

Biological Sciences

Allostery—Role of allosteric ligands in promoting maturation of enzymes—ALPERS and PAULUS (Harvard) 478
Heterochromatin—Localization of region 16qh (.76) linked to α-haptoglobin in man—HECHT, TOLBY, MAGENIS, KIMBERLING, WYANDT and LOVRIEN (Oregon) 480
Hybrid cells—Interpretation of linkage—BATEMAN (Manchester) 480
Cerebellum—Activation of a central noradrenergic projection—SIGGINS, HOFFER, OLIVER and BLOOM (NIMH) 481
Porphyropsin—Regeneration *in vivo*—BAUMANN (Bad Neuheim) 484
L-Asparaginase—An activator and inhibitor of complement in whole serum—DELAGE, SIMARD, LEHNER-NETSCH and BARRY (Quebec) . . . 485
Antitumour substances—A new water soluble polysaccharide with strong antitumour activity—HAMURO, YAMASHITA, OHSAKA, MAEDA and CHIHARA (Tokyo) 486
Juvenile hormone—Terpenoid amines screened for activity—CRUICKSHANK and PALMERE (Princeton) 488
Aggressive behaviour—Possible role of the pituitary/adrenocortical axis—BRAIN (Sheffield) . . . 489

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 447 to 451.

(Continued overleaf)

Unicam SP1800 Ultraviolet Spectrophotometer
Power
Deuterium Lamp
Scale divisions
Absorbance or Concentration
Meter Range
Zero
Concentration
Unicam SP 8000 Ultraviolet Spectrophotometer

Unicam UV Spectrophotometers make light work

What does this mean to you? In research, results you can trust and repeat. In routine applications, error-free operation by the least experienced member of your staff.

When you come to Pye Unicam, even the selection of your UV spectrophotometer is simple. There is a choice of instruments broad enough to meet your every requirement and to match your budget, with a staff of experienced application specialists to back them up.

Just look at what is available . . .
SP500 Series 2 and **SP600 Series 2** quantitative single beam spectrophotometers, **SP700** for research, **SP1800** for low-cost double-beam operation, **SP3000** automatic digital instrument, **SP8000** for the ultimate in flexibility, and the **SP1300 Series 2** low-cost colorimeter.

Write or 'phone for free information today. That's simple too, isn't it.

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

PYE UNICAM

Page

Myoid conduction—in the siphonorphore *Nanomia bijuga*—SPENCER (British Columbia) 490

Corrosion—Effect of sulphate reducing bacteria—KING and MILLER (UMIST) 491

Schistosomes—Infection with Gram-positive Cocci—BASCH (California) 492

Thermophilic bacteria—Physiology of non-sulphur bacteria from hot springs—BROCK and BROCK (Indiana) 494

Sodium hypochlorite—Potential carcinogenicity—HAYATSU, HOSHINO and KAWAZOE (Tokyo) . 495

Pregnancy tests—No evidence for neural tube malformations—LAURENCE, MILLER (Cardiff), VOWLES (Exeter), EVANS and CARTER (Institute of Child Health) 495

Smoking—Reduction by extracts of *Avena sativa*—ANAND (Glasgow) 496

—Possible relationship with peptic ulcer—ROBERT, STOWE and NEZAMIS (Michigan) . 497

Page

Visual masking—Effect of orientation of mask grating on discrimination of letters—HENDERSON, COLES, MANHEIM, MUIRHEAD and PSUTKA (Guelph) 498

Pollution—Organochlorine pesticide residues found in harbour porpoises—GASKIN, HOLDRINET and FRANK (Ontario) 499

Human remains—Earliest find from India—DUTTA (Calcutta) 500

Dowsing—Further information on experiments—SMITH (Ministry of Defence) 501

—Foulkes criticized—MERRYLEES (Suffolk) . 502

BOOK REVIEWS

by Elizabeth Perrott, Don. R. Arthur, Marian Dawkins, Malcolm Lader, D. W. Moss, Martyn Berry, Kathleen Lonsdale, John Walker 503

CORRESPONDENCE

Italian Science (Giorgio Marcuzzi)/Suckling Etymology (A. T. Cowie) 507

Next Monday's issue will include

β-Mg_2SiO_4—Geometric refinement of the crystal structure—BAUR (Chicago).

Oscillating chemical reactions—Variation of temperature with time—BUSSE (Stöckheim/Braunschweig)

Vadose cement—Morphology and composition of Ordovician sample—FREEMAN (Missouri)

Laser-Radar—Suppression of spurious signals—UJIMARA (Tokyo)

Next Wednesday's issue will include

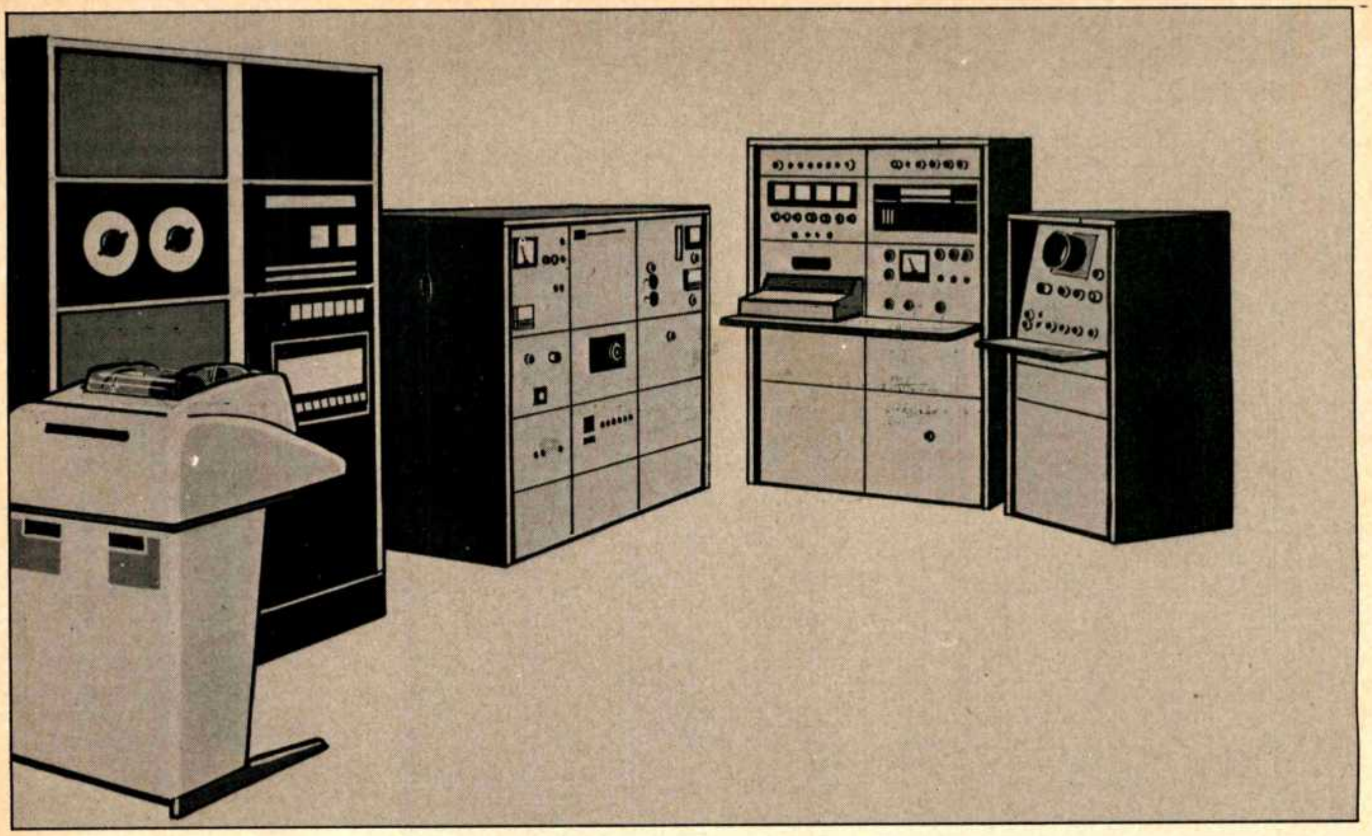
Anti θ sera—Contaminated by antibodies—GREAVES and RAFF (NIMR)

Concanavalin A—Immunofluorescent evidence for binding sites on both transformed and untransformed cells—MALLUCCI (Hammersmith)

***a*-Amanitin**—Inhibition of adenovirus replication in human embryo kidney cells and of adenovirus transformation of hamster cells—LEDINKO (Vermont)

Heart—Effects of diazoxide and dipyridamole derivative on nucleotide content—MAXWELL (Adelaide)

Pure Chemistry Library
SCIENCE COLLEGE
Upper Circular Road
CALCUTTA



We are Hooking the LKB 9000 to a COMPUTER

NOW the world-famous LKB 9000 Combined Gas Chromatograph-Mass Spectrometer has been linked to a computer system. The vast amounts of data supplied by the LKB 9000 can be processed quickly and with a minimum of manual effort. The computer data system can control mass scanning, data acquisition and display of the mass spectrum of any portion of the gas chromatogram. The program will allow on-line data acquisition from the LKB 9000 and simultaneous data handling without the use of internal mass spectra standards. Scans for the Combined GC-MS operations can be handled or scanned as often as you wish. Scan speeds for m/e 0—1000 of 3.5 seconds can be handled comfortably. Routine data will be available as a teletype readout and/or a plotted mass spectra with all background removed, and the data can be stored for future reference on either DEC tape or paper tape.

LKB IN THE SERVICE OF SCIENCE

LKB INSTRUMENTS LTD. • LKB HOUSE • 232 ADDINGTON ROAD
• S. CROYDON, SURREY. CR2 8YD • TEL: 01-657-0286
SALES AND SERVICE THROUGHOUT THE WORLD: STOCKHOLM, WASHINGTON, THE HAGUE, COPENHAGEN, ROME, VIENNA

UK 31

[illegible] Che[illegible] Library
SCIENCE COLLEGE
92 Upper Circular Road
CALCUTTA

NATURE

MACMILLAN JOURNALS LTD

No. 5321 FRIDAY OCTOBER 22 1971 Vol. 233

LONDON
4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633

WASHINGTON
711 National Press Building, Washington DC 20004. Tel: 202 737-2355

Page

Waiting for the Other Shoe to Fall 509

Technology Assessment has a Long Arm . . . 510

Whose Head is in the Sand? 510

OLD WORLD

Science Policy/Research Councils/EMBO/Nobel Prize/Food/French Science/Architectural Association/Museums/Research Associations 512

NEW WORLD

Rogers May Yet Win Cancer Stakes/Hereditary Disease/Cancer Research 516

NEWS AND VIEWS

Time Lost in Air Travel/A New Theory of Muscle Contraction/Glycine/Tropical Ecology/Oceanic Ridges/Proteins/Crystal Growth/Fish Biology/Ultrasonics . 519

Aeronautics before 1919—TAYLOR (Cambridge) . 527

The Perennial Dilemma of Science Policy—WERSKY (Edinburgh) 529

Muscle—Proposed mechanism for the generation of force—HUXLEY and SIMMONS (London) . . 533

Neanderthal Man—Hand morphology—MUSGRAVE (Brsitol) 538

LETTERS TO *NATURE*

Physical Sciences

Deep-sea sediments—Deposition of extraterrestrial nickel—YAMAKOSHI and TAZAWA (Kyoto) . . . 542

Stratospheric water vapour—Oxidation rate of CH_4 compared with possible production by SSTs—SINGER (Virginia) 543

Mesozoic palaeomagnetic reversals—Suggestion that Pecherski's zone names should be adopted—CREER (Newcastle upon Tyne) 545

Oil pollution—Identification of origin of spilled crude oils by gas chromatography—COLE (Esso Research Centre) 546

Ocean basins—A model for the formation and evolution of marginal basins—SLEEP and TOKSÖZ (MIT) . 548

Polywater—Disproof of the model which seemed to explain observed properties—ALLEN and KOLLMAN (Princeton) 550

Biological Sciences

Polynucleotides—New method for synthesizing defined sequences—MACKEY and GILHAM (Purdue) . 551

Cornea—Application of computer modelling—FRIEDMAN (Johns Hopkins) 553

Chromosome aberrations—Non-identity in human lymphoblastoid cell lines—STEEL (MRC, Edinburgh) 554

Leukaemia—Antigens in human serum and embryos—HARRIS (Manchester), VIZA, TODD, PHILLIPS, SUGAR (Searle), JENNISON, MARRIOTT and GLEESON (St Mary's Hospital, Manchester) . . 556

Transplantation—Modification of hyperacute rejection of sheep kidney heterografts in dog by trypsin inhibitor—GHILCHIK and MORRIS (London) . 557

Burkitt's lymphoma—Presence of Epstein-Barr virus antibodies in sera from wild chimpanzees—LEVY (California), LEVY (New England Medical Center Hospitals), HIRSHAUT (Sloan-Kettering Memorial Hospital), KAFUKO (East African Viral Institute) and PRINCE (New York Blood Center) . . . 559

Myoglobin—Possible structural and functional differences between the two conformers with respect to the haem–haem interaction—ATANASOV (Bulgarian Academy of Sciences) 560

5S RNA—Non-coordinated accumulation and synthesis by ovaries of *Xenopus laevis*—FORD (MRC, Edinburgh) 561

Lead—High absorption from the intestine of newborn rats—KOSTIAL, SIMONOVIC and PISONIC (Institute for Medical Research, Zagreb) . . . 564

Spanish moss—Sensor for lead—MARTINEZ, NATHANY and DHARMARAJAN (Louisiana State) 564

Ageing—Zoxazolamine paralysis *in vivo* and metabolism *in vitro* in mice—BAIRD, SAMIS and MASSIE (Masonic Medical Research Laboratory, New York). 565

Drug resistance—Possible mechanism at the cellular level—BOSMANN (Rochester) 566

Red fox—Anal gland secretion—ALBONE and FOX (Washington, St Louis) 569

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 522 to 526.

(Continued overleaf)

OXFORD BOOKS FOR STUDENTS

** Just published* *** Forthcoming*

* An Introduction to the Study of Man

J. Z. YOUNG

Professor of Anatomy, University College, London

The main aim of this book is to provide the basic facts of human biology. The agents controlling human activity are traced from their biochemical basis to the highest levels of consciousness. There are sections on heredity and reproduction, growth, intelligence testing, ageing, and population growth. Human evolution is traced from the origin of life to the beginnings of human culture. These facts provide background knowledge on many urgent problems, such as individual development, aggression and war, racial conflict, population control, and pollution. A main theme is that knowledge about the brain is revolutionizing our understanding of ourselves and the world. 256 line and half-tone illustrations. £6

** Lectures on Biostatistics

An Introduction to Statistics with Applications in Biology and Medicine

D. COLQUHOUN

Lecturer in Pharmacology, University College, London

This book is based on lectures given to medical science students with elementary mathematical knowledge and no statistical knowledge. The aim has been to include those topics which are of interest to laboratory workers, the discussion being based mostly on medical, physiological, and pharmacological problems. An attempt has been made to convey critical understanding while keeping the mathematics elementary, except in the appendices. 70 text figures £5·50 paper covers £2·75

* The Structure of Lebesgue Integration Theory

G. TEMPLE

Formerly Sedleian Professor of Natural Philosophy,
University of Oxford

This work provides an introductory account of Lebesgue integration for undergraduates, with many exercises and special emphasis on the motivation of the theory. The theory is reduced to its essentials—the techniques of 'bracketing' and of monotone sequences. The necessary theory of sets of points is simplified by using 'characteristic functions'. The Lebesgue-Stieltjes integral is briefly described. 4 text figures £3

ALGOL in Brief

A Short Practical Guide

J. F. RACTLIFFE

Senior Lecturer, Lanchester Polytechnic

In this rapid course of programming the scientific language ALGOL, the emphasis is on early program writing. The treatment is, as far as possible, informal. The style and approach is such that the student should be able to progress with only occasional help from his tutor. The general structure of programs is established in the opening pages by a detailed study of a number of simple programs; more advanced techniques are gradually introduced. 3 text figures paper covers 80p

Fungal Spores

Their Liberation and Dispersal

C. T. INGOLD

Professor of Botany, Birkbeck College, London

The remarkable range of structure in fungi is largely related to the production and liberation of spores. This book discusses the take-off of spores from a dynamic and experimental point of view and also considers the problems of further dispersal. Topics discussed include the aerobiology of fungal spores with special reference to methodology; dispersal by insects and the larger animals; seed-borne fungi; and the special features of dispersal in aquatic fungi. 8 plates 199 text figures £4

* Experimental Biology

A Practical Handbook

T. W. KIRBY and H. P. CLARK

This course follows the modern shift in emphasis from the intensive study of selective types to the extensive study of phenomena characteristic of life. The student is encouraged by experiments, realistic in their demands on apparatus, materials, and time, to conduct careful observation, accurate recordings and analysis, and interpretation over the whole field of modern biology to genetics and evolution. Spiral binding, laminated boards £1·25

* Introduction to Phase Transitions and Critical Phenomena

H. EUGENE STANLEY

Associate Professor of Physics,
Massachusetts Institute of Technology

This is the first book to be devoted to the interdisciplinary area of critical phenomena which, although having a long history, has only recently become an extremely active field of interest to physicists, mathematicians, chemists, and materials scientists. The simple mathematics makes the book useful supplementary material in undergraduate courses in thermodynamics. However, the book also provides a foundation for a short course for postgraduate students, and should prove essential for research workers entering the field. 10 plates 50 text figures £5 *International Series of Monographs on Physics*

* Stationary States
The Nature of Atoms
Bonds Between Atoms

ALAN HOLDEN

These three connected books were written for the Commission on College Physics in the United States to provide 'multi-level' instructional materials. Each is arranged in several sections of increasing sophistication in order to facilitate independent use as a textbook for existing courses and to offer bases for new kinds of courses. Their treatments bring together the viewpoints of physics, chemistry, and crystallography. SI units are used. Paper covers 55p, 75p and 85p respectively

OXFORD UNIVERSITY PRESS

Page

BOOK REVIEWS

by J. W. Mullin, J. E. Jones, J. H. Holloway, S. Tolansky, Raymond M. Fuoss, D. Vazquez, Irvine H. Page, Edward J. Johns, Edward Hitchcock 571

Page

CORRESPONDENCE

Rapid Publication (Julian Scheer) 575

Announcements—University News/Miscellaneous . 575

Next Monday's issue will include

Ice melting electrification—Its dependence on earlier freezing rate—HUTCHINSON and MARTIN (Durham)

Hydrogen—New type of sorption by graphite–potassium intercalation compounds—WATANABE, SOMA, ONISHI and TAMARU (Tokyo)

Spectrophotometry—Transient electric dichroism of macromolecules—JENNINGS (Brunel) and BAILY (London)

Next Wednesday's issue will include

SV40—Direct isolation and characterization of "flat" SV40-transformed cells—SCHER and NELSON-REES (US National Institutes of Health)

Tubulin—Heterogeneous structural subunits—FINE (MRC, Cambridge)

Malaria—Isolation of *Plasmodium berghei* parasites by ammonium chloride lysis of infected erythrocytes—MARTIN, FINERTY and ROSENTHAL (US National Institutes of Health)

CAMBRIDGE
UNIVERSITY PRESS

Nitration and Aromatic Reactivity

J. G. HOGGETT, R. B. MOODIE, J. R. PENTON and K. SCHOFIELD

A summary of the present understanding of the nitration process for various types of nitrating systems, with special consideration of those systems in which mechanistic studies have established the identity of the agent through which nitration is effected.

The monograph will be used by graduate students and research workers in universities, institutes of technology and industry.

£5.00 net

The Principles of Chemical Equilibrium

K. G. DENBIGH, F.R.S.

The third edition of this well-known textbook provides a comprehensive guide to the thermodynamics of chemical equilibrium at undergraduate level. The first part of the book outlines the general principles of thermodynamics which are applied, in the second part, to systems in chemical equilibrium. The statistical mechanical approach is introduced in part three.

Cloth £4.40 net
Paperback £1.50 net

SYMPOSIA OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY: 25

Control Mechanisms of Growth and Differentiation

Edited by D. D. DAVIES and M. J. BALLS

The proceedings of the twenty-fifth symposium of the Society for Experimental Biology held jointly with the British Society for Developmental Biology.

The symposium papers deal first with models and simple systems of development such as those found in bacteria and slime moulds. A series of papers then consider the formation and control of pattern in differentiation and development in more complex organisms. A discussion of the theoretical aspects of control mechanisms and of growth and differentiation is included at the end of the volume.

£8.00 net

A Guidebook to Biochemistry

M. D. YUDKIN and R. E. OFFORD

A new introductory text for students in schools, colleges and universities who are beginning courses in biochemistry, either by itself or as a background for work in biology, medicine, agriculture or pharmacology. It is based on Kenneth Harrison's successful *Guidebook to Biochemistry*, but has been completely revised and rewritten.

Cloth £3.20 net
Paperback £1.00 net

Modern Cosmology

D. W. SCIAMA

The exploration of the universe, as conducted by astronomers, cosmologists and physicists, is one of the greatest intellectual adventures of the mid-twentieth century. The author puts this work into perspective, summarizing present knowledge and theories, for senior undergraduate and graduate students and interested physicists.

£3.60 net

NATURE

MACMILLAN JOURNALS LTD

No. 5322 FRIDAY OCTOBER 29 1971 Vol. 233

LONDON

4 Little Essex Street, London WC2R 3LF. Tel: 01 836 6633 Telex: 262024

WASHINGTON

711 National Press Building, Washington DC 20004. Tel: 202 737-2355 Telex: 64280

Page

Should Reproductive Biology be Encouraged? . . . 577

Poor Report for the Post Office 578

OLD WORLD

Research and Development/Education/Research and Development/Soviet Science/Scientific Information/Archaeology/Museums 579

NEW WORLD

New AEC Broom Begins to Sweep/Space/Comsat/Tuberculosis 582

NEWS AND VIEWS

Cholinergic Receptor Sites in Muscle/Evolution/Animal Breeding/Gobies/Plant Pathology/Lipids/Estuaries/Peat/Plant Growth 585

Outlook for Controlled Fusion Power—TUCK (Los Alamos) 593

Muscle—Acetylcholine receptors in muscle fibres—MILEDI and POTTER (University College) . . 599

Molecular evolution—Descent of man—GOODMAN, BARNABAS (Michigan), MATSUDA (Nagasaki) and MOORE (North Carolina) 604

LETTERS TO *NATURE*

Physical Sciences

Krypton-85—Tropospheric increase since 1959—SCHRÖDER, MÜNNICH (Heidelberg) and EHHALT (Colorado) 614

Page

Interstellar molecules—Amino-acid synthesis in the laboratory—WOLLIN and ERICSON (Columbia) . 615

Charged particle detectors—High precision detector using noble liquids—DERENZO, SMADJA, SMITS, ZAKLAD, ALVAREZ and MULLER (Berkeley) . 617

Homogenization—Role of accelerated flow—PHIPPS (National Institute for Research in Dairying) . . 617

Biological Sciences

Taste—New compound with strong sweetness—TAHARA, NAKATA and OHTSUKA (Japan) . 619

Complement—Formation of lesions in lipid membranes—HESKETH, DOURMASHKIN, PAYNE, HUMPHREY and LACHMANN (National Institute for Medical Research) 620

Lantern fishes—Luminescent system similar to that in *Cypridina hilgendorfii*—TSUJI (Pennsylvania) and HANEDA (Japan) 623

BOOK REVIEWS

by T. R. Miles, Edward Hitchcock, W. R. A. Muntz, D. Prys Thomas 625

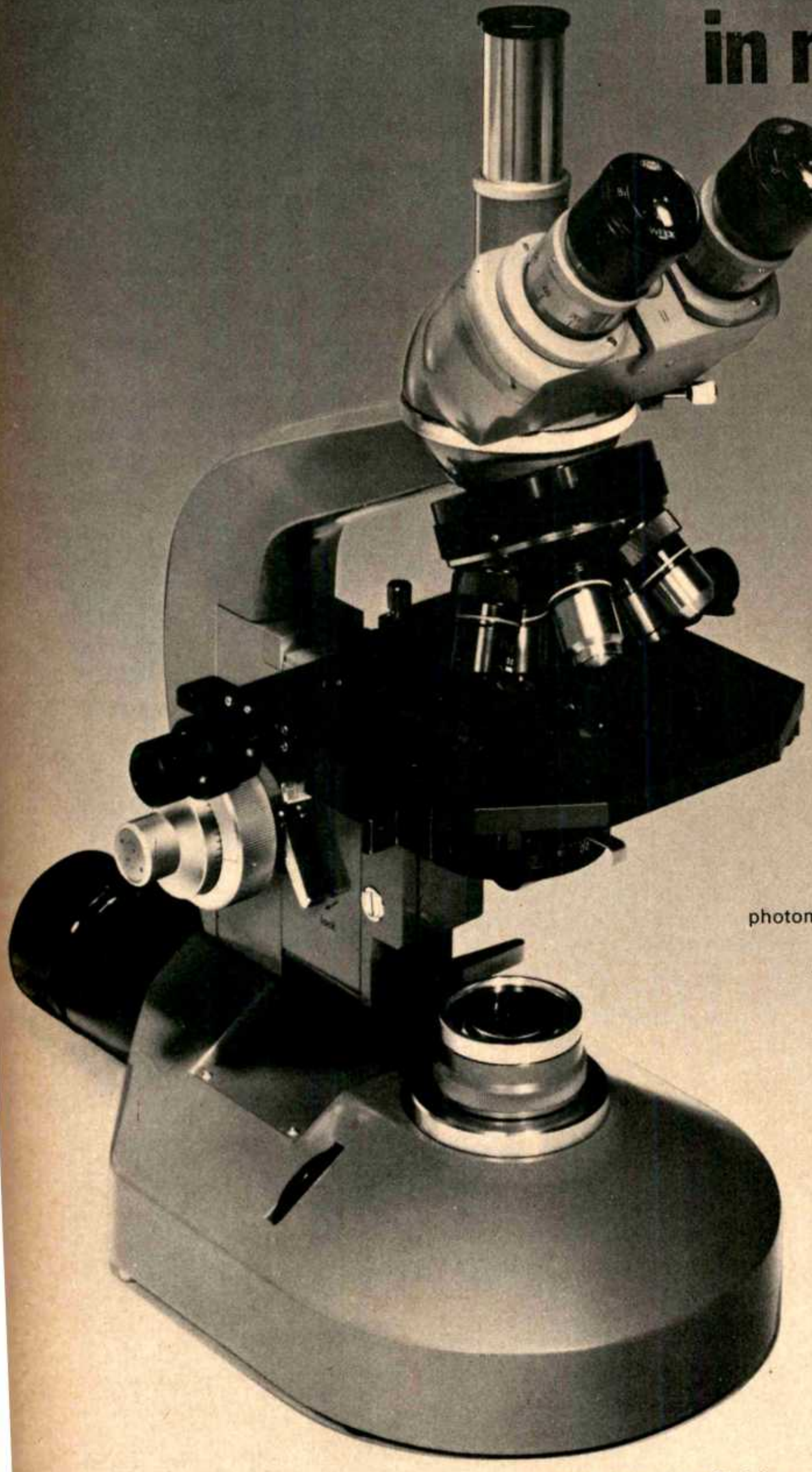
CORRESPONDENCE

Skuas (George A. Llano) 627

Notes of articles appearing next week in *Nature Physical Science* and *Nature New Biology* will be found on pages 588 to 592.

(Continued overleaf)

The microscope that works for you in multiple ways



This is the FHA, in its basic form a valuable addition to the field of microscopy. When full play is given to its versatility it becomes a super deluxe instrument — with enough interchangeable lenses to take you through practically any kind of research programs, a built-in illuminator, focusing down to as fine as 0.0005mm and heads that can deliver a superwide field of view or a full photomicrographic system. And the magnification range jumps right from 28× to 2000×. There are 8 models of the FHA, each one with a different eyepiece / objective / head / condenser arrangement, one just right for your application. With the standard turret mounted plan achromatic objectives you get super corrected components which make color and spherical aberration and field curvature almost non-existent, resulting in ideal images for both microscopy and photomicrography. Images are further guaranteed clarity by an N.A. 1.40 achromatic / aplanatic condenser. Added to this top microscope value is a full range of Olympus accessories — apochromatic objectives, photographic apparatus, exposure meter, phase contrast attachment, micro viewing screen, a darkfield condenser, a fluorescent light source, and more. Please write for a catalogue to your local distributor or to any of the addresses below:

Seeing beyond man's vision

OLYMPUS TOKYO OLYMPUS

We put light to work for science and society through a wide range of optics, microscopes, cameras, endoscopes, lasers and precision measuring instruments.

OLYMPUS OPTICAL CO., LTD. 43-2, Hatagaya 2-chome, Shibuya-ku, Tokyo, Japan
OLYMPUS OPTICAL CO., (EUROPA) GmbH. 2 Hamburg 1, Steindamm 105, Germany
OLYMPUS CORPORATION OF AMERICA 2 Nevada Drive, New Hyde Park, N.Y. 11040, U.S.A.

Next Monday's issue will include

Oceanography—Upwelling in Ghanian coastal waters explained by evaporation—POPLE and MENSAH (Ghana)

Soils—Dissolution of strengite—WILLIAMS and PATRICK (Louisiana)

Strain in solids—Craze formation in amorphous polymers—FOX and FULLER (Cavendish Laboratory)

Carbon fibres—Row nucleation of isotactic polypropylene—HOBBS (General Electric)

Next Wednesday's issue will include

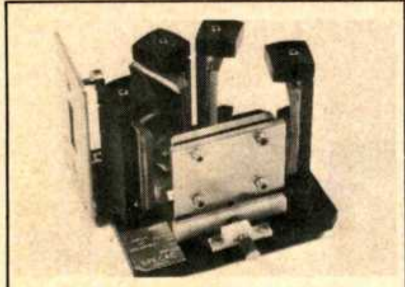
Histones—Selective *in vitro* methylation of rat chromatin histones after partial hepatectomy—BENJAMIN (Pennsylvania)

Cell fusion—Loss of human genetic markers in man–Chinese hamster hybrids—WESTERVELD, VISSER (Rijswijk), MEERA KHAN (Leiden) and BOOTSMA (Rotterdam)

Photoreceptors—"Dark" current generated passively—ZUCKERMAN (Rochester)

Chlorella — Centrioles and microtubules — ATKINSON, GUNNING, JOHN and McCULLOUGH (Belfast)

SPECAC



MODEL 11.000 ATR UNIT

SAMPLE HANDLING ACCESSORIES FOR SPECTROSCOPY

Enhance the utility of your spectrophotometer with SPECAC accessories.

Fixed and Variable Path Length Cells, KBr Pellet Dies, Mills and Presses.

Microsampling devices
Cell for gas analysis

Custom built accessories

Optical components in any of the IR and UV transmitting materials

01-317 8396

Spectroscopic Accessory Co.
134, Timbercroft Lane,
London S.E.18, England

GRIFFIN for STATISTICS

Statistical Monographs

THE NEW TITLES

28	**Pringle & Rayner:** Generalized Inverse Matrices	£2.75
29	**Newman & Odell:** Generation of Random Variates	£1.60
30	**Ord:** Families of Frequency Distribution *Early 1972 c.*	£3.20
31	**David:** First Course in Statistics *2nd Edn.*	£1.90

THE LAST TEN

18	**Huitson:** Analysis of Variance	£1.10
19	**Seber:** The Linear Hypothesis	£1.20
20	**Leser:** Econometric Techniques	£1.30
21	**Fisk:** Stochastically Dependent Equations	£2
22	**Vajda:** Patterns in Finite Spaces	£1.60
23	**Vajda:** Mathematics of Experimental Design	£1.50
24	**Van Dobben de Bruyn:** Cumulative Sum Tests	£1.20
25	**Ottestad:** Statistical Models	£1.30
26	**Guttman:** Statistical Tolerance Regions	£2.25
27	**Mardia:** Bivariate Distributions	£2.10

GRIFFIN

18 20

Descriptive catalogue from Chas Griffin & Co. Ltd., 42 Drury Lane, London WC2

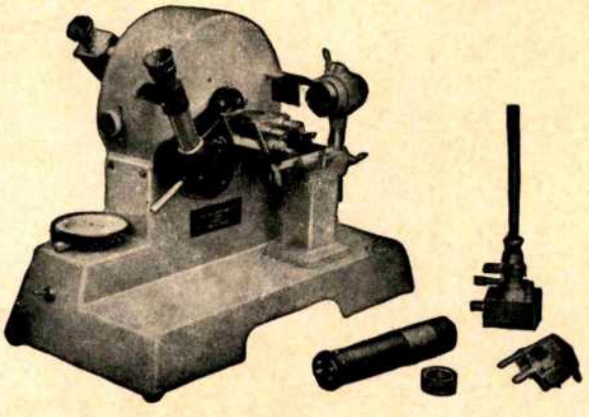
BS Precision Instruments

The Improved Type Pulfrich Refractometer

This instrument, in its modern styling, still embodies the requirements laid down by Mr. Guild of the National Physical Laboratory and the advice of research workers in various laboratories.

* CAN BE SUPPLIED WITH A DIFFERENTIAL CELL COMPLETE WITH COLLIMATOR AND SLIT FOR MEASUREMENT OF REFRACTIVE INDICES OVER THE RANGE 1·26 TO 1·95.

The interchangeable prisms, each covering a recommended range of refractive index, are of 60° refracting angle and are mounted in individual water jackets.



Please ask for Details 'N'.

We now have capacity for aluminising certain types of mirrors, at short notice.

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

BELLINGHAM & STANLEY LIMITED BS
61, MARKFIELD RD. LONDON N.15. TEL. 01-808 2675

NATURE Volume 232 JULY 16 1971

Whose Rate for the Job?

THE British Government seems well on the way to making a nonsense of its labour relations with the scientists on its payroll. In the past few weeks, even those in the scientific civil service whose inclinations lie more in scholarly research than towards the corridors of power have been offended by the parsimony with which the Civil Service Department has dealt with the most recent investigation of the Pay Research Unit into the relationship between salaries in the scientific civil service and salaries of scientists elsewhere. Briefly, the members of the scientific civil service who are above the recruitment grades in status have been awarded no pay rise in the current year (see *Nature*, **232**, 76 ; 1971). To be sure, many of those still working their way up the promotion ladders will have something extra in their pay packets, but there is little doubt that the settlement which the government has proposed, and which may well be modified by the compulsory arbitration to which it will now be subjected, represents a considerable injustice for many senior civil servants. One danger is that the bitterness evoked by this dispute will impede many of the reforms which have seemed to be in prospect in recent months as part of the painfully slow implementation of the Fulton report. More seriously, it is now possible that the government may wreck the whole basis on which it has proved possible in the past to persuade scientists to work in the public service.

What has gone wrong ? To begin with, it is a great misfortune that the government's pay offer should be based on an apparently confidential piece of research carried out by the Pay Research Unit. Why not make this public ? For objective comparisons between the salaries of civil servants, living as they do on a rigid salary structure, and scientists working in industry or commerce, are bound to be extremely hazardous. For one thing, the spread of salary in the civil service is much more closely linked with age than it is in industry. For another, no amount of arithmetic can accurately evaluate the fringe benefits which civil servants enjoy, especially holidays and pensions. After all, the importance of a lush but non-transferable pension scheme is that it persuades civil servants, especially the more senior of them, to put up with financial indignity rather than to move elsewhere.

The comparison between the civil service and industrial life is, however, most of all vitiated by the way in which promotion only rarely carries a scientist in the civil service into other kinds of activities. This was one of the complaints of the Fulton commission about the present organization of the civil service. Briefly, a man or woman who joins the scientific branch is likely to stay there until he or she retires. In other walks of life, and especially in industry, the scientific laboratories are increasingly (and rightly) regarded as stepping stones to administrative or managerial jobs. The result, of course, is that attempts to compare industry with the civil service are comparisons of quite different populations. There may be something in the view, recently put forward by the Confederation of British Industries (see *Nature*, **230**, 344 ; 1971), that the national interest would be better served if there were greater mobility between scientific research and administration, as indeed the Fulton commission has proclaimed. The fact that there are at present so few opportunities of this kind is not, however, the fault of the scientists concerned but of the government. It is entirely understandable that the scientists should feel hard done by.

However solemnly the advice of the Pay Research Unit may have been followed in the negotiations so far, the impression remains that the wellsprings of the government's meanness lie in its appraisal of future needs in the government research laboratories. With the continuing decline of defence research and with the doctrine of non-intervention as a guide, nobody will be surprised that the present British government should be more embarrassed than its predecessor by the numbers of scientists in the scientific civil service. It follows that in its pay negotiations, the government is bound to feel it has the whip hand. But is it really sensible to hope that a sufficient show of meanness will make that other problem go away by persuading young people not to work for the scientific civil service and by persuading more senior members to look elsewhere for new careers ? Such arguments are certain to be recipes for continuing trouble. If the scientific civil service does have too many people, why not work out constructive ways of making fuller use of them?

The underlying question is the extent to which the British Government intends to make constructive use of scientists and their craft. So far, after more than a year in office, the government has had hardly anything to say on the subject. Although there is a sense in which, by comparison with the all too strident utterances of Mr Anthony Wedgwood Benn and Mr Harold Wilson on the subject, silence is a virtue, yet the lack of a policy in such an important field is indefensible. Moreover, there is no prospect that the government's problems will be solved at one fell swoop when Mrs Margaret Thatcher, the minister for education and science, has had a chance to mull over Sir Frederick Dainton's report on the organization of civil science within the government, for it will still be necessary to decide on what scale the government should attempt to strengthen the competitiveness of British industry by means of publicly sponsored research and development. In passing it must be said that on what seems to be the threshold of membership of the European Economic Community, it will be absurd if the British Government chooses to eschew one of the few then permissible methods of stiffening domestic industry. In short, and whatever it may think at present, the British Government is going to need not merely the efforts of its scientists but their goodwill as well.

What Future for Euratom?

ONE of the least dazzling parts of the British Government's policy declaration last week in favour of British membership of the European Economic Community is the passage dealing with what is formally known as the European Atomic Energy Community, Euratom for short. The case for a British link with the EEC is by now so self-evident that it hardly needs to be made, yet the case for membership of Euratom is so meagre as to be non-existent. Even the more enthusiastic supporters of the European venture should acknowledge that, if only so that there shall be no great disappointment over what happens—or, more likely, does not happen—in the years ahead. For the truth is that the issue of Euratom membership is so nearly an irrelevance as to be an embarrassment. It would be in everybody's interest if the prospect of four extra members were now taken as a sign that the time has come to put Euratom on a sounder footing than in the past ten years.

The greatest disappointment has, of course, been the failure of Euratom, even when supported by the full political weight of the European Commission, to arrange for seemly collaboration in nuclear power development on a European basis. To begin with, in the late fifties, there seemed every chance that an organization such as Euratom would have useful work to do. Pre-Gaullist governments of France, urged on by a liberally minded Commissariat de l'Energie Atomique, were all for international collaboration, while the then government of Germany regarded Euratom as a means of making up for time lost during the long postwar uncertainties. In the late fifties, the pace of industrial development in Italy had begun to quicken in fields such as petrochemicals and plastics, so that there too the chance of moving ahead quickly in nuclear energy seemed entirely welcome. In the event, French hankering after nuclear independence in the military field proved to be a serious stumbling block. So too, by the mid-sixties, had become the sneaking suspicion or at least greed in the heart of every European government that there were rich pickings to be had in the design, construction and sale of nuclear reactors. In retrospect, it is just possible that these centrifugal tendencies could have been beaten off by a sufficiently imaginative and forceful organization in Brussels, but it is now even more clear than it appeared to be at the time that the first plans laid by the Euratom organization were too ambitious and too hastily prepared. Schemes like that for developing a reactor cooled by organic liquids were imaginative, it is true, but it would have been more prudent if in its early days Euratom had cut its teeth on projects more likely to be successful. More modest plans would also have been more winsome, especially for those national organizations threatened by the then prospect that Euratom might succeed in all it tried to do. But in the event, things have turned out quite differently. The research programme has for the most part failed.

To be sure, there are some redeeming features, the chief of which is the cooperative programme for research on thermonuclear fusion. Evidently it is easier for nations to collaborate with each other when their national interests appear to be less directly threatened. The investment of Euratom countries in the Dragon reactor will probably be, in the long run, a benefit, hard though it may turn out to be to quantify it. The most serious complaint against the way in which money has been spent on collaborative research in the past fifteen years is that Euratom has conspicuously failed to prevent the separate development of fast reactor projects in France, Italy, Germany and—now—the United Kingdom. As a monument to that failure, however, there remains the large research centre at Ispra (in Italy) with a staff of close on 2,000 and with no clear continuing reason for existence. In the circumstances, it is no wonder that the European Economic Community is making heavy weather of the task of working out a forward plan for Euratom. Each year since 1969, there has been talk of a long-term budget within which Euratom and its research stations could settle down to a useful job, and as a part of that process, governments, officials and industries have made what seems to be a sport of trying to suggest what kinds of work Euratom should now undertake. Some would make Ispra into a centre for environmental research. Others would turn it into a kind of international standards laboratory. But somehow the five year budget never materializes. If Mr Harold Wilson and those who oppose British membership of the EEC will allow the concept of British membership to become real, there is every chance that the negotiations now under way in Brussels within the EEC will lead to a three-year plan to tide over the period between now and the point at which Britain would be a full member of Euratom at the end of 1973.

What needs to be accomplished? The most valuable and certainly the most durable of the functions of Euratom is the safeguards system by means of which the community attempts to keep track of nuclear materials within the member countries. It is true that the safeguards system has never been quite the thing it might have been since the Government of France declared that some method must be found of excluding military explosives from the safeguards provisions of the Euratom treaty. Since then, it has never been quite clear to what extent Euratom is allowed access to French production plants on the understanding that the information gathered should not be publicized. Another possibility is that there are substantial parts of the French military explosives programme which are never included in the safeguards net. Moreover, it is not clear at present how much the Euratom safeguards will be transformed by the negotiations now under way with the International Atomic Energy Agency with a view to substituting Euratom controls for those that will in due course be administered from Vienna. The result is that nobody can safely predict how British membership of the EEC would affect the operations of British nuclear establishments. Would, for example, the fusion plants at Capenhurst now making enriched uranium for civil purposes be subjected to international inspection even though Britain is formally exempted from the provisions of the Non-Proliferation Treaty on the grounds of being or having been a nuclear power? These and many other questions like them are at present exceedingly obscure. The Euratom safeguards system, by all accounts the best feature of the community's activities, is plainly in need of making clearer and simpler if it is indefinitely to survive.

No Jobs for the Boys

A GREAT many active researchers in the United States are still able to remember and even to recall with a mixture of bravado and nostalgia how they were first compelled to find jobs in unlikely and sometimes unsuitable occupations—schoolteaching, salesmanship or shopkeeping. In part, these hard luck stories are an illustration of how deeply the depression of the thirties left its mark on all kinds of professional people then beginning their careers; when jobs are scarce, the newcomers inevitably find themselves at the back of the queue, with the result that whole cohorts of trained people may occasionally find themselves doing jobs for which they were not trained and which sometimes require no skill at all. But the way in which the tales of old heroism are remarked on is also a sign of the professionalization of science in the past few decades. It has now become less easy for men and women trained as scientists to earn their livings in other jobs. For one thing, training lasts so much longer that it counts as a valuable investment of time if not of money to new entrants to the labour market. Second, there is a real sense in which an interrupted career in professional science may be irreparably damaged.

The figures for unemployment in the United States now published by the National Science Foundation (see page 150) fit neatly into the pattern of employment prospects to be expected at times of economic recess. Among scientists as a whole, the rate of unemployment has increased—almost doubled—in a year but is still much better than that for the labour force as a whole (6.5 per cent in the first quarter of 1971). Skill is evidently an advantage still, which is borne out by the way in which people with PhD degrees had one of the lowest unemployment rates of all—1.4 per cent in the first quarter of the year compared with 3.5 per cent for less highly qualified scientists. Evidently one moral to be learned from this is that a person who has embarked on a career as a scientist will not benefit his employment prospects by refraining from completing a PhD degree.

True to the pattern of recession, the statistics also show that newly qualified people are the hardest hit, and there is nothing in the statistics to show that American employers have accepted the demands of the Women's Liberation. (In the beginning of 1971, the unemployment rate was twice as high among women scientists as among men scientists.) Predictably, many of the unemployed had been shaken out of defence and aerospace. The sobering tendency in the statistics now published is that which shows the great duration of the period of unemployment—an average of seven months by the beginning of June this year. With the next crop of graduates about to tread on the heels of those still left over from 1970, it is plain that the present recession must leave a lasting scar.

What solution can there be? No amount of vigorous action, such as that intended by the host of bills now bemusing Congress with proposals for spending money on the retraining of scientists and engineers in the gentle causes of the fight against pollution, will make up for the interruption of young people's careers and the destruction of their expectations that the present recession has caused. Unhappily, however, it helps very little to do what the Administration has done and to argue that, if there are not enough jobs to go round, the best solution is to restrict substantially the numbers of stipends available for those wishing to enter graduate schools. On the face of things, the incidence of unemployment is greatest among the age groups from 25 to 35. Only some of these, at most a third or something like 1,000, will be people with PhD degrees who have left graduate schools in the sixties. Moreover, most of these are people trained in chemistry or physics—the incidence of unemployment in other fields is much more modest. Alarming though the figures may be for the individuals concerned, they are extremely small compared with the annual production of PhDs in the United States in recent years—18,000-odd in all the sciences and engineering in the academic year now finishing. But in any case, as the figures themselves show, a PhD degree is an advantage, not a handicap, in looking for a job, at least in comparison with a lesser qualification. The case for reducing the level of support for graduate schools is, in short, entirely independent of the present employment.

---

100 Years Ago

NATURE

LETTERS TO THE EDITOR

[*The Editor does not hold himself responsible for opinions expressed by his Correspondents. No notice is taken of anonymous communications.*]

Cotteau's "Echinides de la Sarthe"

A NOTICE of Cotteau et Triger's *Echinides de la Sarthe* in a recent number of NATURE (June 15, p. 120) is likely to convey a false impression of the accuracy of M. Cotteau, and throws considerable doubt on the value of his work. It is not often that French scientific men are as conscientious as he is in the examination of authentic types. There is hardly a collection of fossil Echini which M. Cotteau has not examined; and his thorough acquaintance with all that has been written on his subject, as well as his intimate correspondence with the principal echinologists, is a sufficient guarantee that no important memoir (such as Wright's monograph) could have escaped him. Anyone who will take the trouble of turning to Cotteau's work (p. 111) will find, under *Pseudodiadema hemisphæricum*, a notice of Dr. Wright's figure of the same species (so much superior, with many others, to Cotteau's?) and a reference to his description. Nor is this an isolated case. Throughout the work M. Cotteau discusses and criticises more or less the results of this very monograph, said to have been overlooked by him. The mistake Cotteau is accused of making of assigning to Desor instead of Agassiz the specific name of *Pseudodiadema hemisphæricum* is entirely unfounded. Referring again to p. 111., we find, as a synonym, *Diadema hemisphæricum* Agass. M. Cotteau, like many continental and American writers, does not interpret the notation of species as is required by the laws of the British Association, but for that reason he should not be accused of committing mistakes which his own writings show him not to have committed. M. Cotteau, in common with others, looks upon nomenclature simply as a matter of registration; and when M. Desor transfers to *Pseudodiadema* the *Diadema hemisphæricum* Agass., M. Cotteau writes, therefore, *Pseudodiadema hemisphæricum* Desor, and not Agassiz; he may be wrong, according to the principles of the writer in NATURE, but he has not, either in this instance or in the other cases alluded to, committed a mistake through ignorance of the subject.

A. AGASSIZ

From Nature, 4, 220, *July* 20, 1871.

OLD WORLD

SCIENTISTS' PAY

Continuing Dispute

THE date for arbitration of the pay dispute between the members of the scientific civil service, represented by the Institution of Professional Civil Servants, and the Civil Service Department has been fixed for August 12, and, if necessary, August 13, at Church House, Westminster. The proceedings of the arbitration inquiry will not be held in public, even though the Institution of Professional Civil Servants had been hoping to arrange either that the inquiry would take place in a hall sufficiently large to accommodate some hundreds of its members, or, alternatively, that the proceedings would be relayed to a room elsewhere.

Between now and the arbitration date, the institution has also arranged a series of public meetings which are expected to provide good business for the shrunken telegraph service of the British Post Office Corporation in the shape of messages to members of parliament. With an eye to the staff of the Atomic Energy Research Establishment at Harwell, a meeting has been arranged for Friday, July 16, at the Fitzharris School, North Court Road, Abingdon (8.15 p.m.). It is hoped to arrange a meeting at Portsmouth on Monday, July 19, at the Civil Service Club and a meeting at Reading on Wednesday, July 21, at the Great Western Hotel (6.30 for 7.00 p.m.) and at the Cooperative Hall, Western Road, Weymouth, on Thursday, July 22 (7.00 for 7.30 p.m.).

The Institution of Professional Civil Servants intends to bring its campaign of publicity to a climax with a public meeting at the Central Hall, Westminster, on Tuesday, August 10.

The statistics available to the Civil Service Department do at least give the lie to the suspicion that the pay dispute has arisen at a time when the strength of the scientific civil service is declining. Since 1965–66, for example, the number of Principal Scientific Officers has increased from 1,530 to an estimated 1,748 in the current financial year. Similarly, the number of Senior Scientific Officers has increased by almost 20 per cent to a total of 1,244 and there is an even larger proportionate increase in the number of Senior Principal Scientific Officers (whose salaries are determined by a different procedure from that which has occasioned the present dispute) from 541 in 1965–66 to 668 in the current financial year. During the same period, the numbers of Senior Experimental Officers have increased even more dramatically, from 1,471 to 2,090. By comparison, the numbers of scientists in the recruitment grades have increased less quickly over the years, suggesting that the managers of the civil service have done what they can to lessen the pressure on pay by increasing the numbers of comparatively senior posts available.

MINERAL RESOURCES

Metals or Mountains?

THE announcement last week by Sir John Eden, Minister for Industry, of grants totalling up to £50 million to aid mineral exploration of non-ferrous metal ores, fluorspar and potash in Great Britain is certain to be welcomed by the mining companies and equally certain to be regarded with reserve by the conservation lobby. The minister, in announcing the scheme, in which the government will contribute up to 35 per cent of the cost of exploration and evaluation, stressed that the normal planning controls under the Town and Country Planning Acts would still apply. In spite of these reassurances, such a scheme, with full ministerial backing, is bound to increase the pressure to grant planning approval in any part of the country.

The confrontation with the conservationists arises because these minerals are found in older rocks which make up the mountains of Britain. These are also the areas where most of the National Parks are found. Before the present scheme was announced, the Rio Tinto-Zinc Corporation applied for permission to prospect within the Snowdonia National Park. The inquiry into their application was held last March and the decision of the Secretary of State for Wales is expected soon.

The announcement of the scheme comes at a time when the richer and more easily mined ore fields in other countries are being worked out. There has been no large scale mining in the UK since the end of the nineteenth century when the low quality of ore coupled with the mining expansion abroad eventually made it uneconomic to continue mining. But developments since then in mining techniques as well as the high cost of importing these minerals (£600 million a year) have made it economic to mine low quality ores.

Some lead and zinc are being produced as by-products of fluorspar mining in Derbyshire but the only non-ferrous metal currently being mined on any scale in Britain is tin in the west of England. There are, however, known to be deposits of copper, tungsten, gold, barium and uranium. The extent and quality of the known deposits are mostly unknown and the aim of the grants is to encourage mining companies to prove the mineral resources of Britain.

The government will recover the grant with accumulated interest if a successful mine results from the exploration. Sir John also pointed out that as well as the economic advantages of new mines, chiefly on the balance of payments, there would also be more jobs in parts of the country where they are most needed. Such advantages must, however, be weighed against the possible damage to the countryside if wholescale mining were allowed to take place.

Stars in Rock

by our Soviet Corresponden

ANCIENT rock carvings in Armenia (dating from 8000–1000 BC are reported to have revealed a considerable degree of astronomical knowledge in their makers. The carvings, which have been found at various sites in the Armenian highlands, indicate that the then inhabitants of the region divided the year into twelve parts, revealing a fairly good understanding of the length of the year and of the lunar month.

The most detailed astronomical carving is one in the Martuni region of the Sevkar foothills. Here the carving is exceptionally deep and a great deal of detail is preserved. It shows, on the left, the three bright stars of *Cygnus* in a line, with the sizes of the stars roughly proportional to their apparent brightness. Beside them is a pictogram of two men holding a serpent above their heads. This, says Dr B. Tumanyan, Director of the Observatory of the State University of Erevan, indicates the great antiquity of the constellation name of the Serpent-Bearer (*Ophiucus*).

In the top left hand corner of the picture, there are numerous dots and crosses representing the stars of *Delphinus* and the part of the Milky Way between *Aquila*, *Delphinus* and *Sagitta*. At the top there are pictograms of a sword, a shield and two crosses and a triangle, in the section where *Cygnus*, *Lyra* and *Vulpecula* should lie. The layout makes it quite clear that the pictograms represent the stars of this area, and thus indicate very primitive groupings and names for these stars.

According to Dr Tumanyan, detailed study of the carving suggests that it dates from c.3000 BC.

NEW WORLD

Cancer Compromise Almost Unites Senate

by our Washington Correspondent

THE tussle between Senator Edward Kennedy's Health subcommittee and the Administration over the organization of cancer research ended last week in an atmosphere of mutual back-slapping. A compromise bill in the name of the Administration went through the Senate with only one lonely voice raised in dissent, and if the bill survives the rest of the Congressional mill in its present form, hundreds of millions of dollars a year will be poured into an independently funded cancer agency based on the present National Cancer Institute. The compromise, worked out behind the scenes of the Kennedy Committee (see *Nature*, **231**, 414; 1971), seeks to set up an agency unfortunately and misleadingly called the Conquest of Cancer Agency, geographically located within the National Institutes of Health, but with a separate budget and with a director who is responsible directly to the President. A moot point, and the one on which Senator Gaylord Nelson of Wisconsin based his opposition to the bill, is whether the agency will in practice operate within the NIH, or whether the administrative arrangements that have been worked out for it will take it essentially outside the confines of the other nine institutes which together make up the NIH.

But one of the more disturbing aspects of last week's debate—if a seventy-nine to one split can be termed a debate—is that the Conquest of Cancer Agency has been sold to the Senate and the public as a measure which has a good chance of leading in the near future to a cure for cancer. The suggestion is that a massive infusion of capital into a NASA-style agency charged with the task of finding a cure for cancer is all that is needed to come up with an answer to the cancer problem. That was certainly the impression unashamedly put out by Senator Jacob Javits of New York, a member of the Kennedy committee and a co-sponsor of the bill, when he told his fellow Senators that "Senators Dominick, Kennedy and I, and a majority on the committee, feel that we are so close to breakthroughs in cancer; that cancer represents to the people such an awesome disease or illness, that we must, as a national effort, quite apart from the scientific question, undertake a crash programme under a unified direction, to make the final breakthroughs. . . . The country finds the death rate from cancer unacceptable because it thinks it is imminently avoidable. That is the question we think we are close enough to breakthroughs on, and that one final drive will do it."

There is a real fear in the biomedical community that if the cancer cure agency is sold to the public in these terms, and fails to come up immediately with the answers, scientists and science alike will be discredited. The fact that a so-called cure for cancer is probably not as attainable as the Moon could come as a rude shock to those who will be looking for the proposed agency to produce the right answers at the mere wave of the financial wand. These sentiments were eloquently expressed only last month by Albert Sabin, founder of the oral polio vaccine, who has been widely tipped to head the new cancer agency. Speaking at a meeting in Brussels, Sabin said that researchers working on such targeted research are worried because they have been charged with a task which to the public seems to be a question merely of finance, but which in practice is much more complicated.

Those sentiments apart, the passage through the Senate last week of the Administration's bill is an object lesson in Congressional politics. The tussle between President Nixon and Democratic Congressmen led by Kennedy has already been well documented in *Nature*, but which side has in practice won the battle will probably not become clear until the proposed agency has been working for some time. At the root of the disagreement is whether or not the Conquest of Cancer Agency will remain in practice as well as in theory a part of the National Institutes of Health. Kennedy originally sponsored a bill in the Senate (numbered S34) which proposed in essence that the National Cancer Institute should be taken outside the National Institutes of Health and raised to the status of an independently-funded agency reporting directly to the President. But the Administration later introduced a bill (numbered S1828) which proposed instead that the National Cancer Institute should remain as a bureau within the NIH, and that it should have a separate budget. The bill which was passed by the Senate last week was something of a compromise between these two positions, although it retained the number S1828, meaning that it was the Administration's bill.

Kennedy's proposals met with the almost unanimous disapproval of the scientific community, whose witnesses before the Health subcommittee deplored the suggestion that the proposed cancer agency should be taken out of the NIH and away from other biomedical research. And it is on this point that Kennedy has been seen to come into line with the Administration's thinking, for the bill which went through the Senate called for the setting up of the Conquest of Cancer Agency within the NIH, but with a director reporting to the President. The bill in particular lays down some specific guidelines for cooperation between the Conquest of Cancer Agency and the NIH. The most important is that the director of the agency is authorized to "take necessary action together with the Director of the National Institutes of Health so that all channels for the dissemination and cross fertilization of scientific knowledge and information existing prior to the effective date of this Act between the National Cancer Institute and the other Institutes of Health shall be maintained between the Agency and the Institutes of Health to ensure free communications between cancer and other scientific, medical and biomedical disciplines".

But these provisions do not satisfy Senator Nelson, who argued and voted against the bill, and Senator Cranston from California, who expressed doubts but voted for the bill. Nelson made it clear that he believes that it is the Administration which has sold out to the original Kennedy proposal rather than the reverse. Referring throughout his remarks to the proposed agency as an institution separate from the NIH, Nelson warned that other institutes within NIH will want similar treatment, as in fact the National Heart and Lung Institute has already requested, and this bill, if it emerges unchanged by the House, will signal the break-up of the NIH itself. "I have repeatedly asked the proponents of this measure—that is, the proponents of creating an independent institute outside of NIH—just what is to be gained by it . . . there is not much of a reason for it except a kind of emotional commitment to do something with an independent institute that they think cannot be done if they remain within the NIH, the finest institution of its kind on Earth," Nelson said.

Another important plank in Nelson's platform is that under the terms of the bill, the Director of the NIH will be responsible for about $100 million worth of cancer-related research within the nine remaining institutes of health, while a further $332 million will be spent by the proposed Conquest of Cancer Agency housed within the NIH but reporting directly to the President. "It does not make much sense at all," he said. "I think that if everybody sat down and thought about it for a while, they would recognize that this is not the best approach to accomplish the end we all seek." Cranston echoed these sentiments, believing that the suggestion for cooperation between the director of the proposed agency and the Director of the NIH is insufficient. "What is needed is statutory authority making clear that the Director of NIH should have the formal responsibility of submitting the cancer programme and budget to the President, along with his evaluation of the cancer programme and appropriate recommendations," he said.

In the event, however, the bill went through with only Nelson recording his dissent, and the nature of the compromise worked out between the supporters of Kennedy's original bill and those who acceded to the Administration's original proposals can be evinced from an extraordinary tale told by Javits. "Many people always think that we are very strong minded and that we want to have our *imprimatur* on everything and will never yield a single prerogative or permit the name of anyone else to get on a bill except our own names, but in this instance we had finally to prevail on the Senator from Colorado (Dominick), who felt he wanted the Senator from Massachusetts (Kennedy) to bring the bill to the floor."

UNEMPLOYMENT

Grim Prospects

by our Washington Correspondent

THIS year's crop of science and engineering graduates who have already found themselves swelling the ranks of the unemployed do not need to be reminded that they have been thrown on to the job market at its most depressed time since the Second World War. The full extent of unemployment at present remains, however, a subject for speculation, but some alarming indications can be gleaned from a survey conducted in May by the National Science Foundation, the results of which have now been made public.

Table 1 Number and Rates of Unemployed Scientists, by Field of Science, 1971

Field of science	Responding	Not employed and not seeking employment*	In labour force	Unemployed	Unemployed rate (%)
Total	253,078	8,003	245,075	6,337	2.6
Chemistry	73,008	2,576	70,432	2,101	3.0
Earth and marine science	18,724	535	18,189	486	2.7
Atmospheric and space science	5,243	198	5,045	142	2.8
Physics	30,220	1,089	29,131	1,126	3.9
Mathematics	19,745	642	19,103	491	2.6
Computer sciences	8,840	223	8,617	309	3.6
Agricultural sciences	12,708	247	12,461	110	0.9
Biological sciences	39,871	855	39,016	656	1.7
Psychology	20,319	731	19,588	304	1.6
Statistics	2,465	92	2,373	51	2.2
Economics	9,767	318	9,449	154	1.6
Sociology	5,512	251	5,261	196	3.8
Political science	4,286	134	4,152	141	3.4
Anthropology	1,029	48	981	13	1.3
Linguistics	1,341	64	1,277	57	4.5

* Includes such groups as retired, students, housewives, and others not in labour force.
Source: National Science Foundation, National Register of Scientific and Technical Personnel.

Based on returns to a questionnaire sent out to 300,000 scientists, the NSF's conclusion is that 2.6 per cent of all qualified scientists were out of work in the spring of 1971. Compared with a national average unemployment rate of 6.5 per cent during that period, these findings may not evoke too much sympathy on the dole queues. But the important point is that they correspond to the period immediately before this year's new graduates came off the university production line, and with the abundant reports of cutbacks by industry in graduate recruitment, they are cold comfort for new graduates seeking employment. The estimates should also be considered as minima, for they are based on replies from scientists who could be reached, and they do not take account of the fact that many scientists were underemployed or had left science for other sectors of employment.

Chief among the findings are:

- 1.4 per cent of all doctorates were out of work this spring, compared with 0.9 per cent last year, while the unemployment rate among nondoctorate scientists was 3.5 per cent compared with 2.9 per cent in 1970.
- Young scientists were the hardest hit, with an unemployment rate of 5.3 per cent.
- The unemployment rate among women was running at 5.2 per cent, compared with 2.3 per cent for men.
- About 45 per cent of unemployed scientists said that their last science-related employment was supported to some extent from government funds.
- Scientists who are not citizens of the United States reported an unemployment rate of 4.2 per cent, compared with 2.5 per cent for US citizens.

Chemists and physicists between them accounted for half the total of unemployed scientists in the survey, and unemployment rates in these two sectors were running at 3.0 and 3.9 per cent respectively, compared with 1.5 and 2.3 per cent in 1970. Sociologists, political scientists and computer scientists also reported unemployment rates greater than 3 per cent (see Table 1). It also seems that unemployed scientists who were previously employed by industry have been most affected by the present job shortages, for in 1970 industry and business employed 31 per cent of the scientists in the national register, but 38 per cent of the unemployed scientists in the NSF survey gave industry and business as their previous employers.

The hardest hit area is California, where 42 per cent of the unemployed scientists were located, and in Seattle, Washington, the unemployment rate among scientists was running at 5.6 per cent in the spring. The Department of Labor has in fact designated 14 areas as being severely affected by unemployment of scientists, engineers and technicians, and the chief factor linking many of the areas is a high concentration of defence and aerospace activities. The areas include Orange County, California, Seattle, Dallas, Long Beach, California, Atlanta and Cape Kennedy.

Short Notes

THE Kitt Peak National Observatory has a new director in the person of Dr Leo Goldberg, director of the Harvard College Observatory. Dr Goldberg will succeed Dr Nicholas U. Mayall on September 1. Dr Goldberg, who is an astrophysicist specializing in atmospheric problems, has divided his career between Harvard and the University of Michigan.

NEWS AND VIEWS

Circular Polarization and Astronomy

THE possibility that circular polarization measurements can provide significant new information about the planets has now been taken a step further. On page 165 of this issue of *Nature*, Kemp and his colleagues, who have been working at the Mauna Kea Observatory, Hawaii, report further measurements of the circular polarization in the light reflected from Jupiter, and the first measurements of circularly polarized light from Mars, Venus, Mercury and the Moon. To begin with, their new study verifies the explanation of the circular polarization from Jupiter that was put forward tentatively when the discovery was announced two months ago (*Nature*, **231**, 169 and 170; 1971). It also seems probable that the circular polarization recorded from the other planets can be explained by the Jupiter mechanism, or a modification of it. But with positive measurements from several planets now under their belts, Kemp and his colleagues allow themselves a little more speculation about the applications of their discoveries to studies of the planets than they did two months ago.

In the case of Mars and the Moon, the occurrence of circular polarization in the scattered light appears to be attributable to their dust-covered surfaces, so that the measurements are unlikely to provide any information that is not already known. The same explanation probably holds for the single observation of circular polarization from Mercury that Kemp and colleagues report, although in this case any information gathered is likely to be more valuable. It remains to be seen whether the well known awkwardnesses that arise in observing a planet so near the Sun will prove to be serious obstacles.

The data from Jupiter and Venus, and the possibility of obtaining data from Uranus and Neptune, are likely to be more important, however. In the case of Jupiter and Venus, the origin of the circular polarization has to do with their dense atmospheres (as is also the case for measurements of Mars at blue wavelengths where the effect of the thin Martian atmosphere seems to become significant). The mechanism that Kemp and colleagues put forward two months ago for the origin of the circular polarization from Jupiter has now been essentially verified by the way in which the circular polarization from the northern and southern hemispheres of the planet altered through the period of opposition (opposition was on May 23). The way is now open, then, for a detailed examination of the variation in circular polarization across the surface of Jupiter when the equipment for examining small areas of planet is available. Already, however, there is interest in the possibility of significant variation in the magnitude of the circular polarization from the vicinity of the Red Spot. The Red Spot is in the southern hemisphere of Jupiter, and Kemp and colleagues report unusual behaviour of the circular polarization averaged over the entire southern hemisphere with which the Red Spot may conceivably be related. Kemp and colleagues have already reported evidence that the leading edge of the Red Spot is more strongly circularly polarized than the trailing edge (*Nature*, **231**, 169; 1971).

The outlook then is promising that circular polarization measurements, particularly of the outer planets, will provide work for some time to come. Historically this prospect is an extension of research that has been carried out by Kemp and others for the past year or two on circular polarization in the light from white dwarf stars. On the face of it, the detection of circular polarization from white dwarfs is at present likely to be of more significance than the planetary work, largely because the virtually featureless spectra of many white dwarfs are a bar to a better understanding of their nature. Through the work of Kemp and others, however (see *Nature Physical Science*, **231**, 21; 1971), it is now clear that the circular polarization that has now been observed in the light of three white dwarfs—Grw + 70° 8247, G195-19, and G99-37—can be related to the strengths of their magnetic fields. The measurements indicate fields of the order of 10^6–10^7 Gauss. This work is important, if only because it lends support to an essential requirement of all pulsar theories—that the rapidly spinning neutron stars which are believed to be the origin of the pulsating signals have the enormous magnetic fields of the order of 10^{12} Gauss that are necessary for an understanding of their electromagnetic emission. Neutron stars are expected to have diameters of the order of 10 km, compared with 1,000 km for typical white dwarfs, so bearing in mind that the magnetic field increases as the inverse square of the radius as a star collapses (assuming the conservation of magnetic flux), there is now encouraging observational support for the view that the collapse of a main sequence star to a neutron star can lead to the required large fields of 10^{12} Gauss.

The circular polarization from white dwarf stars has its origin in the characteristics of blurred spectral lines in a strong magnetic field. To be sure, Jupiter is in many ways surprisingly close to being a white dwarf, but it was clear from the start that the mechanism which accounts for the white dwarf measurements could not explain those from the planets. For one thing, a field of at least 10^3 Gauss would be needed, rather than the 10–100 Gauss which seems to be indicated for Jupiter. So there are several different mechanisms in the air for the origin of the circular polarization, depending on the observed object, with much work still to be done on each of them. In the current *Astrophysical Journal*, for example, Shipman of the Hale Observatories examines Kemp's theory for the white dwarf Grw + 70° 8247, and is able to modify it to explain better the wavelength dependence of the circular polarization measurements (**167**, 165; 1971). Where Kemp predicted that the circular polarization would increase linearly with wavelength, Shipman is able to reproduce, very roughly, the peak in the fractional polarization that is observed for this star at 4000 Å by a re-examination of the conditions in the star's atmosphere. The anomalously large circular polarization that has been reported in the infrared remains a problem, however. Clearly there is much work to be done, both by theoreticians, and at the telescope, to see how general is the form of the spectral dependence observed for Grw + 70° 8247. Together with the interesting data that may come out of the planetary measurements, the circular polarization measurements are a rich seam which will be worked for many years to come.

NUCLEAR FUSION

Tokamak Excels

from a Correspondent

The conference on plasma physics and controlled fusion research organized by the International Atomic Energy Agency at Madison, Wisconsin, from June 17 to 23 was the fourth in the series of meetings which are held at three-year intervals. They constitute the forum for the international exchange of information on progress towards an ultimate power-generating fusion reactor. At present the principal concern is the plasma confinement problem, and in spite of the title the only plasma physics covered was that held to be directly relevant to fusion.

Many different schemes for plasma confinement are under study and a good deal of attention is given at these international meetings to their relative progress and future promise. This latest meeting was fairly unremarkable in the sense that no outstanding new results or concepts were presented; the remarkable feature was that for two successive meetings in the series the Russian Tokamak system has maintained a lead both in performance and promise over rival schemes.

Thus Dr E. Meservey (Princeton University) presented results from the Princeton Tokamak which in broad terms confirmed those obtained earlier by Russian workers on their T-3 machine in Moscow. There are some differences and attention was naturally concentrated on these. For example, the electron temperature is strongly peaked near the axis in the American machine, whereas in the Russian one the profile is relatively flat. There are other differences which seem to be attributable to differences in interpretation of essentially the same experimental data. Thus the Russians think that the containment time for particles greatly exceeds that for the energy whereas the American view is that they are about equal.

Dr V. S. Strelkov (Kurchatov Institute, Moscow) presented results from the latest Russian Tokamak, T-4. This has a higher magnetic field (50 kG) and the stabilizing copper shell is nearer to the plasma than in T-3. The plasma current has thus been increased by almost a factor of two: the ion temperature as deduced from the neutron yield reaches 650 eV. With a current of 110 kA the electron temperature is ~2 keV, the plasma density is 3×10^{13} cm^{-3} and the containment time is 10 milliseconds. These are impressive numbers but they fall short by about one order in both temperature and density and two orders in containment time over the requirements for a fusion reactor. It should be emphasized that although many features of the Tokamak discharge are not understood there are fairly clear, if expensive, lines along which the next experiments should proceed.

Whether some tortoise will eventually overtake the Tokamak hare is an interesting question. The stellarator which is closely related to the Tokamak, but much more expensive and difficult to build, seems to be undergoing a random walk rather than progress. Dr P. Reynolds (UKAEA, Culham Laboratory) presented results from his small stellarator which agree very well with the theory of classical containment. By contrast Dr K. Myamoto (Nagoya University) finds fluctuations and anomalous losses in his very similar machine. For the largest stellarator at present operating in the world, the Uragan machine at Kharkov, Dr K. N. Stepanov reported electron temperatures up to 400 eV, density ~5×10^{12} cm^{-3} and containment time ~300 µs.

Research on high beta toroidal confinement is still in an early stage as groups previously working on linear theta pinches move into the field. Typical results on screw pinches reported from Jutphaas, Culham and Garching were electron density ~10^{16} cm^{-3}, plasma temperature ~100 eV and containment time ~10 µs. This configuration relies for its equilibrium on toroidal current flow in the outer low density region. The rapid decay of this current is believed to be the limiting factor and Dr P. C. T. van der Laan (Institute for Plasma Physics, Jutphaas) discussed a variety of possible explanations without reaching any definite conclusion. Dr H. A. B. Bodin (UKAEA, Culham Laboratory) presented results on the diffuse pinch configuration which were encouraging in the sense that the configuration could be set up and its stability agreed roughly with theory but discouraging in that the temperature was limited by impurity radiation to ~10 eV. Results from a segment of the Scyllac torus reported by Dr F. L. Ribe (Los Alamos Scientific Laboratory) showed control of the toroidal drift by the addition of $l=0$ and $l=1$ fields.

In mirror research the chief result was that reported by Dr F. H. Coensgen (Lawrence Radiation Laboratory, Livermore) who finds that plasma is lost from his 2X machine at about ten times the classical rate. This fast loss is accom-

---

Mixed RNAs in Transformed Cells

In *Nature New Biology* next Wednesday, Wall and Darnell report a series of elegant DNA.RNA hybridization experiments which establish once and for all that sequences of SV40 RNA occur in large molecules covalently linked to sequences of host cell RNA in the nuclei of mouse cells transformed by SV40.

In 1968 Sambrook and his colleagues showed that, in stably transformed cells, SV40 DNA molecules are integrated into DNA molecules of the host genome. Since then, although the number of integrated SV40 genomes in transformed cells has remained a matter of dispute, numerous groups have found that SV40 RNA is present in both the nucleus and cytoplasm of transformed cells. Further, it is generally agreed that SV40 RNA occurs in the nucleus in molecules which are longer than a single complete SV40 genome and in the cytoplasm the SV40 RNAs associated with polysomes are no longer than a single SV40 genome. There is every reason to believe that these polysomal SV40 RNAs are derived by cleavage from the large nuclear RNA molecules which contain SV40 sequences, but how do these large nuclear RNAs themselves arise?

There are two obvious possibilities which have been canvassed. If several SV40 genomes are integrated in tandem in each transformed cell they might be transcribed as a single unit to yield RNA molecules longer than a single SV40 genome and containing nothing but SV40 sequences. Alternatively, the SV40 genomes may be integrated singly in such a way that they are always transcribed together with some of the adjacent host DNA; in which case the size of these RNAs would depend on how much of the host genome is transcribed and they should contain host sequences either at the 5′ or 3′ or both sides of the SV40 sequences.

To decide this issue, Wall and Darnell first hybridized to SV40 DNA RNAs from the nucleus and cytoplasm of transformed and untransformed cells. They then recovered the hybridizing RNA from the hybrids, which must contain SV40 sequences, and tested to see if it would also hybridize with cellular DNA from untransformed cells. They detected nuclear RNA molecules from transformed cells which hybridized with both SV40 and cellular DNAs. Wall and Darnell conclude therefore that in transferred cells at least some SV40 DNA molecules are transcribed together with some host DNA to give large and mixed RNA molecules. And although their results do not prove the idea that these mixed molecules are precursors of the small SV40 RNAs associated with polysomes they do not contradict it. By further refining the hybridization techniques and ultimately by fingerprinting these various RNAs it should be possible to decide if they are related as precursors and products and to learn something of the mechanisms which control transcription and messenger RNA maturation in eukaryotes.

panied by evidence of instabilities with frequencies near the ion cyclotron frequency. Because a mirror reactor requires near-classical confinement this result is not encouraging; it is possible, however, that the necessary quiescence can be achieved by careful machine design and control of the particle velocity distribution.

Theoretical work presented at the conference dealt chiefly with plasma confinement and stability in the Tokamak. There is now a fair measure of agreement on the classical theory of confinement in symmetric low beta systems. Attention is now paid to the effect of residual fluctuations on such systems and contributions from Dr S. Yoshikawa (Princeton University) and Drs A. A. Galeev and R. Z. Sagdeev (Kurchatov Institute, Moscow) reached similar conclusions by rather different routes. This work emphasizes the role of the poloidal magnetic field in plasma confinement, the much stronger toroidal field performing only an auxiliary function. On the question of stability, the situation is not clear; a contribution by Drs D. Pfirsch and H. Tasso (Institute for Plasma Physics, Garching) which showed the Tokamak to be unstable against a simple mode will undoubtedly be the subject of much critical attention.

For the first time at these meetings a session was devoted to reactor systems. The broad conclusion from this session was that within the necessarily very wide margins of error, fusion as a power source might be competitive with other systems and might offer substantial environmental advantages.

RNA

Messenger's Burden

from our Molecular Biology Correspondent

ONE of the strangest molecules that explorers of the eukaryotic nucleus have brought to light is polyadenylic acid, in almost monodisperse form. This observation became more than a mere unexplained curiosity when Lim and Canellakis reported last year that haemoglobin messenger RNA contains a long tract of poly A, which apparently largely accounts for the discrepancy between its observed and expected molecular weight.

The question of how general this phenomenon may be has now been taken up in several laboratories, and a clutch of three articles on the subject is to be found in the current issue of the *Proceedings of the US National Academy of Sciences.*

Lee, Mendecki and Brawerman (**68**, 1331; 1971) have examined the messenger RNA of ascites cells. They find that at *p*H 9, though not under more nearly neutral conditions, a large proportion of the rapidly labelling RNA fraction of polysomes is specifically trapped on a membrane filter. The fraction so obtained leaves after digestion with pancreatic or T_1 ribonuclease a sedimentable core consisting largely or wholly of adenylic acid residues, a label in adenosine being almost completely recovered in this resistant fragment, whereas a label in uridine vanishes. Moreover, the behaviour of synthetic poly A on the membrane filters makes it clear that it is the poly A segment that causes the messenger to be retained. The poly A core recovered from the messenger sediments at about the same rate as tRNA. Another property of the messenger, conferred on it by its poly A, is its tendency, depending on the salt conditions, to enter the wrong phase during phenol extraction.

Very similar results were obtained by Edmonds, Vaughan and Nakazato (*ibid.*, 1336) for the messenger RNA of HeLa cells. The poly A core was shown by polyacrylamide gel electrophoresis to have a very narrow molecular weight distribution, and an estimated length of 150–200 nucleotides. This is in fact rather too high to be accounted for by the estimated poly A content of the messenger, but it does not necessarily follow that some of the messenger is devoid of poly A: a number of other explanations are possible, for example that some of the label goes into other kinds of RNA than messenger.

The chief object of the article, however, is to treat the poly A as an identifying feature, and to look for its presence in the rapidly labelling polydisperse high molecular weight RNA present in the nucleus, which many workers in the field would like to believe is a messenger precursor. Poly A is indeed recovered from this material by digestion, and is of the same size as that found in the messenger. It makes up only some 0.5 per cent of the total. That the poly A is a part of a large RNA molecule was demonstrated by its failure to dissociate and redistribute itself between labelled and unlabelled nuclear RNA after addition of a high concentration of an organic

Acceleration of Cosmic Rays

THE drawback of the venerable Fermi mechanism for the generation of the cosmic ray spectrum is the large energies that the particles need to have before the mechanism comes into play. Although the scattering of cosmic ray particles by irregularities in the magnetic fields of interstellar space will produce an energy distribution which matches the experimental data on cosmic rays, as Fermi suggested the particles have to have initial energies of 200 MeV if they are protons, and higher energies if they are heavier particles.

A process which will raise the energies of particles from thermal energies to sufficiently high energies for injection into the Fermi mechanism is suggested in next Monday's number of *Nature Physical Science.* A group at the University of Bologna argue that particles able to take part in the Fermi mechanism may come from the nucleus of the galaxy, where they have been accelerated from thermal energies by interactions with the magnetic fields of neutron stars.

This notion seems to be inspired by the pulses recorded by Weber's gravitational wave equipment, and whatever the explanation of the data published by Weber, his results have stimulated theoretical work on the conditions in the nuclei of galaxies. Interactions among clusters of collapsed objects at the centre of the galaxy might be a source of gravitational waves, but there are other more prosaic reasons for supposing that the nuclei of galaxies contains dense matter. In any case, the Bologna group consider a cluster of neutron stars, a proportion of which have strong magnetic fields, and show how charged particles can gain energy by being scattered by the fields.

Protons need to be scattered only twice to gain the 200 MeV that is the entry ticket for the Fermi process. Heavier particles may need to be scattered more than twice, but there is no reason why the 1 BeV, 20 BeV, and 300 BeV that are the required injection energies for alpha particles, oxygen, and iron respectively should not be achieved in this way.

Little more can be said without knowing more about the abundances of the elements in the interstellar gas in the galactic nucleus, and little light is likely to be thrown on this aspect of the problem for many years. The hope is, of course, that the abundances, together with the fact that it becomes progressively more difficult to provide the heavier elements with the necessary injection energy, will provide a match with the element abundances observed from measurements on cosmic rays.

The Bologna article is well in tune with recent trends in astrophysics. The beginnings of a realization of the parts played by high energy processes and by collapsed objects are suggesting several ways in which the acceleration of cosmic rays may be accomplished. For example, particles thrown out of the atmospheres of pulsars by their rapid rotation rates may also have high enough energies to take part in cosmic ray processes.

solvent which causes the breakdown of base pairing. When RNA synthesis is stopped by actinomycin D, the poly A is also largely suppressed. Evidently therefore the poly A is transcribed from the DNA as part of the RNA chain, and is not added afterwards.

These results then provide support for the messenger precursor hypothesis, and agree with yet another study with a similar aim by Darnell, Wall and Tushinski (*ibid*., 1321). Working with HeLa cells they too find poly A in the messenger, and in small quantity in the nuclear RNA. They have also hybridized both species of RNA with the endogenous DNA under conditions that lead to the detection only of molecules with sequences multiply "reiterated" in the DNA. The hybridized RNA is then isolated by digesting away the unbound part with ribonuclease. The hybridized messenger is enriched in A, compared with total messenger. It seems then the poly A is a part of, or at least adjacent to, the rapidly hybridizing RNA sequence, otherwise it would be liberated during the nuclease step.

The function of the poly A tract in the messeger is a matter of conjecture. Because the messengers are monocistronic and the poly A is not translated, it is likely that it occupies one end of the RNA chain. From the length of the haemoglobin message and that of the poly A tract, it seems clear that there is no room for the elaborate "leader" sequences that are found in viral RNA messengers, and are of uncertain function. The poly A may be required for translation, or for attachment to the messenger, involving perhaps the group of proteins that appear always to be attached to the messenger, without evidently getting in the way of the translation machinery. This RNA–protein package, in fact, seems to be the form in which the messenger is expelled from the nucleus, and the possibility that the function of the poly A relates to the transport of the messenger into the cytoplasm may also be entertained.

BACTERIOPHAGES

Pathway to a Phage

from our Cell Biology Correspondent

ANYBODY who has looked at high resolution electron micrographs of bacteriophage T4 particles and marvelled at their intricate architecture can readily understand why more than one molecular biologist has been content to devote years fathoming out the genes involved and the steps in their morphogenesis. And the story they have to tell is becoming as fascinating as the T4 phage is intricate. The tally of genes required for T4 morphogenesis has now reached 46, no less, and that is a minimal estimate. In the current issue of the *Journal of Molecular Biology* (**58**, 685 and 693; 1971) two groups, Meezan and Wood at CalTech and King at the MRC Laboratory at Cambridge, describe in detail the functions of just four (genes 48, 54, 19 and 3) of the twenty-one genes involved in the assembly of the phage tail.

Both groups find that in *Escherichia coli* infected with strains of T4 carrying amber mutations in any one of genes 48, 54 or 19 phage baseplates—hexagonal structures with six projecting spikes which in the mature phages are attached to the tail core and its surrounding sheath—accumulate, but complete tails do not form. Because extracts of cells infected with one or other of these mutants complement and yield some infectious phage the proteins specified by these genes are able to catalyse tail formation *in vitro*. Moreover such complementation experiments indicate that the products of these three genes are required in a unique order (gene 48 product first, followed by gene 54 product and finally gene 19 product) for the polymerization of the tail core on the baseplate. Exploiting temperature sensitive mutants, King has also shown that gene 19 specifies the major structural protein of the tail core and the proteins specified by genes 48 and 54, not themselves part of the core, somehow act on the baseplates rendering them a suitable substrate for the polymerization of the gene 19 structural protein.

King has also determined the role of another gene, 3; its product is not involved in the polymerization of the tail core, neither is it the structural protein of the surrounding tail sheath (that is specified by gene 18) but the gene 3 product does react with the terminal annuli of the tail core in the absence of the sheath. It seems that the gene 3 product acts on the free end of the core and converts it to a substrate for the attachment and stabilization of the sheath protein. And once these four genes have acted in the correct order and the baseplate, core and sheath have assembled, the product of yet another gene, 15, can form the actual joint linking the sheath to the tip of the core.

As King takes pains to point out, the assembly of the tail of T4 depends on a sequence of interactions between particular gene products and a macromolecular precursor structure; apparently the various structural proteins, unable to interact strongly when in solution, strongly interact with the macromolecular substrate produced by the preceding step in the morphogenetic pathway. The gene 15 protein, for example, which joins the terminal subunits of the tail core and sheath does not react in solution with the proteins specified by genes 18, 3 and 19, but strongly interacts with them once they are assembled into a sheath and core attached to the baseplate. Presumably during assembly these proteins undergo either allosteric or chemical changes which make them suitable substrates for, and able to interact with, the next protein to be polymerized. Such a highly regulated pathway, which may well be the evolutionary consequence of the fact that the phage is assembled at sites distant from those where the

Sequence and Synthesis of Substance P

IT has been known for forty years that a substance, identified only recently as a peptide, and present in a variety of tissues, possesses hypotensive and a variety of other physiological activities. Appreciable amounts of this material are hard to come by, but in view of its broad neural activity, which is quite different from those of the hypothalamic peptide hormones, the determination of its structure is of considerable interest. The complete sequence is reported by Chang, Leeman and Niall in next Wednesday's *Nature New Biology*. The sequence is arg-pro-lys-pro-gln-gln-phe-phe-gly-leu - met - NH_2. Preparations derived from three different bovine tissues were found to have the same sequence. Substance P is inferred to occur throughout the central nervous system.

The sequence is also compared with those of two other peptides of similar activity, one, physalaemin, from the skin of an amphibian, the others eledoisin, from the salivary glands of a cephalopod. These also have eleven residues, of which four, including the last three at the N-terminus, are identical in all three molecules. In most other positions there are conservative substitutions. Physalaemin and eledoisin have pyrrolidone carboxylic acid as the C-terminal residue.

In a second article Tregear *et al.* describe the synthesis of substance P by the solid-phase Merrifield method. The product is active in respect of the various functions associated with natural substance P. This work provides a good example of the value of modern peptide synthesis technique, for it makes available in relatively large quantity a biological material, which can otherwise only be isolated in very small amounts. The authors point out the possibilities now open for its biological characterization. The synthetic material will be used to prepare antibodies, with the aid of which it should be possible to locate and assay it in nervous tissue and elsewhere.

structural proteins are synthesized, has obvious selective advantages. Phage heads, for example, cannot attach to tails unless they are complete; heads cannot therefore be wasted in incomplete and uninfectious particles. Likewise sheath protein cannot polymerize on baseplates until the baseplates have initiated the polymerization of a tail core. With every successive step rigorously dependent on all its predecessors the phage assembly line is optimized for the production of complete and infectious progeny.

CONTINENTAL DRIFT

Ice and Gondwanaland

from our Geomagnetism Correspondent

Gough's recent novel suggestion (*J. Geophys. Res.*, **75**, 4475; 1970) that the Carboniferous ice cap may have been the agent which initiated the break-up of Gondwanaland is now severely criticized by Meyerhoff and Teichert (*J. Geophys. Res.*, **76**, 4038; 1971), who rather unexpectedly take the view not that the ice cap was incapable of doing any such thing but that the idea of a large continuous Carboniferous ice sheet is not valid in the first place.

The gist of Gough's original contention was that the stresses produced by a large ice sheet could have fractured the lithosphere and initiated separation of the fragments and could then have caused the flow in the asthenosphere which allowed continental drift to continue. At the same time, the asthenospheric material would have risen in the crustal fractures to form the embryonic ocean floors. To support this idea Gough performed a simple first-order calculation for the stresses produced by an ice sheet 2 km thick having a diameter of 3,000 km and so showed that the stress differences at the surface under the edges of such a load are up to 100 bars, that the largest stress difference under the centre of the load is about 130 bars and occurs at a depth of about two-thirds of the load diameter, and that at the depth of the asthenosphere the stress would be in the range 60–90 bars, far greater than the 10 bars sufficient to produce creep in the mantle.

One of the earlier and most influential authorities for the existence of a large Carboniferous ice sheet was du Toit (*Our Wandering Continents*, Oliver and Boyd, 1937) whose detailed reconstruction showed a slightly curved ice cap 11,000 km long and 4,500 km wide covering a quarter of South America, slightly less than half of Africa, most of Australia and the whole of Madagascar, India and Antarctica. Such a reconstruction has since been accepted by most palaeomagnetists. But Meyerhoff and Teichert no longer believe it. It is their unequivocal view that "at no time during the Carboniferous and Permian did a large ice sheet occupy any part of the southern continents—with the single possible exception of Antarctica". Instead they point out that there is evidence for about thirty separate, unconnected Carboniferous and Permian ice "centres" in the southern hemisphere of which the largest, in Brazil and southern Africa, had maximum diameters of 1,600 km. Furthermore, there were apparently six in India and Pakistan and four in the Urals and north-central Siberia. The evidence for the lack of connexion between these ice centres is, according to Meyerhoff and Teichert, that all but three were mountain ice caps with radial U-shaped valleys and that even the remaining three were surrounded by highlands. Moreover, they quote numerous references which suggest that many of the known Carboniferous and Permian ice caps were not precisely contemporaneous. In short, "the concept of a single, vast, continental ice sheet over a large part of Gondwanaland has been thoroughly disproved".

The second class of objection put forward by Meyerhoff and Teichert raises issues of a rather different kind. Thus when their isolated ice centres are plotted on a reconstruction of Gondwanaland they find that many lie in the

---

Role of Cytotoxic Cells *in vivo*

One of the more interesting developments in cellular immunology in recent years has been the recognition of several sorts of cytotoxic cells. In almost all instances the aggressive activities of such cells have been demonstrated *in vitro*; direct observation has shown that certain kinds of killer cells will seek out and eventually destroy the hapless target cells. Work on these cell populations is now advancing towards a definition of the mechanism by which cell death is brought about and towards delineation of the role of cytotoxic cells in situations *in vivo*. Three articles in the forthcoming issue of *Nature New Biology* relate to this subject.

Alexander and Evans describe how they have brought macrophage populations in contact with either endotoxin or twin stranded RNA. Subsequently, the macrophages proved capable of killing a lymphoma cell population. This intriguing phenomenon is apparently non-specific, but it does require fairly long contact between the lymphoma cells and the endotoxin or ds RNA treated macrophages. The results cannot be explained, according to the authors, by any direct cytotoxic effect of endotoxin or ds RNA on the lymphoma cells, but it could be that a cytotoxin is released from the activated macrophages.

Peter and Dawkins quite specifically invoke a cytotoxin to explain their results. Human adenoidal cells were placed in culture with PHA and the resulting supernatants were found to be cytotoxic for mouse L cells in a manner which was superficially similar to that of a known cytotoxic antibody. The cell array present in these cultures is, of course, less well defined than that of Alexander and Evans, in which an adherent macrophage population can be specifically involved as the significant cell. Nevertheless, Peter and Dawkins conclude that because the production of their cytotoxic effect is dependent on the presence of PHA, which is noted for its capacity to transform lymphocytes, their cytotoxin derives from a lymphocyte population. It is intriguing to note that the type of lymphoid tissue placed in culture was derived from a part of the body which is subject to continuous contact with bacteria and presumably with their endotoxins. It would be interesting to learn whether lymph node cells from "quieter" parts of the body were similarly effective.

The third article, by Harding *et al.*, deals with a more specific phenomenon. Allogeneic (same species, different strain) target cells can be destroyed in culture by immune thymus-derived (T) lymphocytes (Cerottini *et al.*, *Nature*, **228**, 1308; 1970), by cells which produce cytotoxic antibody but which are not directly cytotoxic or by cells which can only attack antibody coated cells (the antibody itself is not lytic in the absence of complement). Harding *et al.* are concerned with the origin of this last kind of cell. They conclude that it is not a cell of thymic derivation because it is still present in normal numbers in animals with much reduced numbers of T cells. These authors also say that their cytotoxic cell presumably attacks not the antigens on the target cell but the antibodies attached to these antigens; it is possible that this phenomenon is specific only in the sense that the antigen-antibody complex is specific.

All these articles add to the information which is accumulating about the reactivity patterns of cells derived from the lymphoid and reticuloendothelial systems. It is to be hoped that eventually they can be placed against a wider context to include consideration of the activities of mast cells, granulocytes and eosinophils, all of which can have non-specific more or less toxic activities.

interior of the supercontinent where they suppose that the influence of moisture-bearing wind currents could not reach. And no moisture means, of course, no glaciation. Similarly with coal deposits. Coal apparently can only form in areas where the minimum annual rainfall is 1,500–2,000 mm ; and yet coal is found in the interior of Gondwanaland. The obvious conclusion then, according to Meyerhoff and Teichert, is that Gondwanaland could not have existed in the late Carboniferous and Permian. This contention is also supported by the presence of marine evaporites in Gondwanaland's interior within 15° of the Carboniferous and Permian poles. Although they do not say so explicitly, Meyerhoff and Teichert's position apparently conceals an anti-continental drift philosophy or at least one which does not allow ancient supercontinents.

It is the view of Meyerhoff and Teichert that a revolution in the concepts of southern hemisphere glaciation has been taking place simultaneously with the revolution in the Earth sciences but that the Earth scientists have been too busy to notice it—and thus one of the props of the new global tectonics has been whipped away. Clearly this is to overestimate the importance of glaciation in the continental drift context. Until Gough's recent article appeared the concept of the large Gondwanaland ice sheet was largely of historical interest, important at a time when evidence for drift was sparse but later superseded by far more convincing evidence. This apart, however, it would seem that the conflict between Meyerhoff and Teichert on the one hand and Gough on the other is essentially one of apparent irreconcilability of different disciplines —palaeontology and geology versus geophysics.

In his reply Gough wryly admits that "as a geophysicist, [he is] an outsider in these geological matters", but nevertheless proceeds to demolish his critics with the confidence of somebody who firmly believes in seeing wood for trees. Thus the existence of the thirty centres of glaciation in their respective positions leads him, with du Toit, to the interpretation that large ice sheets existed over part of Gondwanaland and not to Meyerhoff and Teichert's negative conclusion. The mountain-top ice caps and U-shaped valleys are easy to explain because if ice forms at all in a continent of rugged topography it will do so first on mountain tops. The resulting glaciers would thereby produce radial U-shaped valleys and, if rainfall and freezing continued, possibly continuous ice sheets. Thus the ice movement would be relatively inhibited such that few permanent traces of ice action would be left in the lowlands.

As for the question of rainfall in the Gondwanaland interior, Gough takes the view that Meyerhoff and Teichert are formulating the problem the wrong way round. The starting point must be the existence of Gondwanaland, evidence for which is now overwhelming. The global tectonic evidence was not mentioned by Meyerhoff and Teichert. The presence of interior ice caps does not disprove the reality of Gondwanaland but only the idea that interior rainfall is impossible. The real problem therefore is to decide how, and under what conditions, the interior ice caps formed. In any case it is not clear to Gough that rainfall cannot occur far from continental margins as long as there is suitable topographical relief.

But there is a final irony. In his original article Gough considered for simplicity a continuous ice sheet 2 km thick and 3,000 km in diameter. The fact is that the same mass of ice distributed in lumps over the same area will produce the same stresses except very near the surface. Thus a continuous ice sheet is not necessary at all! Moreover, an ice sheet 2 km thick and only 1,000 km in diameter will produce the same stresses in the lithosphere and the same stresses below its centre but at a lower depth. Thus the smaller sheet actually produces greater stresses in the asthenosphere where the tectonic flow takes place.

This whole argument, of course, is indicative of the sort of exchange which is likely to become increasingly common. The fact is that the revolution in the Earth sciences has been based almost completely upon geophysics, and many geologists feel that their discipline has not always received the consideration it deserves. The job now is to reinterpret several hundred years of geology in terms of the new global framework ; and some friction is only to be expected.

Advances in Calcitonin Research

A RENAISSANCE in calcium metabolism research has taken place since the discovery of calcitonin nine years ago. Underlying this effort is the interest in the treatment of some fifty million people in the world today with bone-losing diseases. These diseases are loosely referred to as "osteoporosis".

The current investigative approach to the treatment of bone loss is the use of substances that inhibit bone resorption. The most popular of these is calcitonin, a hormone from the thyroid. Calcitonin acts directly and immediately on osteoclasts, the cells which resorb bone to inhibit their action. Because bone is constantly undergoing remodelling and replacement, prevention of bone loss, while new bone is continuing to be laid down, should lead to more bone. In next Wednesday's *Nature New Biology,* two letters contribute to the discussion of whether or not this hormone will prove of therapeutic importance.

The first of these contributions, by Anderson and his colleagues in Urbana, Illinois, reports the failure of moderately long-term treatment with porcine calcitonin to alter the retention of strontium-85. This isotope is unique in that its uptake in and release from bone is similar to that of calcium. Anderson *et al.* have measured whole body retention in dogs of this isotope, as a function of bone mineral turnover; and found that calcitonin did not delay its loss. The results are surprising in view of work done elsewhere (for example at the Hammersmith Hospital, London), which has demonstrated that calcitonin increases metaphyseal bone in rats and promotes positive calcium in patients with Paget's disease. In contrast to both growing rats and Pagetic patients who have a rapid rate of bone turnover, bone turnover in normal dogs, however, is less and therefore more closely approximates the rates observed in osteoporosis in man. If the observations reported in next week's issue are true, it may be that calcitonin will not prove effective in preventing bone loss in patients whose rates of bone resorption are not abnormally high.

But in a second letter, Potts and his group at the Massachusetts General Hospital in Boston report that salmon calcitonin is 20–2,000 times more potent than the porcine hormone used in the previous study. Two explanations for this difference are tenable: either the salmon hormone has a greater affinity for receptor sites or it is more resistant to degradation. Potts *et al.* present evidence which supports the second explanation. As measured by radioimmunoassay, salmon calcitonin was found to have a slower rate of disappearance in the dog.

Salmon and porcine calcitonin differ in fifteen of their constituent amino-acids. That the hormones from different species have different rates of destruction introduces a new concept. This concept seems to be as follows: the activity of a peptide hormone is dependent not only on those amino-acids which confer biological activity but also possibly on other amino-acids which affect its rate of destruction. It follows from this that synthetic analogues which further delay degradation may lead to even more potent preparations than are found in nature.

Transport in the Phloem

D. C. SPANNER

Department of Botany, Bedford College, London NW1 4NS

Exactly how nutrient materials travel through the green plant is still a matter for controversy. This review highlights some of the current points of interest.

MANY readers, not themselves botanists, will be aware of the old dictum "up the wood, down the bast". This draws attention to the two long-distance transport processes in higher plants: the movement of water up the wood from the soil supply to the transpiring leaves, and the independent movement of the products of photosynthesis down from the leaves to the roots. Strictly, this second movement, which is chiefly of sugars, occurs rather from regions of supply to regions of utilization, and so over any given portion of the plant axis is not invariably in the same direction. Broadly, however, the dictum is true. It is with the bast or phloem movement that this article is concerned.

The transport of sugars and other assimilates down the trunks of trees—to consider a concrete case—takes place in files of elongated cells making up the phloem. The most important of these cells are the sieve elements. These are living cells with very little content of living substance, disposed end-to-end like drain pipe sections. They are very narrow (typically about 20-40 µm in diameter), with a length about ten times as great as their width, and with the junction of one element and the next not unconstricted (as in earthenware drainage systems) but crossed by the fused end walls of the two elements. The transverse obstructions, which are about 1 µm thick, are pierced by numerous holes of diameter roughly 0.5–1 µm. For this reason they are aptly referred to as sieve plates, and the long files of cells which they cross at intervals of about 0.5 mm as sieve tubes. Down these tubes the assimilate stream flows at the relatively high velocity of about 50 to 100 cm per hour Already it can be seen that here is a difficult problem in fluid dynamics, at least in the case of tall trees; but the problem has in the past fifteen years been made much more acute by the revelations of the electron microscope. Observations have shown that the most obvious content of the sieve elements (at least in the Angiosperms, and perhaps universally) is a fibrous protein, the P protein, which superficially resembles certain preparations of collagen or actin. Furthermore, this material appears in most careful preparations to occupy, fairly compactly, the pores in the sieve plates. If these plates constitute in themselves a considerable obstruction to flow, it is easy to see the problem which arises when their pores appear densely occluded with fibrous material (Figs. 1 and 2).

The discoverer of sieve tubes (Hartig, 1837, 1860) suspected that they were the conducting channels and although controverted, this view has steadily gained ground until today it is almost unchallenged. Recently autoradiography of thin sections has been the most potent factor in the strengthening of this view. Starting with the earlier work of Biddulph[1] using ^{35}S and ^{32}P, improved resolution has been obtained, first with $^{14}CO_2$ incorporated naturally into assimilates, and then with tritiated sugars applied externally to the leaf[2-4]. The difficulty of the technique arises largely from the water solubility of the compounds concerned, but this has been overcome to such an extent that very recently[5] natural ^{14}C-assimilate has been autoradiographed in resin-embedded material cut no thicker than 1 µm. In both the tritiated material (cut at 6–8 µm in paraffin wax) and this ^{14}C material, convincing evidence has been presented that transport is confined to the sieve tubes. Incidentally, labelled and unlabelled sieve tubes occur in juxtaposition[2], suggesting that to some extent they act independently.

Rate and Pattern of Movement

Recent work by Zimmermann[6] has confirmed the generally accepted view of the velocity of transport. The alternation of day and night would be expected *a priori* to produce a rhythmic change in the transport of assimilates, and Huber *et al.* (1937) measured a sinusoidal variation with height in the sugar concentration in the phloem of trees and were thus able to derive a velocity of movement; it was three or four times larger than currently accepted values. Zimmermann reasoned that changes in water potential in the xylem (which is very close to the phloem) would independently affect the sugar concentration. In certain trees he has demonstrated that sucrose, the most usual sugar found in the transport stream, undergoes in the

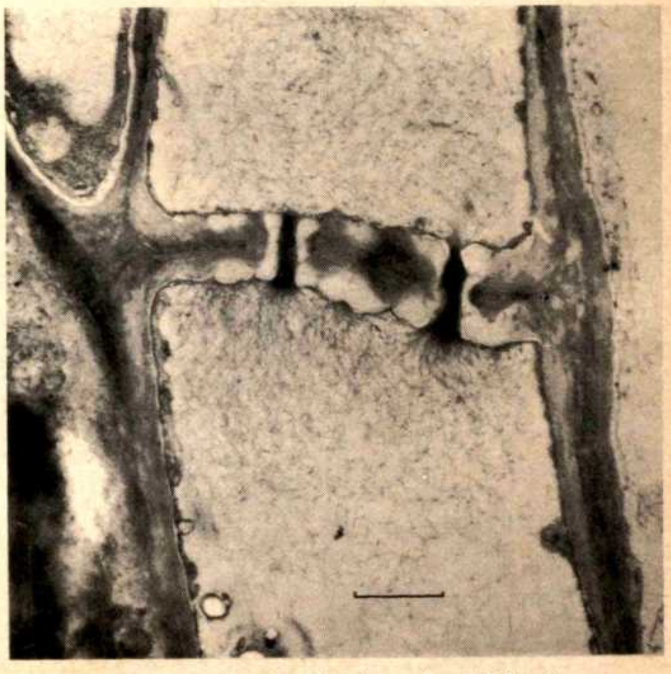


Fig. 1 Longitudinal section of a sieve tube of *Helianthus annuus* showing sieve plate. Fibrils of P protein occupy the lumens and densely occlude the pores. By courtesy of A. W. Siddiqui. (Index line 1 µm.)

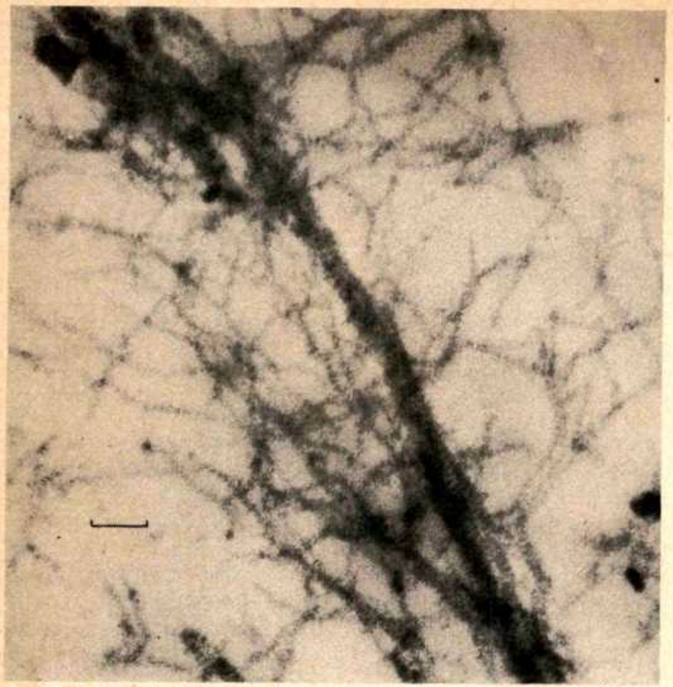


Fig. 2 Banded fibrils of P protein from sieve tube of *Salix caprea*. Note that the bands tend to align themselves transversely. By courtesy of U. Mishra. (Index line 0.1 µm.)

sieve tubes some degree of inter-conversion with the higher sugars raffinose and stachyose. By measuring at various heights simultaneously the ratios between these three sugars he showed that they varied sinusoidally. The velocity of movement implied by these "ratio waves" (30–70 cm h^{-1}) fits well into the accepted range.

On the disputed question of whether or not the sieve tubes are able to conduct in both directions at once (certain suggested mechanisms would allow them to), the past two or three years have provided evidence but no settlement. Eschrich[7] in 1967 reported work on broad bean in which ^{14}C naturally assimilated by one leaf and fluorescein artificially applied to another both appeared in the exudate from an aphid feeding on the stem between them. Because aphids are known to tap single sieve tubes, the evidence was strong for bidirectional movement. But Eschrich himself noted that another explanation was possible: opposite unidirectional movement in parallel sieve tubes with lateral transfer between them. Ho and Peel[8], using ^{14}C and tritiated sugars in willow segments, obtained rather similar results, as did Trip and Gorham[9] using microautoradiography instead of aphids to localize the tracers. But strong contrary evidence turned up in the work of Peterson and Currier[10]. Applying fluorescein at the mid-point of an internode and allowing only a short period (one hour) for translocation, they found on sectioning the stem above and below the mid-point that the dye had moved both upwards and downwards, but only in different bundles. When a longer time was allowed for translocation, however, single bundles were labelled in both directions, almost certainly as a result of the lateral movement of the tracer at the nodal anastomoses. Reluctantly, it must be recognized that this crucial problem, on which considerable effort has been expended, is still unresolved.

Factors affecting the Rate

Although inhibitors have long been used for investigating physiological processes, they have provided a singularly unsatisfactory picture of translocation, and the past few years have confused it rather than otherwise. The chief difficulties arise from the fact that it is hard to discriminate between the transport process itself and the terminal processes in which assimilates are loaded into or unloaded from the conducting channels. In addition the proximity of the xylem, itself capable of long distance conduction, and the propensity of the sieve plates for rapidly sealing themselves off with callose, constitute further difficulties in interpreting inhibitor results in such a way as to decide unambiguously between mechanisms. Harel and Reinhold[11] ensured in soyabean that the vein-loading process was not involved by cutting off the ^{14}C-treated leaflet before the introduction of the inhibitor dinitrophenol (DNP). They found, however, that not only was the transport not inhibited, but downward movement to the roots was promoted. This evidence contradicts previous conclusions. In a well argued case the authors interpret their results as support for the Münch hypothesis (discussed later), though it is not clear how the opening of sieve tubes at their upper ends could leave the pressure gradient (on which the Münch theory relies) unimpaired; nor how they decided against movement throughout the xylem to the roots, a distinct possibility with the likely disturbance of root function through DNP and an overhead water supply. It is an indication of the complexity of the problem that at least two other factors need to be considered before the results can be interpreted confidently—the vascular anatomy, and the effect of the treatment on sieve plate callose. For strangely, in certain circumstances the laying down of callose can accelerate transport[12], a possible consideration in Harel and Reinhold's results.

Low temperature is another factor which has been investigated fairly recently. A striking feature of the phenomenon is that it only requires cooling of a short length of axis (say 2 cm) to reduce drastically the mass transfer rate[13]. Furthermore, if the low temperature is maintained, the subsequent behaviour of the plant varies. With plants acclimatized to cold regions the rate may recover to its previous value fairly quickly, whereas with plants from warmer regions it remains low. In the latter case, restoration of the cooled portion to ambient temperature is followed by recovery in mass translocation rate after a certain time. Geiger considers possible mechanisms for these effects. With naturally cold climate plants the recovery in rate when the axis is held at a low temperature (0.5° C) takes no longer when 8 cm of axis is cooled than when 2 cm is; he argues that this tells against the recovery being due to a build-up of the concentration differential over the cooled region, presumably because a bigger differential would be required over the longer region, and this would take longer to establish. But over the small distances involved and with sugar transport as large as it is, this argument is not so cogent as it seems at first.

It would be natural to suggest that the slow-down caused by chilling is due to a decrease in the rate of the metabolic energy supply. This interpretation would favour the electro-osmotic theory, since unlike the Münch hypothesis its energy supply is mediated all along the route, and not merely at the ends. Geiger, however, suggests an alternative explanation: that the resistance of the transport channels is raised by the chilling process by changes in the sieve tube organelles. But a ten-fold increase is required, and it seems unlikely that the sparse contents could manage this unless the changes took place in the vicinity of the sieve plates. Additional callose, and a denser plugging of the pores with P protein, seem obvious claimants for investigation. It remains possible, however, that the primary cause is a decrease in the rate of supply of metabolic energy. The electro-osmotic theory would then explain recovery in terms of a re-adjustment of the pore mechanism.

Sieve Tube Contents

The contents of the sieve tubes have remained a source of interest, especially those elements which can be considered part of the mechanism, rather than part of the translocation stream. The content of ATP, already known to be fairly high from the work of Kluge and Ziegler[14], has been confirmed using aphid stylet extract of willow[15], and more recently by measurements on the copious phloem exudate of *Yucca* inflorescence[16]. All reports give a fairly high concentration

(0.6–1 mg ml.$^{-1}$ or about 0.002 M). No doubt this is significant for the callose closing reaction, and perhaps also for the flow mechanism. Phosphatases have been further reported on. In view of the slightly alkaline reaction of sieve tube sap the incidence of alkaline phosphatase in mature *Pisum* sieve tubes[17] is interesting, though most of the reports have been of acid phosphatases. Zee[18] found no acid phosphatase activity in the "slime" of *Pisum* (contra a report[19] for *Tilia*) though there was activity in the plasmalemma and endoplasmic reticulum of mature tubes. Robards and Kidwai[20] reported ATPase activity at *p*H 7.2 in immature phloem and ray cells; lead deposits occurred on both the inner and outer edges of the plasmalemma as well as elsewhere. Yapa (unpublished) has found ATPase activity at *p*H 7.2 in the plasmalemma of mature sieve tubes.

But of all the sieve tube contents the one of major interest has continued to be the famous slime, now appropriately renamed P protein[21]. Its proteinaceous nature has been inferred from staining reactions, ultrastructural appearance, enzyme digestion (Yapa, unpublished results) and chemical analysis of phloem exudate[22], and there seems little doubt that the banded fibrillar material so often seen in electron micrographs is pure protein, probably of a rather chemically resistant nature. It seems to be the case that ontogenetically it originates from "slime bodies" often composed of bundles of more or less parallel tubular elements, resembling microtubules but distinct from them[23]. There seems to be some diversity in the tubular-type slime bodies, and the slime bodies may in some plants be composed of fibrils from the first[24]. The sieve tubes may be the sites of P protein production for some time after maturity sets in; and P protein, or something similar to it, may be produced in ways other than from typical slime bodies. For example, it may come in addition from protein crystals in Leguminosae[25] and from sieve tube plastids in certain Caryophyllales[26,27]. In primary tissue the P protein seems to be connected with peculiar cell organelles called "spiny vesicles"[28]. Later studies seem to have confirmed this idea[29]. If so, then it would seem to be the case that in protophloem, adjacent parenchyma cells assist in the production of P protein for the sieve elements. Furthermore, the occurrence of typical P protein in the plant kingdom seems to have been extended more firmly to the monocotyledons[30], so that when everything is considered, the conviction that this material fulfils some essential role in the translocation process has been strengthened.

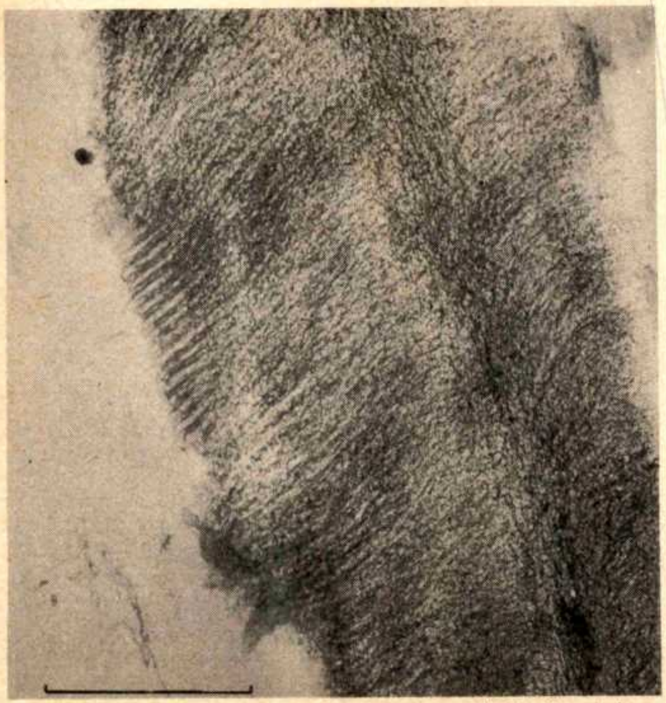


Fig. 3 Membrane stacks in close contact with the sieve tube wall in *Nymphoides* petiole. By courtesy of R. L. Jones. (Index line 0.5 μm.)

Of the other constituents of the sieve tube I shall mention only the endoplasmic reticulum (ER). Recent studies have confirmed this to be a fairly abundant element of the cytoplasm in mature tubes[31]. It is frequently present in parietal "stacks", with the constituent membranes lying either parallel to the tube wall or set almost at right angles to it (Fig. 3). Often it occurs in curious convoluted configurations, like papier-mâché egg boxes stacked within one another[32]. It usually seems to be anchored closely to the wall, and sometimes when the membranes stand perpendicular to the latter they seem to be continuous with dark lines within it. Certainly the thick sieve tube wall, the cellulose fibrils of which probably run in shallow spirals and which seem to contain little cementing substance, often has pronounced radial striations in longitudinal section. These appearances have led to the suggestion[33] that the ER stacks are really plasmalemma which in the turgid cell is buried in the cellulose wall, but which emerges more or less rapidly (hence the variety of configurations) into the lumen on collapse of turgor. Whether this unorthodox idea can be substantiated remains to be seen. Attempts to fix and section plasmolysed sieve tubes have not so far yielded supporting evidence.

The Question of Mechanism

In this short review I shall consider only two mechanisms (though others are being canvassed); the Münch or pressure flow hypothesis[34], which considers movement in the sieve tubes to be due to a gradient of hydrostatic pressure; and the electro-osmotic hypothesis, which invokes electrical forces called into play at the sieve plates by the active secretion of potassium ions[33,35]. Both involve mass flow, and both therefore have a similar stake in the observation (or otherwise) of simultaneous bi-directional movement, which was discussed inconclusively earlier. One important issue on which the theories differ is in their relation to the state of the pores in the sieve plate. If the natural functioning condition of these is as Fig. 1 indicates, then simple calculation shows that the pressure gradient required by the Münch hypothesis is far too great to be realizable in practice—of the order of several hundred atmospheres per metre[36]. On the other hand, there is no problem here for the electro-osmotic theory—it positively requires that the pores be occluded in some such way as the micrograph shows. On the other hand, this theory runs into difficulties of its own, particularly that it seems to imply a very high current density for potassium ions, perhaps as high as 1 A cm^{-2} of sieve tube cross-section. In this connexion the suggestion of a greatly expanded plasmalemma in the sieve tube is important, for secretion of the ion across this membrane would be the resistive as well as the energy-transducing step.

The conflict between the two hypotheses centres largely on whether micrographs such as Fig. 1 are faithful representations of the functioning sieve plate. It must be emphasized that the sieve tubes have high turgor pressure coupled with relative ease of longitudinal movement; thus any attempt to cut into them or manipulate them for examination in the electron microscope runs the risk of causing violent axial surges. P protein being present in the lumen, such surges will arguably carry it to and through the sieve plates. The supporters of the Münch hypothesis argue that the pores in the functioning tube are unoccluded with P protein. Fig. 1 accordingly shows an artefact; it is a difficult matter (though micrographs have been obtained) to record faithfully the functioning condition. That is why the pores so rarely appear open. The supporters of the electro-osmotic theory take a different view. They maintain that much of the evidence adduced to support the "artefact" view in fact does almost the opposite. Even the very narrow and exposed sieve tubes of small leaf veins, so narrow that the sieve plates may have only one pore, show the same sort of plugging[37]. The same can be said, they point out, of the short curved sieve tubes of callus tissue[38]. Of course, every device of chemical or physical manipulation (such as freeze drying, freeze etching and prior wilting or boiling) has been pressed into service to resolve the issue, but neither side seems disposed to give way[39,40].

It is often urged that the use of macroscopic equations of viscous flow is misleading in the case of channels which might be no more than 100 Å wide, so that the argument about the state of the pores is for the moment pointless. But some evidence indicates that in very fine channels the viscosity of water does not drastically fall; on the contrary it seems to rise. Churayev, Sobolev and Zorin[41] found an increase of 40% in quartz capillaries of 0.04 mm radius. Thus the problem is a real one.

The special evidence for the Münch hypothesis is not particularly strong. It turns mostly on its simple physical character. Also, it can allow the ready transport in sieve tubes of large particles like viruses[42]. On the other hand, the electro-osmotic theory has numerous arguments of an indirect nature to recommend it. It is much more physiological, and can take in its stride any well authenticated evidence for an effect of poisons, anoxia or low temperature. It offers a major role for potassium, hitherto without one; and it also makes substantial suggestions for the functions of P protein, membrane stacks, companion cells and the thick sieve tube wall. More direct (though hardly decisive) is the experimental evidence for a saw-tooth profile of electrical potential[43] in the sieve tubes, as required by the theory; and that potassium deficiency too small to cause visible symptoms apparently has a primary effect in lowering translocation[44].

Two recent approaches to the investigation of mechanism should be mentioned. Fensom and his collaborators[45] have applied Nomarski interference optics to the cinematography of functioning *Heracleum* phloem. No actual translocatory movement of particles was visible across the sieve plate, but an unusual type of particle movement (probably of starch grains) was reported. But in view of the reports that the release of starch grains from plastids in sieve tubes is probably an injury effect one needs evidence that the sieve tubes recorded were functioning normally. Milburn[47], in what he described as a "lunatic fringe" experiment, followed up a clue from Bose and tried the effect of simple massage (squeezing gently between finger and thumb) on the stem of young plants of *Ricinus*. Repeated at intervals three times a day for several days, this had the remarkable effect that when an oblique incision was made into the bark the phloem exuded copiously, up to 1 ml. of sap being obtainable from a single incision in a plant 20 cm tall, with little progressive decrease in concentration. Milburn interprets this phenomenon as follows. The sieve plate pores are normally open. Massage triggers an automatic sealing mechanism which operates to block the pores (and so serves in severed plant organs a function analogous to blood clotting). Subsequently an anti-sealing mechanism frees the pores and enables translocation to re-start. Repeated massage exhausts this double reaction so that when an incision is finally made the plant is defenceless and bleeds phloem sap profusely. Milburn rejects the obvious suggestion that massage cumulatively disrupts a natural state of affairs illustrated by Fig. 1, on the grounds that if a plant which has been induced to bleed profusely is massaged at the point of exudation, the exudation stops within minutes or seconds. Temporary application of pressure 2.5 cm from the incision on both sides acts similarly. My interpretation would be, on the contrary, that the whole phenomenon supports the *prima facie* understanding of Fig. 1. Massage ejects the P protein plugs from the sieve pores, and repeated massage prevents their re-establishment and furthers the dislocation. In the rapid exudation following subsequent incision the disorganized P protein is being hurried through the sieve plate pores; temporary slackening of the flow by finger pressure enables it to build up in front of one or more sieve plates so that on release an effective blockage remains. This interpretation seems less contrived than invoking a sealing–unsealing mechanism of unspecified character which, thrown out of gear by massage, can nevertheless recover sufficiently when exudation is in full operation to respond to pressure of the fingers.

Future Developments

Taken by itself it is hardly disputable that the electron microscope evidence supports the view that the sieve plate pores are plugged in a carefully structured fashion with P protein fibrils. The difficulty of reconciling this picture with the physiological facts leads to the impasse. Thus after more than a century of investigation there is no agreed answer to the question of how the sieve tubes conduct. What developments may be expected in future work on the problem? On the anatomical side we may anticipate a wider range of comparative studies with the electron microscope, including more emphasis on perennial monocotyledons and the lower vascular plants, and on freeze etching and other recent techniques. The sieve tube wall will probably be examined carefully. On the biochemical side, the chemical and physiochemical characterization of P protein will be an obvious target, and also the nature of the callose-triggering reaction and the metabolic function of high energy compounds in the sieve tubes. Microelectrode techniques and the movements of potassium will certainly fit into the picture. Finally, on the level of whole plant physiology, quantitative data for the movement of tracers of different kinds will probably be combined with computer analysis of more adequate model systems. With patience we may hope to see fuller light on this intriguing phenomenon.

[1] Biddulph, S. F., *Amer. J. Bot.*, **43**, 143 (1956).
[2] Trip, P., and Gorham, P. R., *Canad. J. Bot.*, **45**, 1567 (1967).
[3] Schmitz, K., and Willenbrink, J., *Planta*, **83**, 111 (1968).
[4] Schmitz, K., *Planta*, **91**, 96 (1970).
[5] Fritz, E., and Eschrich, W., *Planta*, **92**, 267 (1970).
[6] Zimmermann, M. H., *Planta*, **84**, 272 (1969).
[7] Eschrich, W., *Planta*, **73**, 37 (1967).
[8] Ho, L. C., and Peel, A. J., *Ann. Bot.*, **33**, 833 (1969).
[9] Trip, P., and Gorham, P. R., *Plant Physiol.*, **43**, 877 (1968).
[10] Peterson, C. A., and Currier, H. B., *Physiol. Plant.*, **22**, 1238 (1969).
[11] Harel, S., and Reinhold, L., *Physiol. Plant.*, **19**, 634 (1966).
[12] Eschrich, W., Currier, H. B., Yamaguchi, S., and McNairn, R. B., *Planta*, **65**, 49 (1965).
[13] Geiger, D. R., *Ohio J. Sci.*, **69**, 356 (1969).
[14] Kluge, M., and Ziegler, H., *Planta*, **61**, 167 (1964).
[15] Gardner, D. C. J., and Peel, A. J., *Nature*, **222**, 774 (1969).
[16] Kluge, M., Becker, D., and Ziegler, H., *Planta*, **91**, 68 (1970).
[17] Flinn, A. M., and Smith, D. L., *Planta*, **75**, 10 (1967).
[18] Zee, S.-Y., *Austral. J. Biol. Sci.*, **22**, 1051 (1969).
[19] Lester, H. H., and Evert, R. F., *Planta*, **65**, 180 (1965).
[20] Robards, A. W., and Kidwai, P., *Planta*, **87**, 227 (1969).
[21] Esau, K., and Cronshaw, J., *Virology*, **33**, 26 (1967).
[22] Kollmann, R., Dorr, I., and Kleinig, H., *Planta*, **95**, 86 (1970).
[23] Wooding, F. B. P., *Planta*, **85**, 284 (1969).
[24] Cronshaw, J., and Esau, K., *J. Cell Biol.*, **38**, 25 (1968).
[25] Laflèche, D., *J. Microscopie*, **5**, 493 (1966).
[26] Falk, H., *Planta*, **60**, 558 (1964).
[27] Behnke, H.-D., *Planta*, **89**, 275 (1969).
[28] Newcomb, E. H., *J. Cell. Biol.*, **35**, C17 (1967).
[29] Esau, K., and Gill, R. H., *Protoplasma*, **69**, 373 (1970).
[30] Behnke, H.-D., *Protoplasma*, **68**, 377 (1969).
[31] Esau, K., and Cronshaw, J., *J. Ultrastruct. Res.*, **23**, 1 (1968).
[32] Johnson, R. P. C., *Planta*, **84**, 68 (1969).
[33] Spanner, D. C., and Jones, R. L., *Planta*, **92**, 64 (1970).
[34] Münch, E., *Die Stoffbewegungen in der Pflanze* (Fischer, Jena, 1930).
[35] Spanner, D. C., *J. Exp. Bot.*, **9**, 332 (1958).
[36] Weatherley, P. E., and Johnson, R. P. C., *Intern. Rev. Cytol.*, **24**, 149 (1968).
[37] Trip, P., and Colvin, J. R., *Ann. Bot.*, **34**, 1101 (1970).
[38] Wooding, F. B. P., *Planta*, **85**, 284 (1969).
[39] Cronshaw, J., and Anderson, R., *J. Ultrastruct. Res.*, **27**, 134 (1969).
[40] Siddiqui, A. W., and Spanner, D. C., *Planta*, **91**, 181 (1970).
[41] Churayev, N. V., Sobolev, V. D., and Zorin, Z. M., *Faraday Soc. Special Discussion* (1970).
[42] Cronshaw, J., and Esau, K., *J. Cell. Biol.*, **34**, 801 (1967).
[43] Bowling, D. J. F., *Biochim. Biophys. Acta*, **183**, 230 (1969).
[44] Hartt, C. E., *Plant Physiol.*, **44**, 1461; *ibid.*, **45**, 183 (1970).
[45] Fensom, D. S., Clattenburg, R., Chung, T., Lee, R., and Arnold, D. C., *Nature*, **219**, 531 (1968).
[46] Esau, K., *The Phloem*, 88 (Borntraeger, Berlin, 1969).
[47] Milburn, J. A., *Planta*, **95**, 272 (1970).

The Space Scars of Earth

PETER M. MILLMAN
National Research Council of Canada, Ottawa, Ontario

There is some evidence for a meteoritic origin of craters in the Earth's surface. In this article the characteristic features of impact scars are discussed with the help of some examples.

THE two planetary bodies whose surfaces can be seen best from Earth and from spacecraft are the Moon and Mars. A dominant characteristic of both surfaces is a complex of circular craters of widely varying size and there is now considerable evidence that most of these craters resulted from the infall of the smaller, and less massive, bodies of the solar system—asteroids, meteoroids and possibly comets. We see the primary and secondary impact scars of these collisions with material from interplanetary space. It can be argued that the Earth should have suffered a similar celestial bombardment, and evidence of this is now growing rapidly, but because the Earth's surface is subject to many changes initiated by the atmosphere and oceans, the impact craters often have to be untangled from a maze of other features that may hide them from all but the persistent investigator.

Increasing Attention

It is rather surprising that in the past the detection of impact scars on Earth attracted little attention from most geologists. This situation is now changing rapidly and new information is becoming available in such profusion that any review of the subject rapidly becomes out of date.

Near the end of the last century there were tentative suggestions that the formation of certain depressions on Earth might have a connexion with meteoritic impact. G. K. Gilbert, for example[1], discussed this possible origin for the crater in Arizona—then known as Coon Butte—and for Lonar Lake in India. He discarded the hypothesis, chiefly because of a lack of understanding of the tremendous explosive force inherent in an impact at typical interplanetary collision speeds (15 to 25 km s^{-1}).

The scientific study of terrestrial impact features can properly be dated from the presentation of two papers[2,3] before the Academy of Natural Sciences in Philadelphia on December 5, 1905. Both Barringer and Tilghman put forward in detail numerous reasons why Coon Butte should be considered a meteoritic crater and not the result of volcanic action or a steam explosion. In the face of considerable opposition to his theory, Barringer followed up his original paper with a continuing study of the Arizona crater until his death in 1929; in recognition of his pioneering work this feature is now officially named the Barringer Crater. (Barringer also has a crater on the Moon named after him.)

It was not until 1926 that the crater at Odessa, Texas[4,5], was suspected to be of meteoritic origin and in the next few years impact features were recognized in a number of other sites[6]. Spencer[6] reviews accounts of twenty-eight craters in seven locations, all of which are now considered to be meteoritic in origin and to contain associated meteoritic material. Spencer also mentioned the Tunguska event in Siberia on June 30, 1908, but this is not generally considered to have produced recognizable impact craters on the Earth's surface[7].

Cryptovolcanism

The second phase in the study of terrestrial impact scars commenced with a series of papers[8-10] by J. D. Boon and C. C. Albritton who gave reasons why certain geological structures, previously called cryptovolcanic, should be considered of impact origin. In this case the argument was not as direct as for the craters listed by Spencer. The ages of the cryptovolcanic features were measured in hundreds of millions of years so that little remained of the original form, and any meteoritic material that might have been scattered in the area had long since disappeared. A common characteristic of these generally circular structures is the evidence of strong explosive action at a period that did not coincide with the age of the rocks involved. A wide acceptance of the views of Boon and Albritton came only after recent advances in the knowledge of highly shocked minerals. Eight of the nine structures listed by these authors as possibly meteoritic are now generally accepted as impact scars.

Canadian Studies

The third phase in the study of meteoritic craters did not start until 1950 when V. B. Meen of the Royal Ontario Museum led an expedition to Northern Ungava, Quebec, to investigate a circular lake that seemed to be a geological anomaly in the peninsula[11]. This feature, now named the New Quebec Crater, was pronounced meteoritic by Meen. Other discoveries among the ancient rocks of Canada were made soon afterwards, many as a result of a systematic search of air photographs organized by C. S. Beals[12] at the Dominion Observatory, Ottawa. Beals also introduced a new technique in the study of these ancient structures; various geophysical measurements were carried out systematically in a coordinated programme and then combined to give an overall picture. A good example is the investigation of the Brent Crater[13], which included aerial photography, topographical survey, surface geology, deep core drilling, and surveys by magnetic, seismic and gravity techniques. Brent has been used as a type example for comparing and assessing data secured in various ways. About twenty Canadian features are now being studied by these comprehensive geophysical methods[14].

The latest developments in the study of meteoritic impact features have arisen from the recognition of several definitive

Table 1 Identification Criteria for Impact Craters

Form and structure	General circular form, depression with raised rim, radial fracturing and rim strata uplift, central uplift.
Meteoritic material	Presence of meteorites, nickel–iron spherules, iron shale of meteoritic origin.
Rock breccia	Impact produced breccia—on surface with fragments approaching metre sizes or larger, in drill cores with fragments of centimetre sizes, in rock samples as micro-breccia or suevite.
Shatter cones	Cone shaped formations found in a variety of rock types.
Mineralogical shock evidence	Numerous small scale and microscopic characteristics ranging from planar features and deformation discontinuities to fused glassy fragments with vesicles and streaks, forming a pumice-like material with lechatelierite.
Impact melts	Igneous materials—rocks, lavas, glasses, formed by impact melting.

characteristics, both microscopic and macroscopic, of highly shocked materials. As early as 1946 Dietz[15] pointed out the value of shatter cones as shock indicators and since 1960 further knowledge of shock metamorphosis has accumulated rapidly[16] as a result of X-ray analysis and work with the microscope and the electron microprobe. The application of these new techniques to samples recovered from suspected impact sites has made it much easier to distinguish between structures that have only a superficial resemblance to impact craters and those that are genuine impact scars.

Geophysicists, geologists and astronomers now have at their disposal a wide variety of criteria for deciding the origin of a possible terrestrial impact feature. Some of the more important of these are shown in Table 1 but the list is by no means exhaustive. The overall picture of any site and the possibilities of origins other than impact must be considered carefully in coming to a conclusion in any given case.

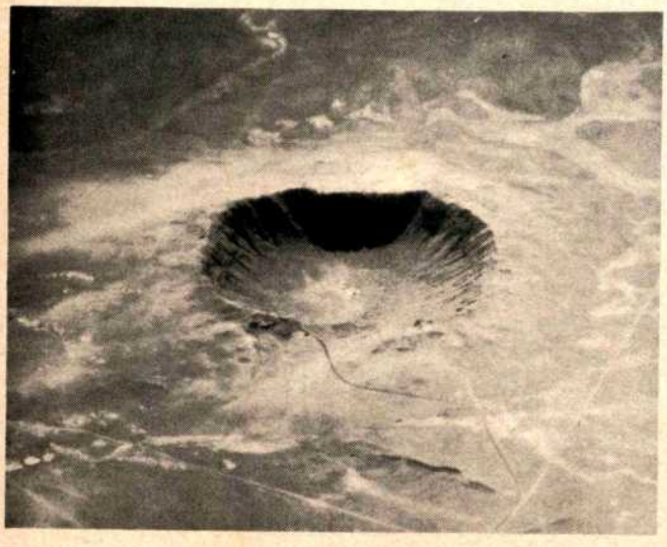
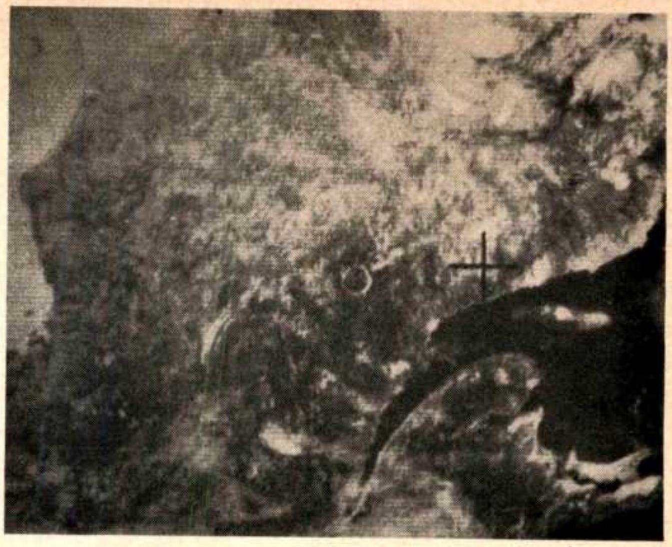


Fig. 1 The Barringer Crater, Arizona, diameter 1.2 km, photographed from the north near local noon on January 29, 1967. The polygonal form of the crater rim, seen in so many lunar craters, is quite evident.



[Photo: Canadian Meteorological Service]

Fig. 2 The Manicouagan Crater, Quebec, diameter 60 km, photographed from the ESSA-8 satellite on March 18, 1969. The crater appears at centre while a much larger feature, the Hudson Bay arc, is at upper left. This arc is still on the list of suspected impact scars[34].

Energy Release

The shape of a newly formed impact crater is caused by the sudden release of the kinetic energy of the impacting mass within a small volume somewhat below the original impacted surface. Matter will be ejected approximately equally in all directions and the impacting meteoroid will be broken up, pulverized and partially vaporized in the process. The result is a bowl shaped depression with a raised rim and, in some cases, a central uplift. Typical energies range from $\sim 10^{20}$ erg for a crater of diameter about 1 km to $\sim 10^{30}$ erg for a diameter of 50–100 km.

The form of relatively recent terrestrial impact craters corresponds closely with the youngest craters on the Moon and craters produced by artificial explosions on the Earth. The best example of a comparatively fresh impact scar is the Barringer Crater (Fig. 1) which has been preserved by the arid climate of Arizona from excessive erosion and sedimentation during the 30,000 or more years since it was formed. The outward tilting of the normally horizontal rock strata is very evident, even to the casual observer, and one-sixth or more of its original depth has probably been filled up. In the case of the much older Canadian craters in the same size range, the original depression has often been completely filled by sediments tens or hundreds of millions of years old. Geophysical methods introduced in the 1950s make it possible to outline the original crater depth and the brecciated volume beneath the crater floor in these instances. The old craters of a category in which the original diameter was more than several kilometres normally show a central uplift. In many cases, the original crater has been filled and the rim eroded, but the central uplift is the one dominant feature which remains clearly visible. Some details of the central uplift in terrestrial craters are not yet completely understood but, in broad terms, the uplift may be ascribed to a combination of post-impact slumping of the crater walls, isostatic adjustments, and elastic rebound in the subsurface rock layers[17]. The mechanisms of central uplift will have a greater effect in the larger craters, since here the energy of impact is in a higher ratio to the energy required to deform the geological strata. One of the large Canadian impact scars, Manicouagan (Fig. 2), demonstrates well the peripheral trough which is often the most conspicuous structural feature in deeply eroded ancient craters.

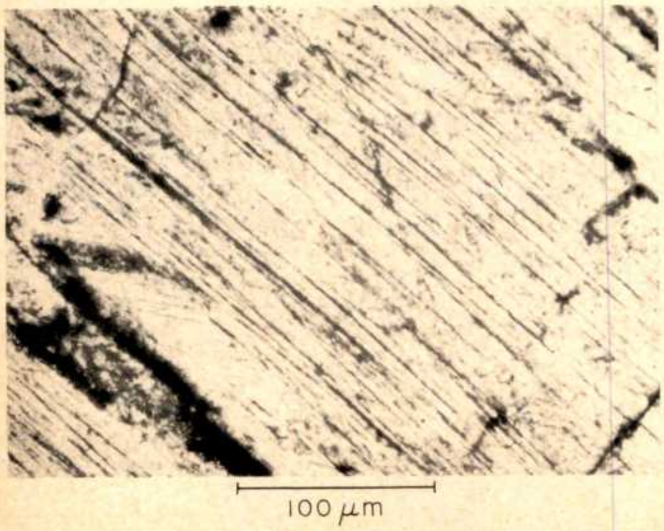
Meteoritic Evidence

The presence of meteoritic material was once considered essential for the positive identification of an impact crater, and meteorites have been associated with all the smaller and younger impact features. Nickel–iron spherules of several types in the millimetre size range were first found in 1946 by Nininger[18,19] at the Barringer Crater, and have been found more recently at a number of sites where meteorites have not yet been located. The manner of formation of these spherules is still a matter for discussion but there is no doubt that they are a product of the original meteoroid[20]. In the case of the very old impact features identifiable meteoritic material has not often been found. Calculations of the diameters of the meteoroids producing these craters have varied from 1/20 to 1/30 of the crater diameter for a stony meteoroid, so the meteoroid volume would have been much less than 10^{-4} times the volume of terrestrial rock involved. Surface meteoritic material would not have remained *in situ* for millions of years, and probably only the nickel–iron portion of this material would be identified in the drill core sampling. Only a very small fraction of the total volume is studied in drill core methods and it is not surprising that there is little direct evidence of the original meteoroid.

Evidence of the shattering force of space impact may be seen in the broken rock fragments commonly associated with space scars. Barringer[2] called early attention to the large-scale debris scattered around the crater that now bears his name. Drill cores, such as those from the Holleford Crater[21], illustrate vividly the type of rock breccia that is found below the crater floors. Breccias of an even finer grain size can be identified with a microscope[22].

The work of R. S. Dietz on shatter cones has already been mentioned and he has recently reviewed their occurrence at terrestrial impact sites[23]. These cone-shaped structures are formed by shock in a variety of rock types at pressures from 20 to 100 kbar, much greater than the pressures usually ascribed to terrestrial volcanic mechanisms.

Other mineralogical shock evidence is relevant to even higher pressures[24] and at shock pressures between 100 and 300 kbar planar features (closely spaced fine parallel lines) develop in quartz, plagioclase and other minerals. An example of this microscopic structure in quartz is shown in Fig. 3. Apart from the planar features there is other evidence of deformation, for example, slip bands, kink bands and deformation lamellae. At pressures from 300 to 500 kbar there are still planar features and also the highly shocked phases of SiO_2, coesite and stishovite. In this pressure range undisturbed



[Photo: M. R. Dence]

Fig. 3 A thin section of quartz from the central uplift of Lake St Martin, Manitoba, showing one dominant set of planar features and several other faint sets.

Table 2 Locations of Impact Sites

North America	Canada	18
	United States	14
South America		2
Europe		10
Asia		3
Africa		8
Australia		7
	Total	62

Table 3 Selected List of Terrestrial Impact Sites

	Latitude	Longitude
Aouelloul, Mauritania	21° 15′ N	012° 41′ W
Barringer, Arizona	35° 02′ N	111° 01′ W
Bosumtwi, Ghana	06° 32′ N	001° 23′ W
Boxhole, N. Territory, Australia	22° 37′ S	135° 12′ E
Brent, Ontario, Canada	46° 05′ N	078° 29′ W
Campo del Cielo, El Chaco, Argentina	27° 28′ S	061° 30′ W
Carswell, Saskatchewan, Canada	58° 27′ N	109° 30′ W
Charlevoix, Quebec, Canada	47° 32′ N	070° 18′ W
Clearwater, Quebec, Canada	56° 10′ N	074° 20′ W
Crooked Creek, Missouri	37° 50′ N	091° 23′ W
Dalgaranga, Western Australia	27° 45′ S	117° 05′ E
Decaturville, Missouri	37° 54′ N	092° 43′ W
Deep Bay, Saskatchewan, Canada	56° 24′ N	102° 59′ W
Dellen, Sweden	61° 50′ N	016° 45′ E
Flynn Creek, Tennessee	36° 16′ N	085° 37′ W
Gosses Bluff, N. Territory, Australia	23° 48′ S	132° 18′ E
Haviland, Kansas	37° 37′ N	099° 05′ W
Henbury, N. Territory, Australia	24° 34′ S	133° 10′ E
Holleford, Ontario, Canada	44° 28′ N	076° 38′ W
Howell, Tennessee	35° 15′ N	086° 35′ W
Jänisjärvi, Karelia, USSR	61° 58′ N	030° 55′ E
Jeptha Knob, Kentucky	38° 06′ N	085° 06′ W
Kaalijarv, Estonia, USSR	58° 24′ N	022° 40′ E
Kentland, Indiana	40° 45′ N	087° 24′ W
Köfels, Austria	47° 13′ N	010° 58′ E
Lac Couture, Quebec, Canada	60° 08′ N	075° 18′ W
Lappajärvi, Finland	63° 10′ N	023° 40′ E
Liverpool, N. Territory, Australia	12° 24′ S	134° 03′ E
Lonar, Maharashtra, India	19° 59′ N	076° 34′ E
Manicouagan, Quebec, Canada	51° 23′ N	068° 42′ W
Manson, Iowa	42° 35′ N	094° 31′ W
Middlesboro, Tennessee	36° 36′ N	083° 36′ W
Mien, Sweden	56° 25′ N	014° 55′ E
Mistastin, Newfoundland, Canada	55° 53′ N	063° 18′ W
Monturaqui, Chile	23° 54′ S	068° 18′ W
New Quebec, Quebec, Canada	61° 17′ N	073° 40′ W
Nicholson, NWT, Canada	62° 40′ N	102° 41′ W
Odessa, Texas	31° 48′ N	102° 30′ W
Pilot, NWT, Canada	60° 17′ N	111° 01′ W
Pretoria Salt Pan, South Africa	25° 30′ S	028° 00′ E
Ries, Germany	48° 53′ N	010° 37′ E
Rochechouart, France	45° 50′ N	000° 56′ E
Roter Kamm, South West Africa	27° 45′ S	016° 17′ E
St Martin, Manitoba, Canada	51° 47′ N	098° 33′ W
Serpent Mound, Ohio	39° 02′ N	083° 25′ W
Sierra Madera, Texas	30° 36′ N	102° 55′ W
Sikhote-Alin, Siberia, USSR	46° 07′ N	134° 40′ E
Siljan, Sweden	61° 05′ N	015° 00′ E
Skeleton, Ontario, Canada	45° 15′ N	079° 27′ W
Steen River, Alberta, Canada	59° 31′ N	117° 38′ W
Steinheim, Germany	48° 02′ N	010° 04′ E
Strangways, N. Territory, Australia	15° 12′ S	133° 35′ E
Sudbury, Ontario, Canada	46° 36′ N	081° 11′ W
Talemzane, Algeria	33° 18′ N	004° 06′ E
Temimichât, Mauritania	24° 15′ N	009° 39′ W
Tenoumer, Mauritania	22° 55′ N	010° 24′ W
Vredefort, South Africa	27° 00′ S	027° 30′ E
Wabar, Saudi Arabia	21° 30′ N	050° 28′ E
Wahnapitae, Ontario, Canada	46° 44′ N	080° 44′ W
Wells Creek, Tennessee	36° 23′ N	087° 40′ W
West Hawk, Manitoba, Canada	49° 46′ N	095° 11′ W
Wolf Creek, Western Australia	19° 18′ S	127° 47′ E

mineral crystals begin to change to the disordered or amorphous phases and at 500 to 650 kbar glasses containing vesicles and streaks are formed and most of the original texture of the crystalline rocks is destroyed. At pressures approaching 1,000 kbar and temperatures of 5,000 K complete melting occurs.

High temperatures are developed in an impact event, particularly the larger terrestrial features. The surface rocks melt and leave igneous materials in the form of glass in mixed breccias, layers of rock melt, and intrusions into basement rocks below the crater. Dence[25] has noted these melts at about forty impact sites.

General Characteristics

There is no single standard listing of terrestrial impact craters and a number of lists (refs. 26–33 and R. W. Barringer, personal communication) have, for example, been published since 1960. Many suspected sites are now known at which a full scale field study has not yet been possible and most of these are therefore in the doubtful category at present. To illustrate some general characteristics of the Earth's impact scars, I have selected from these lists and from more recent literature 62 sites which include the best authenticated examples of impact craters. Preference has been shown in this selection for evidence of high shock effects. The 62 sites include 112 individual craters with diameters ≥10 m, an arbitrarily chosen lower size limit. The geographical distribution of the 62 sites is summarized in Tables 2 and 3.

Estimates can be made for the original diameter of each crater and for its age. Only small accuracy can be achieved with individual craters, particularly when their age is large, but the overall statistical picture is probably free from serious systematic errors. Diameter and age estimates for the 112 craters have been plotted in Fig. 4—the eight groups of multiple craters have been separately identified.

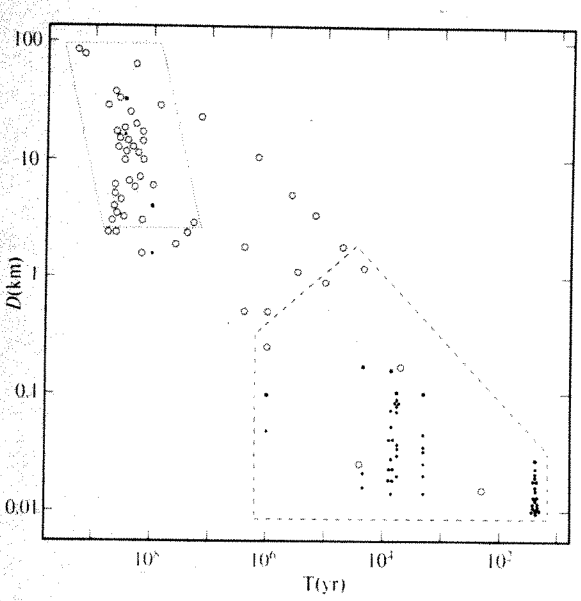


Fig. 4 A logarithmic plot of the estimated original diameter of each crater, *D* km, plotted against the estimated age, *T* yr. Meteorites have been found associated with all craters in the area enclosed by dashed lines. Central uplift is present in three-quarters of the craters in the area enclosed by dotted lines. ○, Single crater; ●, crater group.

One must be careful not to draw unwarranted conclusions from Fig. 4. It has been plotted more to show the distribution in age and size of the recognized impact features on Earth, than to demonstrate any important relation. Even though the logarithmic scale tends to crowd points to the left of the diagram, the apparent lack of large recent craters is probably a real effect; massive space impacts were undoubtedly more common when the solar system was younger. The lack of old small craters is caused by erosion and obliteration.

If the assumption is made that the majority of large lunar craters were formed by impact, it is natural to compare the numbers found per unit area on the Moon with the numbers found on Earth, as both Moon and Earth have been subjected to the same interplanetary environment. An approximate count indicates that the crater density on the Moon is several orders of magnitude greater than it is here but the impossibility of estimating the terrestrial obliteration effects over hundreds of millions of years makes a quantitative comparison almost useless. It can safely be said, however, that lunar crater statistics suggest that many more terrestrial impact features will be found if we look hard enough for them. At a time when a very large effort is being expended on the study and interpretation of lunar surface materials, the analysis of the corresponding materials from terrestrial impact sites is an essential concomitant activity.

I acknowledge many profitable consultations with Mr M. R. Dence.

[1] Gilbert, G. K., *Science*, NS3, 1 (1896).
[2] Barringer, D. M., *Proc. Acad. Nat. Sci. Philadelphia*, **57**, 861 (1905).
[3] Tilghman, B. C., *Proc. Acad. Nat. Sci. Philadelphia*, **57**, 887 (1905).
[4] Barringer, jun., D. M., *Proc. Acad. Nat. Sci. Philadelphia*, **80**, 307 (1928).
[5] Sellards, E. H., *Bull. Geol. Soc. Amer.*, **38**, 149 (1927).
[6] Spencer, L. J., *Geog. J.*, **81**, 227 (1933).
[7] Krinov, E. L., *Principles of Meteoritics*, 131 (Pergamon, London, 1960).
[8] Boon, J. D., and Albritton, jun., C. C., *Field and Lab.*, **5**, 1 (1936).
[9] Boon, J. D., and Albritton, jun., C. C., *Field and Lab.*, **5**, 53 (1937).
[10] Boon, J. D., and Albritton, jun., C. C., *Field and Lab.*, **6**, 44 (1938).
[11] Meen, V. B., *J. Roy. Astron. Soc. Canada*, **44**, 169 (1950).
[12] Beals, C. S., Ferguson, G. M., and Landau, A., *J. Roy. Astron. Soc. Canada*, **50**, 203, 250 (1956).
[13] Millman, P. M., Liberty, B. A., Clark, J. F., Willmore, P. L., and Innes, M. J. S., *Publ. Dominion Observ.*, **24**, 1 (1960).
[14] Dence, M. R., Innes, M. J. S., and Robertson, P. B., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 339 (Mono Book Corp., Baltimore, 1968).
[15] Dietz, R. S., *Popular Astron.*, **54**, 465 (1946).
[16] French, B. M., and Short, N. M., editors, *Shock Metamorphism of Natural Materials* (Mono Book Corp., Baltimore, 1968).
[17] Dence, M. R., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 169 (Mono Book Corp., Baltimore, 1968).
[18] Nininger, H. H., *Science*, **113**, 755 (1951).
[19] Nininger, H. H., *Arizona's Meteor Crater* (American Meteorite Museum, Sedona, Arizona, 1956).
[20] Brett, R., *American Mineralogist*, **52**, 721 (1967).
[21] Beals, C. S., *Pub. Dominion Observ.*, **24**, 113 (1960).
[22] French, B. M., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 383 (Mono Book Corp., Baltimore, 1968).
[23] Dietz, R. S., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 267 (Mono Book Corp., Baltimore, 1968).
[24] von Engelhardt, W., and Stöffler, D., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 159 (Mono Book Corp., Baltimore, 1968).
[25] Dence, M. R., *J. Geophys. Research* (in the press).
[26] Shoemaker, E. M., and Eggleton, R. E., *Terrestrial Features of Impact Origin* (US Geological Survey, Menlo Park, California, 1961).
[27] Monod, T., *Université de Dakar—Institut Français d'Afrique Noire, Catalogues et Documents*, No. 18 (1965).
[28] O'Connell, E., *A Catalogue of Meteorite Craters and Related Features with a Guide to the Literature* (RAND Corp., Santa Monica, California, 1965).
[29] Beals, C. S., and Halliday, I., *International Dictionary of Geophysics*, **2**, 1520 (Pergamon, London, 1967).
[30] Short, N. M., and Bunch, T. E., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 255 (Mono Book Corp., Baltimore, 1968).
[31] Barringer, R. W., *Meteoritics*, **3**, 151 (1967).
[32] Freeberg, J. H., *US Geological Survey Bulletin*, No. 1220 (1966).
[33] Freeberg, J. H., *US Geological Survey Bulletin*, No. 1320 (1969).
[34] Beals, C. S., Halliday, I., and Wilson, J. T., in *Science, History and Hudson Bay* (edit. by Beals, C. S.), 985 (Department of Energy, Mines and Resources, Ottawa, Canada, 1968).

Circular Polarization: Jupiter and Other Planets

JAMES C. KEMP* & RAMON D. WOLSTENCROFT
Institute for Astronomy, University of Hawaii, Honolulu, Hawaii 96822

JOHN B. SWEDLUND
Department of Physics, University of Oregon, Eugene, Oregon 97403

Circular polarization of scattered light from Jupiter has now been studied during a recent opposition of the planet, showing changes in the sign of the north and south polar values indicative of a non-magnetic origin for the polarization. Preliminary observations on Mars, Venus, Mercury and the Moon demonstrate the generality of this effect and its potential usefulness.

SINCE our discovery of circular polarization in the scattered light from Jupiter[1], we have continued the observations through the period of opposition. A reversal in sign of the predominant "polar" effect can now be reported, clearly substantiating a scattering model[2] as opposed to alternatives such as magnetoreflexion. We have also observed what are believed to be analogous effects, although sometimes with differing signs and other interesting features, in the cases of Mars, Venus, Mercury and the Moon. The data in these cases are illustrative only, but they show that the phenomenon is general, that it can be measured and that it provides new information for planetary astronomy.

All these observations except those on the Moon followed a scheme shown in Fig. 1*a*. Fractional circular polarizations Q_0, Q_N, and Q_S were measured in light from the whole planet, the northern hemisphere, and the southern hemisphere respectively. North and south in all cases are heliocentric directions (normal to the plane of the solar system) rather than terrestrial or planet-axis directions. For Q_N and Q_S a large aperture stop was used at the telescope focal plane, two to three times the diameter of the planet image; the stop edge served to bisect the planet approximately as shown. In the earlier Jupiter measurements[1], Q_N and Q_S were measured instead with a small stop, about half the diameter of the image of the planet and centred inside the planet disk near one or the other of the poles. The new choice gave very similar values (as checked on Jupiter) and reduced the problem of guiding, as the principal drift was in right ascension. It should be approximately true that $Q_0 = \frac{1}{2}(Q_N + Q_S)$; in the data reported this is not always so, notably in the case of Mercury, because of telescope guiding errors. Contamination by sky effects was always checked and was possibly significant only in the case of Mercury. Spurious signals due to the indirect effect of linear polarization were cancelled out routinely, as in previous work, by taking the algebraic average of readings made with the polarimeter at relative orientations 0° and 90° about the telescope axis. The bulk of the measurements were made on one of the two 24 inch telescopes at Mauna Kea Observatory; on Jupiter, Venus, and Mars, comparison measurements were made with the 88 inch telescope, with entirely consistent results.

With the exception of Mars we have confined virtually all the measurements to a pass band about 500 Å wide (half-transmission points) centred near 6800 Å, for reasons indicated earlier[1]. Actually the spectral coverage can be readily extended.

Jupiter

The crossover of Q_N and Q_S (hemispheric polarizations) through zero at opposition of Jupiter is apparent in Fig. 2. The abscissa here is the phase angle φ between the Sun-planet and Earth-planet directions. (This angle was denoted ψ in the earlier papers[1,2]; we have changed notation to conform to that of Pospergelis[3], whose laboratory work will be discussed here.) Before opposition, or when the planet is a "morning" object, φ is negative. We observe that Q_N goes almost linearly through zero at $\varphi = 0°$; this is the behaviour expected over the small range of φ represented here. A sur-

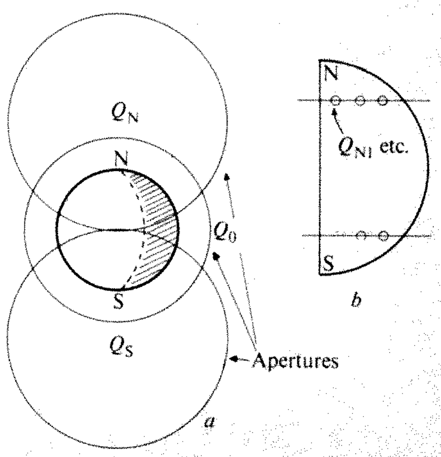


Fig. 1 Observing schemes for measuring the circular polarization of the whole planet Q_0, and hemispheric values. The lighter circles in *a* are telescope aperture stops. *a*, Planet; *b*, Moon.

---

*On leave from Department of Physics, University of Oregon, Eugene, Oregon 97403.

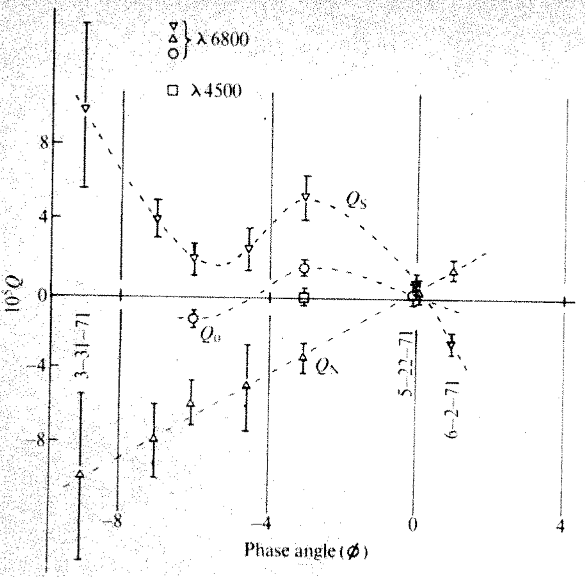


Fig. 2 Fractional circular polarization of Jupiter during the period of opposition, May 23, 1971. Observation on June 15, at $\varphi = +4.5°$, after this figure was drawn, gave $Q_0 = +1 \times 10^{-5}$, $Q_N = +5 \times 10^{-5}$, and $Q_S = -6 \times 10^{-5}$, all at $\lambda = 6800$ Å.

prising peculiarity, however, is seen in Q_S—a kind of S-shaped undulation, superimposed on the overall crossover through zero. This undulation is also reflected in the polarization, Q_0, of the whole planet. The cause of this fluctuation cannot be a general north–south asymmetry with respect to absorption; that would lead merely to a difference in slopes between the Q_S and Q_N curves. One question is whether the Q_S anomaly can somehow be related to the presence of the Red Spot in the southern hemisphere. We did not monitor Q_S and Q_N over a complete cycle of the planet's rotation cycle, at each orbital phase φ. But in one case, the observations of May 10–11 ($\varphi \simeq -3°$), we measured the quantity Q_0 periodically over a five hour period, during which the Red Spot traversed the front of the planet and disappeared behind the west limb. Q_0 did not change by more than the measurement uncertainty of about ±20%. The cause might instead be related to the orientation of Jupiter's spin axis, which is inclined about 3° from the normal to the orbit plane and which, during the period of this opposition, was such that the south pole was tilted toward us by the maximum amount.

In the observations of May 10–11 when Q_0 showed a maximum positive value, we also measured this quantity with a broad blue filter, having a pass band of 1000 Å centred at about 4500 Å. The null result $(0.0 \pm 0.5) \times 10^{-5}$ is consistent with the notion that ellipticity requires absorption. Studies in the near infrared, where there are strong methane bands, will be expected to show large Q values.

No further results were obtained on the interesting localized effects noted earlier in the area of the Red Spot[1]. These must await construction of an improved arrangement for guiding the telescope which will facilitate signal-averaging on small areas of the image.

Venus and Mercury

Having a dense atmosphere as does Jupiter, Venus showed the polar scattering effect, as expected, with the same signs of Q_N and Q_S as Jupiter for the same sign of φ. (In this instance Venus was a morning star, with negative φ as with Jupiter before opposition.) What one might call the elliptical scattering power Q/φ (or $\partial Q/\partial\varphi$) is notably smaller (Table 1) than for Jupiter, at the same measuring wavelength in the deep red.

The single result on Mercury showed the same sign of the polar effect as for Jupiter ($\varphi < 0$) and Venus, but with a surprisingly large asymmetry that is a large Q_0, as shown in Table 1. As with observations of other types on Mercury[4], this measurement was a precarious one which had to be accomplished within a period of less than 30 min while the planet was high enough to be free of horizon effects, yet before sunrise. Seeing conditions were just adequate to permit us to discern the halfmoon-like image, and resolution of the planet into north–south halves (Fig. 1*a*) was done with only fair accuracy. We were aware that an elliptically polarized morning sky could in this case be a spurious cause of the asymmetry, adding, for example, to Q_S and acting to cancel Q_N. A brief test on adjacent sky tended to rule this out, and we believe the asymmetry is real. Whether or not that is so, the polar difference Q_N–Q_S should be independent of sky effects and clearly has the same sign as in the case of Venus. We will comment later on the significance of this sign.

Mars

A striking wavelength-dependent asymmetry was observed in the case of Mars, shown in Fig. 3. In the deep red, the circular polarization seems to approach the ideal polar asymmetry, $Q_N = -Q_S$, although with signs opposite to those of Jupiter and Venus. In the blue, however, the polarization on both poles was overwhelmingly negative. On the basis of the visual appearance of the planet on the dates of the observations, some kind of asymmetry could indeed have been anticipated. The Martian season corresponded to spring in the southern hemisphere, the south pole being tilted toward the Sun with the south polar cap a very dominant feature. A consistent, although quite tentative, explanation for the behaviour of Q_N and Q_S is as follows. At long wavelengths the atmosphere is unimportant, and the scattering is characteristic of a solid surface. That a solid surface can in fact give a polar effect of the sign observed, for the orbital phase angle obtaining here (−40°), will be indicated later. In the blue, on the other hand, there is appreciable atmospheric absorption, the Q values tending to switch over to the "Jupiter" signs. But the albedo of the south polar cap is so large that most of the light from the south comes from solid-surface reflexion, washing out the oppositely polarized flux associated with atmospheric scattering in that region. In the blue, therefore, the atmospheric sign only achieves dominance in the north, where the mean albedo is low.

Uranus and Neptune

Data for Q_0 for Uranus and Neptune are included in Table 1, but are to be taken merely as upper limits. They represent

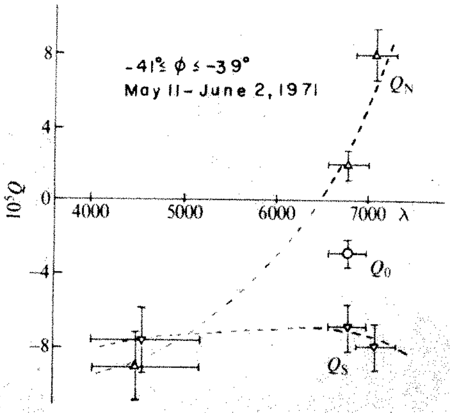


Fig. 3 Crude wavelength dependence of the circular polarization values on Mars. The horizontal error bars are pass bands of the filters used, weighted by the photomultiplier response.

Table 1 Fractional Circular Polarizations in Units of 10^{-5}

	Dates (1971)	Mean φ	Mean λ	Q_0	Q_N	Q_S
Venus	May 20, 23; June 1	$-37°$	6,800	—	$-(7\pm2)$	$+(5\pm1)$
Mercury	June 2	$-67°$	6,800	$+(12\pm4)$	$-(2\pm2)$	$+(18\pm6)$
Moon	May 31	$+90°$	6,800	—	$+(4\pm1)$	$-(3\pm1)$
Uranus	May 23	$+2°$	5,000	(0 ± 1)	—	—
Neptune	June 1	$0°$	5,000	$-(20\pm40)$	—	—

signal averaging over one hour on the 88 inch telescope (Uranus), and about three hours averaging on a 24 inch telescope (Neptune). As far as the polar scattering effect is concerned, if these planets are reasonably symmetric no finite values of Q_0 are expected. It seems feasible to resolve Uranus into north and south halves in a future measurement.

The Moon

The observing scheme in Fig. 1*b* was used to measure Q values on the Moon. Here it was not convenient with the existing polarimeter and telescope optics to make hemisphere measurements. We used an aperture diameter of about 1 arc minute, and selected a sequence of three mirror-image pairs of regions located at complementary northern and southern lunar latitudes of very roughly $\pm45°$, as shown in the sketch. The regions were selected to be as far as possible free of severe relief features. Regions N1 and N3 (and southern counterparts) were near the terminator and the limb respectively, with N2 intermediate. In either hemisphere the Q values for locations 1, 2, and 3 were within a factor of three of each other and had the same sign. The two averages Q_N and Q_S of each of these sets of three values are entered in Table 1; these may reasonably be compared with the hemispheric averages measured on the planets. The phase here was first quarter (half Moon waxing), corresponding to $\varphi = +90°$. The polar values are seen to have the same signs as for Jupiter at positive φ.

Models for the Polarization

The rudimentary model for elliptically polarized scattering at a gaseous surface[2] is consistent with the signs of the polar effect on Jupiter and Venus. What, however, happens with solid surfaces, as on the Moon? Although no theoretical treatment has been given, we may appeal to an extensive laboratory study by Pospergelis[3] of the ellipticity of initially unpolarized light as scattered from a variety of rock and mineral surfaces. For comparison we must point out that our Q equals $-S$ in his notation, where S is the conventional fourth Stokes parameter. His analysis involves certain angles—the phase φ (which has the same meaning as in this article), and the angles γ and ψ giving the orientation of the plane scattering surface. In our context we interpret γ and ψ as the latitude and longitude respectively of a local scattering region on a sphere. (The region on the sphere nearest the observer has $\psi = 0°$.) His results for the circular polarization show, as well as the quite general antisymmetries $Q(-\varphi) = -Q(\varphi)$ and $Q(-\gamma) = -Q(\gamma)$, sign change on variation of φ, not dictated by symmetry considerations. The situation is complicated by the behaviour with respect to ψ; but for scattering from the entire north or south half of the visible surface of a planet at phase φ, there is a mean ψ roughly equal to $\varphi/2$. For $\psi = \varphi/2$, Pospergelis's data for all surfaces investigated (excluding unusual materials with optical activity) indicate that for all φ between 0° and a critical phase φ_c the hemispheric values Q_N and Q_S should have opposite signs from the gas-layer case; $Q(\varphi_c)$ is zero, and for $\varphi > \varphi_c$ the signs agree with those of the gas case. Evidently φ_c depends on the nature of the surface, but the typical value is around 70°. (Another critical value $\varphi \sim 40°$ is mentioned by Pospergelis[3] but that is for $\varphi = 0°$. We have deduced $Q(\varphi)$ for the case $\varphi = \varphi/2$ from families of curves given for a typical surface.) The gas-layer model[2] does not have any such critical angle, $Q(\varphi)$ having one sign over the interval $0° < \varphi < 180°$.

The mechanism for elliptically polarized scattering from condensed matter is different from the gas-layer case. We are considering a model based on double specular reflexions from the facets of crystal grains exposed on a surface. (Equivalently this could be a layer of aerosol particles large compared to a wavelength.) The first reflexion linearly polarizes the ray; the second, from an adjacent grain, elliptically polarizes it by virtue of the material being slightly absorbing. The critical angle φ_c is perhaps related to Brewster's angle for the substance.

The signs of Q_N and Q_S on Mars in the deep red (Fig. 3), at $\varphi = -40°$, are consistent with the conclusions for solid surfaces, for it is fairly clear that the magnitude of φ is less than φ_c. The data for the Moon, at $\varphi = +90°$, are similarly consistent as here we seem to have $\varphi > \varphi_c$. With Mercury the situation is less sure, and little more can be said until investigations at other phases are carried out.

One difference between the data given here and the laboratory results of Pospergelis is in the general magnitudes. His values are of the order 0.1% to 1%, whereas ours are between 0.001% and 0.01%. As noticed empirically[3], the elliptical polarizing power is drastically reduced in a powdered sample as compared to polycrystalline rock. On the face of it this implies that Mars, the Moon and presumably Mercury are largely covered with dust or other fine-textured material, and of course the nature of lunar dust is now well known. The suppression of the polarization is probably because the double reflexion mechanism gives way to multiple reflexions, that is, a ray is trapped in surface cavities before scattering out, randomizing the polarization; or perhaps the particle size becomes smaller than a wavelength and Rayleigh scattering occurs, which cannot produce elliptical polarization by the mechanism described.

What meaning does the polar scattering effect have for astronomy? Given a better knowledge of the origin of the effect, it can give information on planetary surfaces. Even without such knowledge the symmetry aspects alone are of immediate use. A dramatic application should be in the study of asteroids. Here we refer to measurement of Q_0—the integrated effect for the whole body—which is nonvanishing for an asymmetric figure. From Q_0 and its time dependence one can deduce facts about the shape and orientation of the body, complementary to the information furnished by linear polarization and the light curve. Finally, because the effect specifically requires absorption, a spectral curve $Q(\lambda)$ might reveal absorption bands difficult to see otherwise; confusion with absorption bands in the Earth's atmosphere would be almost impossible.

Recognizing the polar scattering phenomenon is necessary before we can search for other causes of circular polarization in light from the planets, such as magnetoreflexion or circular dichroism. The latter is important in the detection of biological molecules. For example, the possibility of Martian plant material having optically active scattering properties comparable to those of green leaves[3] can be tested by searching for the circular dichroism band at 7000 Å. As the circular-dichroic ellipticity neither vanishes nor changes sign at $\varphi = 0°$, such an effect would stand out clearly at opposition. (It happens that our two filter points at 6800 Å and 7100 Å in Fig. 3 approximately straddle that particular band, although they were not selected with such an investigation in mind. The rapid change in Q values between the two wavelengths is likely to be related to the transition from solid-surface to atmospheric scattering, as indicated earlier.) In forthcoming observations a monochromator will be used to study $Q(\lambda)$ with a resolution of about 20 Å on Mars and other planets.

This work is supported by NASA, the National Science Foundation, and Research Corporation.

Received June 21, 1971.

[1] Kemp, J. C., Swedlund, J. B., Murphy, R. E., and Wolstencroft, R. D., *Nature*, **231**, 169 (1971).
[2] Kemp, J. C., and Wolstencroft, R. D., *Nature*, **231**, 170 (1971).
[3] Pospergelis, M. M., *Sov. Phys. AJ*, **12**, 973 (1969); trans. from *Astron. Zh.*, **45**, 1229 (1968).
[4] Morrison, D., *Space Sci. Rev.*, **11**, 271 (1970).

The Evolution of the North-East Atlantic

C. A. WILLIAMS & DAN McKENZIE
Department of Geodesy and Geophysics, Cambridge

Magnetic data taken over a sixteen year period have been analysed and information on the evolution of the North-East Atlantic has been deduced.

It is well known that the evolution of ocean basins can be worked out in considerable detail from magnetic lineations observed at sea level. This technique has already been applied to the Pacific, Indian and South Atlantic oceans, whose evolution is now known in some detail. Very little work has been carried out so far on the North Atlantic, probably because the slow rate of separation of Eurasia and North America has

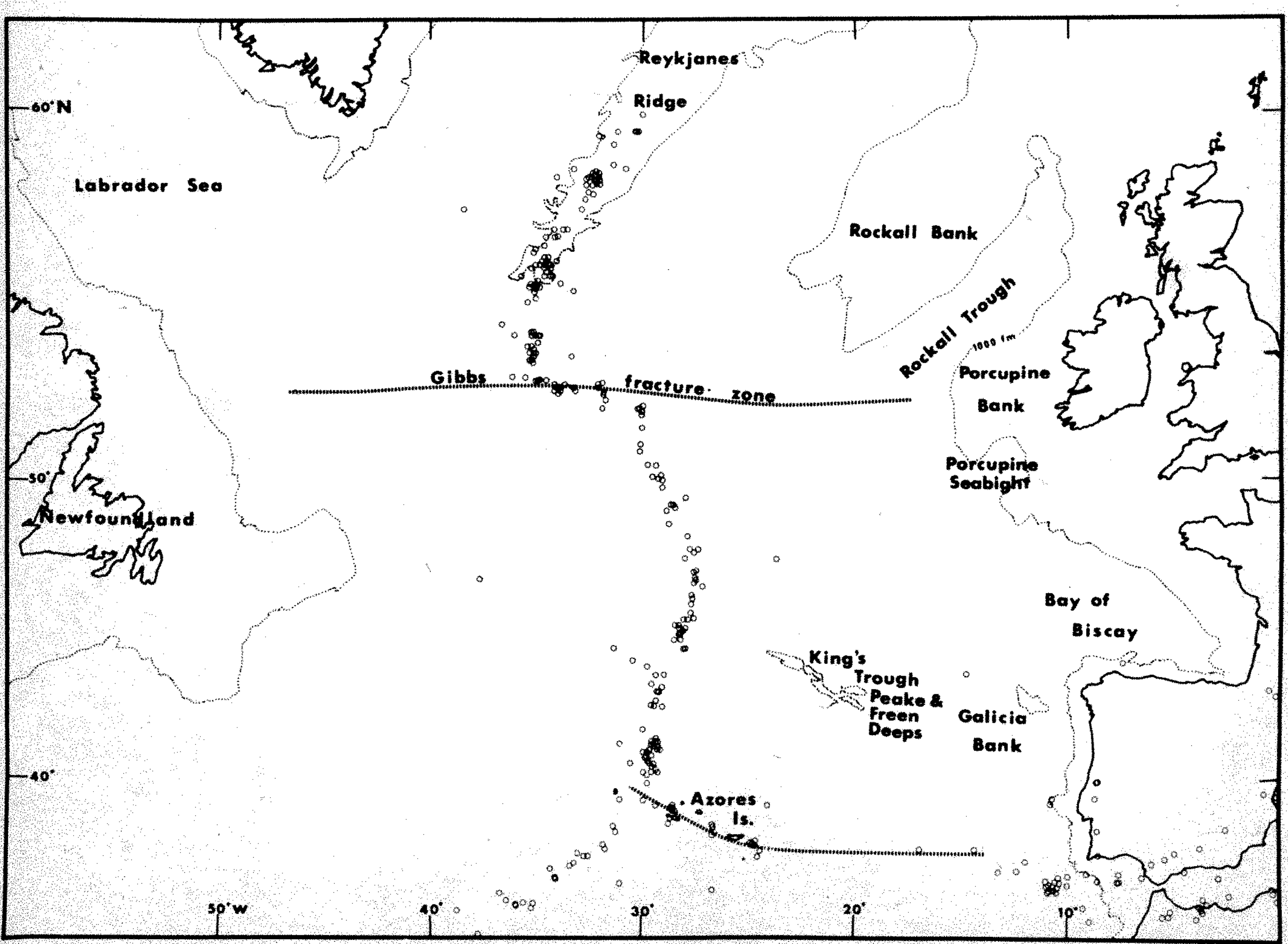


Fig. 1 Location map of the North Atlantic, with USCGS earthquake epicentres.

caused the anomalies to be harder to identify. This is partly because the details of short wavelength anomalies cannot be resolved from the sea surface and also because the seafloor spreading lineations are confused by anomalies from topographic effects and serpentinite intrusions (F. Aumento, personal communication).

Most of our data (Fig. 2) lie within the region bounded by the Azores–Gibraltar Ridge, the Mid-Atlantic Ridge, the Gibbs fracture zone and the continental margin. It is this area with which this article is primarily concerned. It is, however, particularly difficult to apply plate tectonics to a restricted region and our interpretation also depends upon anomalies identified by Pitman and Talwani in the North-West Atlantic[1].

The principal features of the seafloor in this region are the Azores–Gibraltar Ridge, which is seismically active, Kings Trough and Peake and Freen Deeps[2], the Gibbs fracture zone at 53° N and the present ridge crest. The continental margin from southern Spain to 53° N contains four major indentations. The largest of these is the Bay of Biscay. The other three are Porcupine Seabight[3] and Rockall Trough (Fig. 1) and the southern end of Hatton–Rockall Basin[4].

The other major feature on the continental margin is an elongated sediment-filled trough, parallel to the north coast of Spain[5], and characterized by a large negative gravity anomaly. These features must have been formed since the separation of Europe and North America and the evolution of the North Atlantic, recorded by the magnetic lineations, should help to understand their origin.

Magnetic Anomalies

Magnetic measurements have been made in the North-East Atlantic by the Department of Geodesy and Geophysics, Cambridge, since 1956, and wherever possible these original records were used. Other data collected by the British and Dutch Navies[6] and Bedford Institute, Canada (C. A. Williams and D. M. Porteous, account in preparation, and D. I. Ross, Bedford Institute Geophysics report, 1967), were also used, though not in the form of the original records. These magnetic data were digitized and the International Geomagnetic Reference Field[7] removed. The resulting anomaly profiles were plotted at right angles to the ships' tracks on 1 : 1 m scale charts at a scale of 200 γ to 1 inch. The strike of the lineations obtained from these charts was then used and the ship's tracks projected perpendicular to it. The anomalies were identified with the help of synthetic profiles at exactly the same scale and strike. A more detailed description of this procedure can be found elsewhere[8].

The profiles projected at right angles to the strike of the lineations are shown in Figs. 3 and 4 and the lineations themselves in Fig. 6. The synthetic profiles were obtained from the reversal time scale of Heirtzler *et al.*[9] with McKenzie and Sclater's modification of the time scale beyond anomaly 30. A depth to magnetic basement of 3.3 km was assumed from the ridge axis to anomaly 5 and of 5 km beyond anomaly 5. The reduced amplitude of the older part of the synthetic profile is probably due to a palaeolatitude effect since this part of the ridge was then closer to the magnetic equator. Since the lineations change trend in this region the strike of the line perpendicular to them is not constant.

The profiles in Figs. 3 and 4 show that all the anomalies from 1 to 32 are present in this part of the North Atlantic. This conclusion differs from that of Talwani, Pitman and Heirtzler[11] and Pitman, Talwani and Heirtzler[1] who consider that there was a pause or extreme slowing down in spreading between 38 and 9 m.y. ago (anomalies 13–5). Their identification of anomalies at the beginning and end of the sequence agrees with that in Figs. 3 and 4. The main difference between

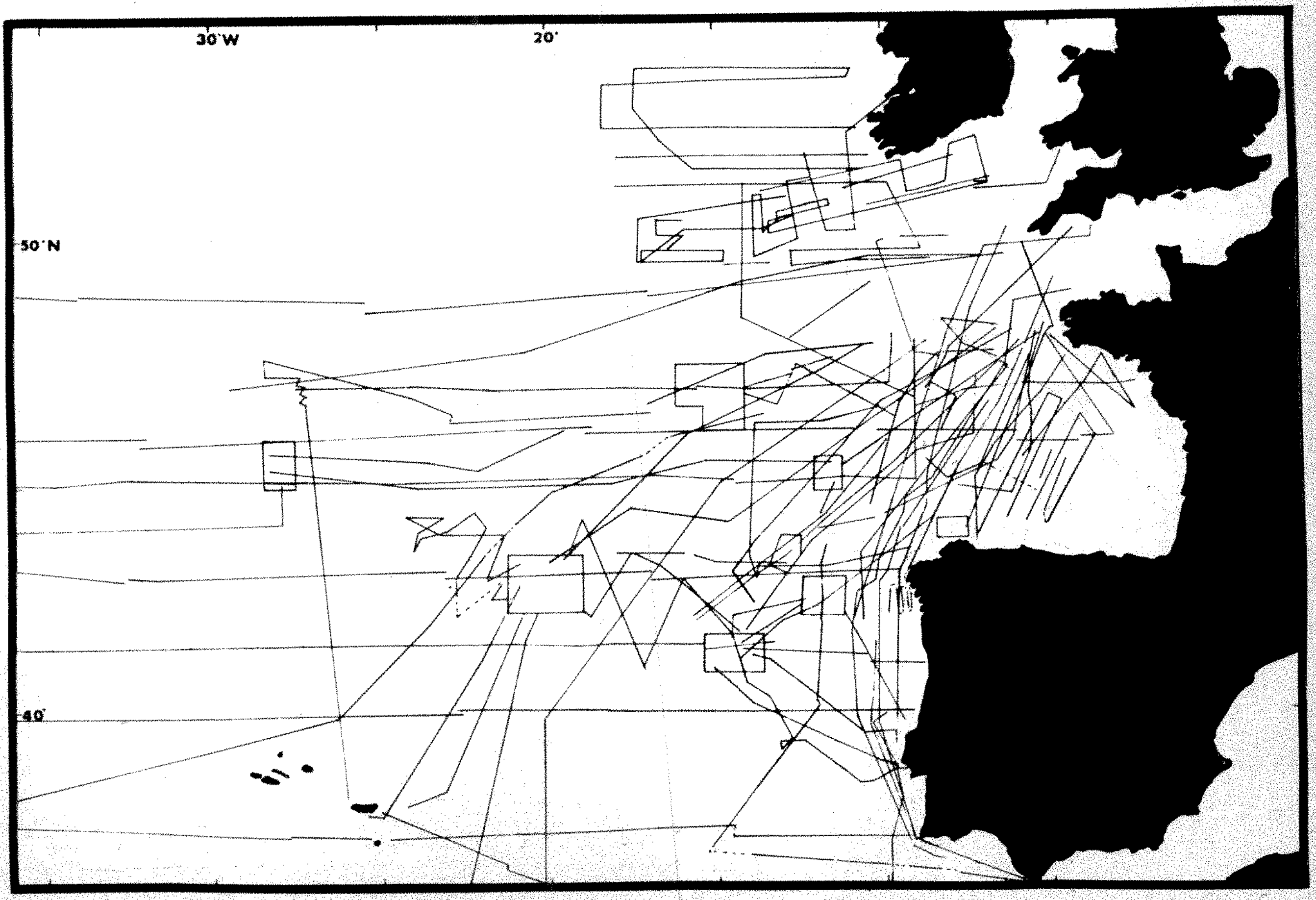


Fig. 2 Ships' tracks along which magnetic data are available. Areas of detailed survey are outlined.

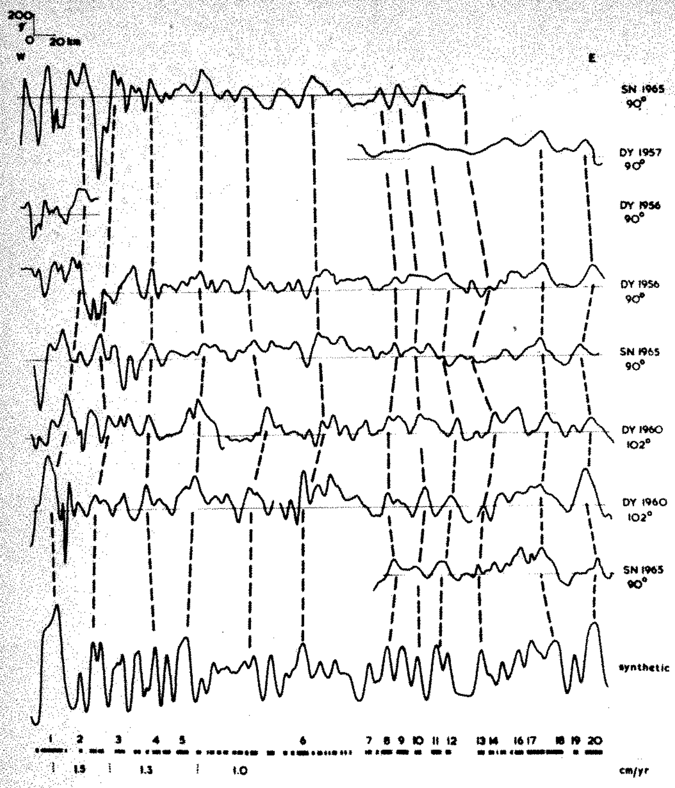


Fig. 3 Projected anomaly profiles from anomaly 1 at the Ridge Crest to anomaly 32. The cruise identification and the directions along which the tracks are projected are shown. The angle is not constant since there is a bend in the lineations in this region. The synthetic profile was calculated after Heirtzler *et al.*[9] and the positively magnetized blocks are numbered according to the convention of Pitman and Heirtzler[10]. The complete sequence of anomalies can be identified. The synthetic profile is calculated at 45° N, except beyond anomaly 24 where a palaeolatitude of 25° N was used. Cruise identifications: Dy, RRS Discovery; Sn, H. Neth, M. S. Snellius 1965 project NAVADO lines J to N; H, CSS Hudson; JM, RRS John Murray; B, CSS Baffin.

these two interpretations depends upon which anomaly is identified as anomaly 13. Fig. 5 shows the two interpretations of this crucial section together with the projected profiles on which the anomalies are most easily recognized. Pitman and Talwani believe 13 to be an anomaly closer to the ridge crest than the one we have identified.

The reversal time scale is not yet known beyond anomaly 32 (79 m.y.). Immediately preceding this there is thought to have been a period of approximately 50 m.y. with no reversals[12]. Seafloor formed during this period is known as the quiet zone in the Pacific. The seafloor east of anomaly 32, where no lineations are observed, was probably formed at this time. It is, however, important to point out that there are other explanations for the absence of magnetic anomalies since lineations are easily destroyed.

On the eastern side of the mid Atlantic Ridge there are two regions where anomalies are found which are not part of the numbered sequence formed since the Upper Cretaceous (Fig. 6). The first region is the Bay of Biscay where a pattern of magnetic lineations fanning from the South East corner of Biscay have been mapped by Matthews and Williams[13]. The strike of these lineations fanning from the south-east corner of Biscay have northern Biscay they appear to be truncated by part of the quiet zone to the east of anomaly 32, suggesting that the Biscay sequence is older than anomaly 32[14]. As there is evidence of reversals here, the seafloor appears to be more than 50 m.y. older than anomaly 32, that is older than 125 m.y. which is lower Cretaceous.

The second region where older anomalies occur is off South-West Spain, where a group of lineations have approximately north–south strikes and fan out in a similar way to those of Biscay (Fig. 6). One of these anomalies is anomaly J and has also been identified on the western side of the Ridge[1].

Evolution of the North-East Atlantic

The south of the North Atlantic began to open in the Late Trias with the separation of North America and Africa[1]. In pre-upper Cretaceous times the spreading ridge extended northwards into Biscay. The fanning of the Biscay anomalies and also those off South-West Spain suggests that both sets were formed synchronously accompanied by the anticlockwise

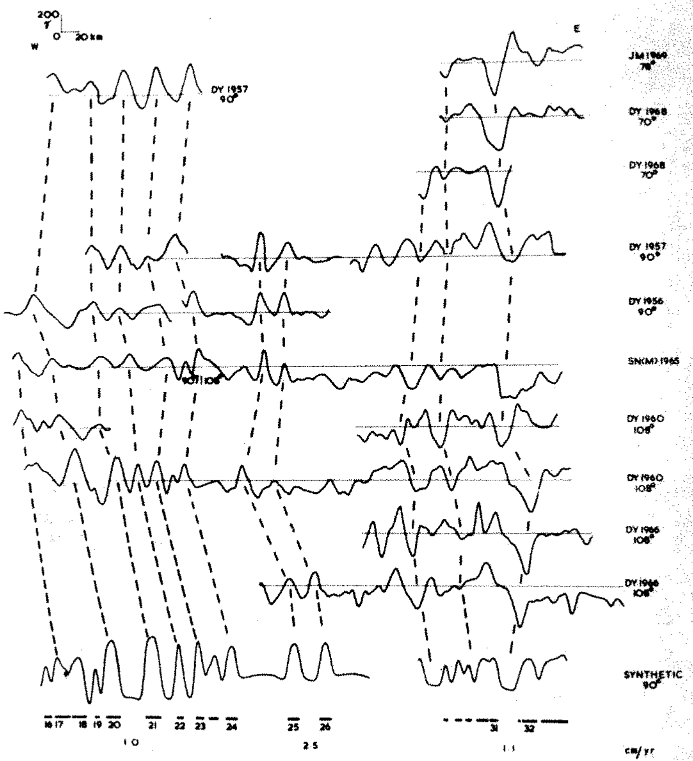


Fig. 4 See caption to Fig. 3.

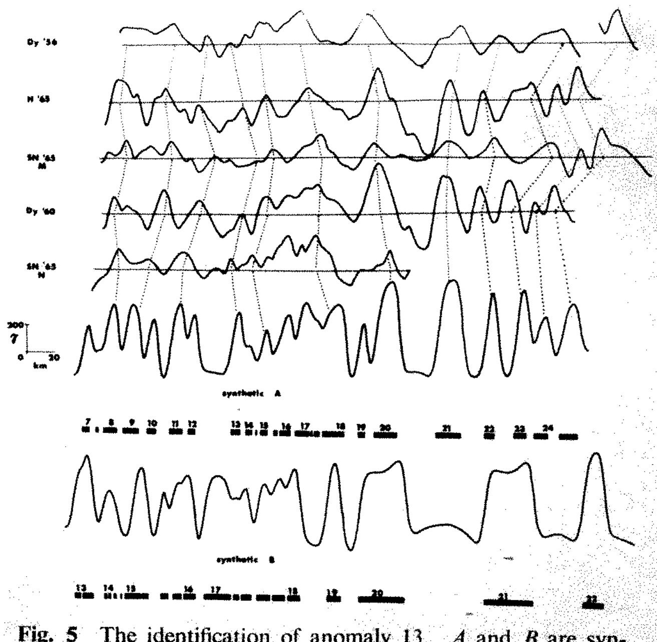


Fig. 5 The identification of anomaly 13. *A* and *B* are synthetic profiles. *A* according to this interpretation with a spreading rate of 1 cm yr^{-1} and *B* according to Pitman and Talwani with a spreading rate of 2 cm yr^{-1} for comparison. Anomalies 24 to 20 are easily recognized and the decrease in spreading rate can be seen across anomaly 24 and in Fig. 4. Cruise identifications as for Fig. 3.

motion of Iberia. If this suggestion is correct, then one of the Biscay anomalies is anomaly J. It seems likely that the Bay of Biscay was formed by the rotation of Spain in the Upper Jurassic or Lower Cretaceous.

By the time of anomaly 32, Biscay had ceased to be active and the spreading ridge extended north-westward to the north of Biscay (Fig. 7). The motion at the northern end was probably taken up by the Gibbs Fracture Zone. By the time of anomaly 31 the ridge extended beyond the fracture zone into the Labrador Sea[15]. The northern part of anomaly 32 is sub-parallel to the continental shelf edge and crosses the mouth of Porcupine seabight. This feature was therefore probably formed before anomaly 32 during the early phases of separation of Europe and North America. The seabight, Rockall Trough and Hatton–Rockall basin are likely to have been formed by the ridge attempting to extend by breaking through the plate to the north. This does not necessarily imply that these are floored by oceanic crust, however, as they may have been produced solely by extension of continental crust.

Anomaly 32 is less easy to identify than the negative anomaly

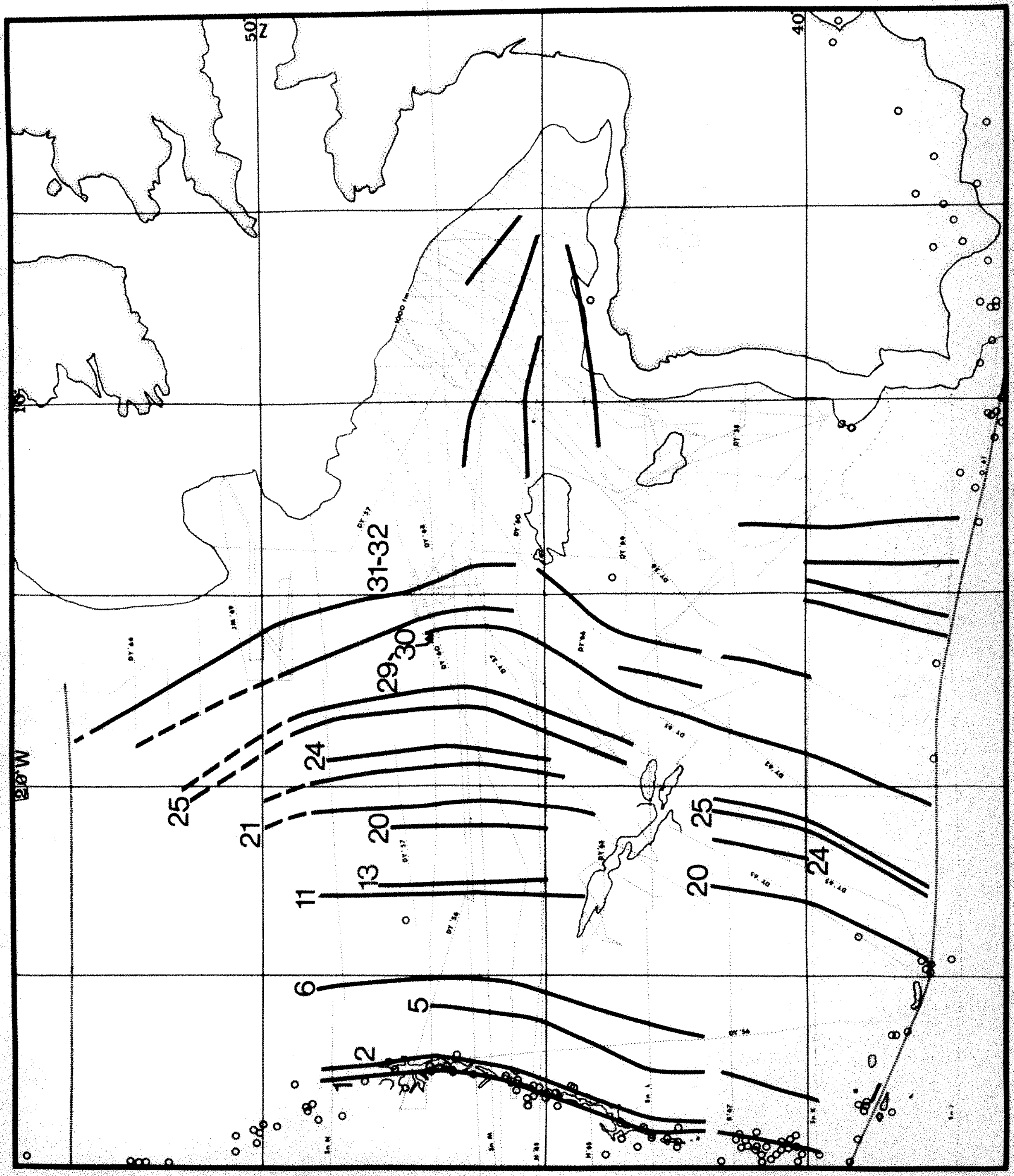


Fig. 6 Magnetic lineations. Only the most prominent ones are shown; the ships' tracks are dotted and indicate the control on the lineations. Cruise identifications as for Fig. 3.

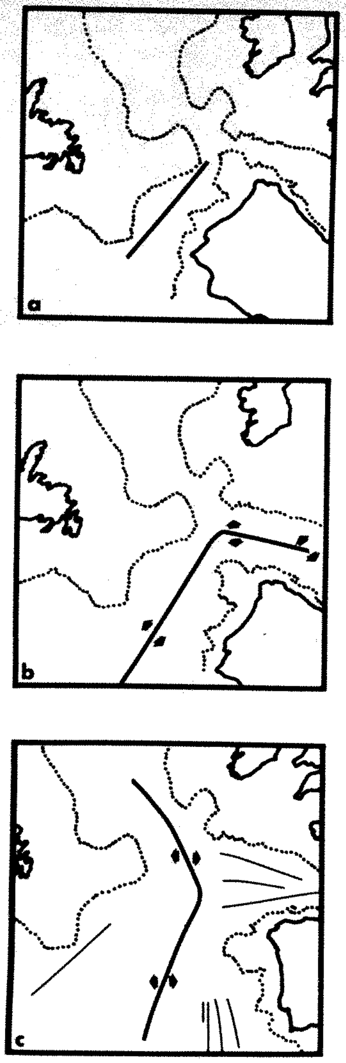


Fig. 7 Diagrammatic reconstruction of early opening of Biscay and the North Atlantic. The thick line represents the Median valley. *a*, Early Mesozoic extension between Iberia and Newfoundland; *b*, extension of the spreading centre into Biscay; *c*, extension north of Biscay about 80 m.y. BP (anomaly 32).

between 31 and 32 (Figs. 4 and 6), which forms a striking feature on most profiles. The 31–32 lineations have a north-easterly trend south of Biscay, which changes off the mouth of Biscay and becomes north-westerly. The adjacent younger lineations also show this changing trend. Similar anomalies have been found in the North-East Pacific, where the lineations forming the Great Magnetic Bight have two straight segments meeting at an angle. Unlike the North Pacific anomalies, however, the eastern north Atlantic lineations young in the concave direction. They are therefore difficult to explain by the evolution of a triple junction similar to the Great Magnetic Bight. Moreover, Pitman *et al.*[1] have identified similar shaped lineations on the opposite side of the Atlantic. If the bend in the lineations on both sides of the ridge is fitted together, the motion required to do so is essentially parallel to the Gibbs fracture zone, and therefore the ridge must have been spreading obliquely to the lineations at this time.

This curious geometry is not easily explained, because if a ridge spreads symmetrically it cannot change its shape. Two possible mechanisms which would account for the observations are that spreading was asymmetric or that the ridge jumped, forming several small offsets. These two possibilities and their consequence are illustrated in Fig. 8. The present density of ship tracks is not sufficient to choose between the two mechanisms, and if there are indeed small fracture zones present in this area the strike of the lineations in Fig. 6 will not be correct. It should be straightforward to do this by a detailed survey, especially if satellite navigation is available. Anomalies west of 31 are presumably 29 to 27, but cannot be numbered with any certainty. Anomalies 25 and 26 are, however, easily recognized on most tracks. Though these lineations are sub-parallel to anomaly 31, careful measurement of their strikes shows that the ridge has become straighter with time. This process continued until anomaly 24, and after anomaly 23 the lineations are straighter on both sides of the Atlantic. These events are closely related in time to the extension of the ridge to the north-east to form a triple junction south of Greenland[16] at anomaly 24 time. There is also a slight change in trend of the lineations in Fig. 6 at anomaly 18 time, which is the time at which the spreading ceased in the Labrador Sea.

The approximate spreading half-rates were 1.1 cm yr^{-1} at anomaly 32 time, between anomalies 26 to 24 spreading increased to 2.5 cm yr^{-1} and slowed to 1 cm yr^{-1} at anomaly 23. Spreading continued at 1 cm yr^{-1} until anomaly 6 when it increased to 1.3 cm yr^{-1}. At anomaly 5 it increased again to the present rate of 1.5 cm yr^{-1}.

Bacon and Gray[17] have suggested that Spain moved eastward relative to Europe after the Bay of Biscay opened. The magnetic anomalies in Fig. 6 do not permit a large strike-slip motion after anomaly 32 was formed, and therefore any such motion must have occurred before the Upper Cretaceous. The most likely explanation for the sedimentary trough north of Spain is that it was produced by a small amount of crustal consumption along this boundary, perhaps during the Eocene when the Pyrenees were folded.

Fig. 6 also shows that there is no major horizontal displacement across Kings Trough and Peake and Freen Deeps. Therefore these features are not the relics of a large transform fault. They cut anomalies from 24 to the first part of 6 and may well be connected with the elimination of the bend in the ridge axis at anomaly 24 time and subsequent small changes in ridge orientation which took place during the period of anomalies 24 to 6.

This study of the magnetic lineations shows that the formation of the North-East Atlantic began with the separation of Spain from North America and Europe in probably the Upper Jurassic and early Cretaceous. The ridge extended northwards as far as the Gibbs fracture zone at 53° N by the Upper Cretaceous and beyond this into the Labrador Sea by anomaly 31 time. Most of the complications of the European continental margin and surrounding seafloor appear to be the result of this intermittent northward extension of the mid-Atlantic Ridge into Biscay, possibly beginning to extend into Porcupine

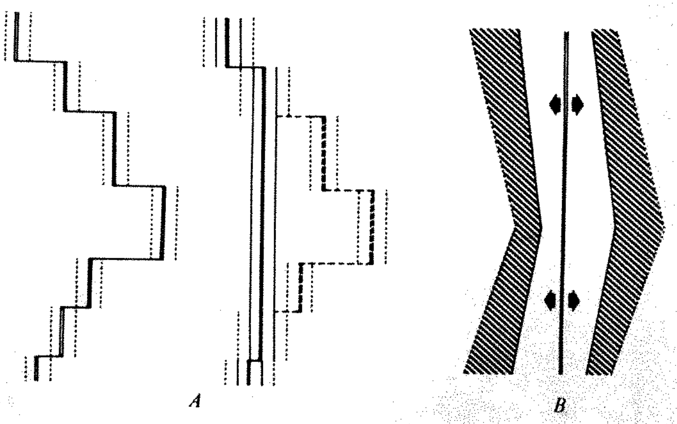


Fig. 8 Two possible mechanisms to account for the shape of the observed lineations. *A*, The ridge straightens by reducing the number of offsets. *B*, Spreading is asymmetric across certain segments of the ridge. This has not been conclusively shown to occur.

seabight, Rockall Trough and Hatton–Rockall basin. Sometimes these extensions were long term and sometimes very temporary. This behaviour is strikingly similar to that of the ridge in the Indian Ocean[8].

We thank Mrs Annette Fahlman and Mrs Alette Lawson who digitized these data and ran all the programs. We also thank the late Dr M. N. Hill and Dr D. H. Matthews under whose guidance the data have been collected and the officers, crew and scientists on board the ships who took part in the data collection. This work was sponsored by the Natural Environment Research Council.

Received June 18, 1971.

[1] Pitman, W. C., Talwani, M., and Heirtzler, J. R., *Earth Planet. Sci. Lett.*, **11**, 195 (1971).

[2] Matthews, D. H., Laughton, A. S., Pugh, D. T., Jones, E. W. J., Sunderland, J., Takin, M., and Bacon, M., *Geophys. J. Roy. Astron. Soc.*, **18**, 517 (1969).

[3] Scrutton, R. A., Stacey, A. P., and Gray, F., *Earth Planet. Sci. Lett.*, **11**, 140 (1971).

[4] Scrutton, R. A., and Roberts, D. G., in *Geology of the Eastern Atlantic Continental Margin* (edit. by Delaney, F. M.), *Inst. Geol. Sci. Rep.*, 70/14, part II (ICSU/SCOR Symp. Cambridge, 1970).

[5] Bacon, M., Gray, F., and Matthews, D. H., *Earth Planet. Sci. Lett.*, **6**, 377 (1969).

[6] Netherlands Hydrographer, Navado III. Bathymetric, magnetic and gravity investigations. H. Neth, M. S. Snelling (1964–1965) (1967).

[7] Cain, J. C., Hendricks, S., Daniels, W. S., and Jensen, J. C., *Computation of the Main Geomagnetic Field from Spherical Harmonic Expansions* (Data Users' Note NSSDC 68–11, Greenbelt, Maryland).

[8] McKenzie, D. P., and Sclater, J. G., *Geophys. J. Roy. Astron. Soc.* (in the press).

[9] Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).

[10] Pitman, W. C., and Heirtzler, J. R., *Science*, **154**, 1164 (1966).

[11] Talwani, M., Pitman, W. C., and Heirtzler, J. R., *Trans. Amer. Geophys. Union*, **50**, 189 (1969).

[12] Helsley, C. E., and Steiner, M. B., *Earth Planet. Sci. Lett.*, **5**, 325 (1969).

[13] Matthews, D. H., and Williams, C. A., *Earth Planet. Sci. Lett.*, **4**, 315 (1968).

[14] Williams, C. A., *Magnetic Survey West of Biscay*. Symp. sur l'histoire structurale du Golfe de Gascogne, Paris (in the press).

[15] Le Pichon, X., Hyndman, R., and Pautot, G., *J. Geophys. Res.* (in the press).

[16] Laughton, A. S., *Nature* (in the press).

[17] Bacon, M., and Gray, F., *Earth Planet. Sci. Lett.*, **10**, 101 (1970).

Establishment of Symbiosis between *Rhizobium* and Plant Cells *in vitro*

R. D. HOLSTEN, R. C. BURNS, R. W. F. HARDY & R. R. HEBERT

Central Research Department, E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898

A symbiotic nitrogen-fixing relationship has been established in culture between soybean cells and the bacterium *Rhizobium*.

SYMBIOTIC fixation of atmospheric nitrogen by leguminous plants is perhaps the most quantitatively important agency by which this element is incorporated into the biosphere, and is the principal process for maintaining nitrogen fertility in agriculture. The interaction of plants of the family Leguminoseae and specific bacteria of the genus *Rhizobium* in this symbiotic relationship is not yet well understood, probably because of the absence of a suitable defined experimental system. Other workers have used isolated plant roots in combination with the appropriate bacteria[1–4], and, more recently, plant cell and tissue culture[5]. Although stimulation of plant cell differentiation and lignification apparently resulted, no evidence for intercellular bacterial symbiosis could be found.

We have attempted to establish a symbiotic N_2-fixing relationship with plant cells and microorganisms *in vitro*, using the techniques of aseptic plant cell culture in conjunction with the sensitive acetylene–ethylene assay for nitrogenase activity. We consider that such a system can be used further to elucidate the factors which control nodulation processes and N_2-fixing activity *in vivo*.

Soybean Root Cell Cultures

Seeds of soybean (*Glycine max* var. Acme) were surface sterilized by immersion in a commercial hypochlorite solution ('Zonite', Chemway Corp., Wayne, New Jersey) for 15 min, and rinsed with sterile distilled water. Seed was placed on the surface of an agar (1% w/v) solidified medium composed of the mineral salts (MS medium)[6] supplemented with sucrose, 30 g/l., for germination in the dark at 26° ± 1° C. All media were sterilized by autoclaving at 121° C for 15 min.

When the primary root was approximately 4 cm long, it was excised aseptically, and a 5 mm explant was removed 2.5 cm behind the growing tip. This explant of root origin was placed on the surface of 50 ml. of agar-solidified MS medium supplemented with 15% whole coconut milk (CM, Grand Island Biological Co.) and 2 mg/l. 2,4-dichlorophenoxyacetic acid (2,4-D) contained in a cotton stoppered 250 ml. Erlenmeyer flask. The medium was adjusted to *p*H 6.0 before autoclaving. The explanted root section was incubated at 26 ± 1° C in the dark.

An actively growing mass of undifferentiated cells (callus) was produced on the root explant in 10–14 days. The callus was then aseptically removed and portions subcultured to fresh MS medium supplemented with 15% CM and 2 mg/l. 2,4-D. In these and subsequent subcultures, we used a liquid medium similar to that described, with 225 ml. of medium in a modified 1,000 ml. flask (Blaessig Glass Co., Rochester, New York)[7], as well as 50 ml. agar-solidified medium in 250 ml. Erlenmeyer flasks. The liquid cultures were incubated on a klinostat rotating at 1 r.p.m.[8] in the dark at 26 ± 1° C. An actively

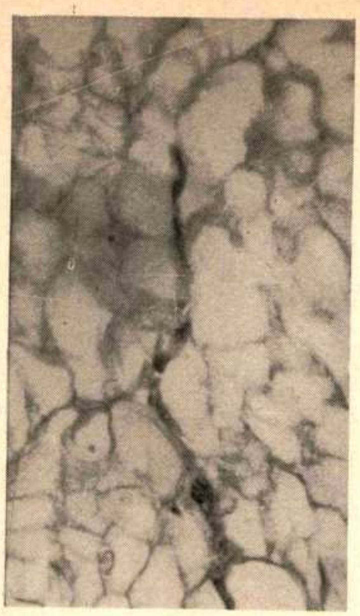


Fig. 1 Paraffin section of a callus culture showing *Rhizobium japonicum* penetration through an infection thread-like structure (× 600).

growing cell and callus population with a cell doubling time of the order of 18 h was established in the liquid environment over a 14–21 day period. Subculture of the liquid system was carried out at 20–30 day intervals to maintain an actively dividing cell population. Cotyledonary tissue was similarly treated to establish cell cultures from this source.

Bacterial Growth

Rhizobium japonicum strains 61A76 (supplied by Dr Joe C. Burton of the Nitragin Co., Milwaukee, Wisconsin) and ATCC 10324 were grown in a liquid culture medium composed of (in g/l.): K_2HPO_4, 1.0; KH_2PO_4, 1.0; $FeCl_3 \cdot 6H_2O$, 0.005; $MgSO_4 \cdot 7H_2O$, 0.36; $CaSO_4 \cdot 2H_2O$, 0.17; KNO_3, 0.70; yeast extract, 1.0; mannitol, 3.0. The medium was adjusted to *p*H 6.4 before autoclaving at 121° C for 15 min. A bacterial inoculum was added to 40 ml. portions of the above medium contained in 125 ml. Erlenmeyer flasks and incubated at $30 \pm 1°$ C with constant shaking. Subcultures of the bacterial cultures were carried out at 7 day intervals to maintain vigorous stocks. At regular intervals aliquots of the *R. japonicum* cultures were examined microscopically and checked for nodulating activity by application to seedlings of Acme or Kent variety soybeans maintained in a Sherer–Gilette CEL 255–6 growth chamber under a 16 h photoperiod.

Establishment of a Symbiotic Relationship

A large culture flask from the klinostat containing 225 ml. of MS medium with 15% CM, 2 mg/l. 2,4-D and containing an actively growing root cell and callus population of approximately 15 g fresh weight was inoculated with 0.1 ml. (260×10^6 cells/ml.) of a liquid suspension culture of actively dividing *R. japonicum* and returned to the klinostat for incubation. After 3–7 days of incubation, the flask contents were poured through sterile cheesecloth. Plant cells and callus pieces retained on the cheesecloth (designated stage 1) were washed with 250 ml. of sterile MS medium, transferred to fresh growth media of varying composition, and incubated in the conditions described. Concomitantly, portions of the cell population inoculated with bacteria, as well as control tissue without bacteria, were assayed for nitrogenase activity using the acetylene–ethylene assay procedure[9]. Dry weight determinations were performed after drying the tissue for 48 h at 60° C.

Cytological Examination of Tissue

Random samples of the soybean root cell cultures from bacteria-inoculated and control flasks were prepared for paraffin sectioning by fixing in formalin : acetic acid : ethanol (5 : 5 : 90 v/v) or in Fleming's osmic acid fixative for 24 h at room temperature. The tissues were then dehydrated through the ethanol series before being embedded in 'Paraplast' (Mh 56°–57° C) for sectioning. Sections were cut at 8–10 µm, stained with safranin-fast green or Van Gieson's Nile blue and examined with a Zeiss microscope fitted with phase contrast optics.

At the same time similar cell samples were overlaid with 5% glutaraldehyde in Millonig's phosphate buffer (*p*H 7.2–7.4) and fixed for 60 min at room temperature. The fixed samples were rinsed for 20 min, post fixed for 60 min at room temperature with 1% OsO_4, washed with phosphate buffer for 20 min, and dehydrated through an ethanol series up to 100% propylene oxide. The samples were then embedded in 'Epon 812', polymerized at 60° C for 24 h, and sectioned at 500 Å using a diamond knife. The resulting sections were stained with uranyl acetate followed by Karnowsky's lead and examined in an RCA EMU-3G electron microscope.

Invasion of Cultured Root Cells

Examination of paraffin sections prepared at the time the bacteria-inoculated liquid plant cell cultures were transferred to fresh growth media (stage 1, day 3–7) revealed the presence of structures resembling the infection threads found in *in vivo* nodulating soybean root systems. These "pseudo-infection threads" of the *in vitro* system were observed to penetrate the undifferentiated cell mass for a considerable distance before entering the cell cytoplasm (Fig. 1). *Rhizobia* were observed within the thread-like structures as they traversed the intercellular spaces within the cell mass. Whenever *Rhizobia* entered a plant cell from the terminus of a "pseudo-infection thread", they multiplied to displace the plant cell contents completely with bacteria (Fig. 2). No other morphologically differentiated cells (for example conducting elements) were found in any of the

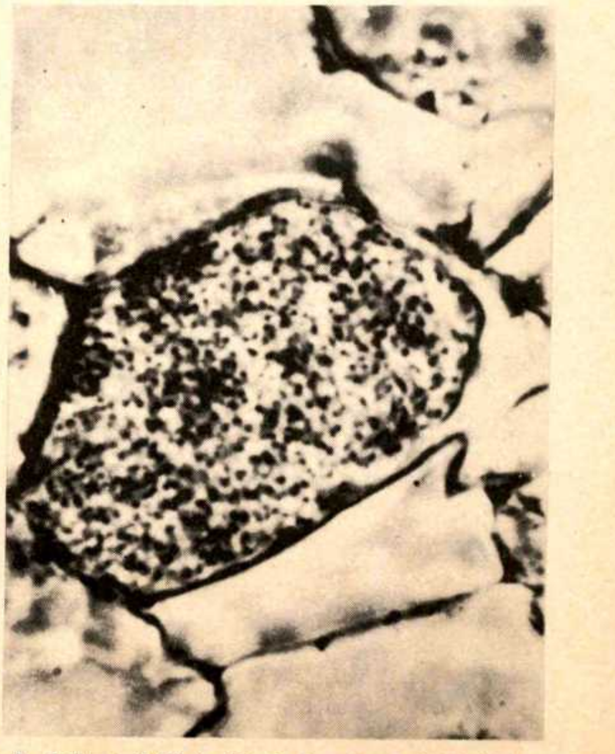


Fig. 2 Callus cell filled with *R. japonicum* after initial penetration.

bacterially populated plant cell masses at this developmental stage.

Continued Development of the Symbiotic System

Following the initial penetration of the plant cells by *Rhizobia* (3–7 days after bacterial inoculation of the cell culture flask), the cell cultures were transferred to fresh media, both liquid and agar-solidified, for development of stage 2. The following media were utilized: (1) MS, (2) MS plus 10–15% whole CM and (3) MS plus 10–15% whole CM and 2 mg/l. 2,4-D. Control cultures of soybean root cells (without bacteria) were transferred to similar media and incubated in the same conditions. All cultures were incubated for 7–20 days after transfer before being examined for bacterial penetration and for nitrogenase activity. Light-grown cultures received approximately 500 foot-candles of light from Duro-Test Optima lamps (Duro-Test Corp., North Bergen, New Jersey).

Fig. 3 shows that the degree of rhizobial invasion after transfer of the cultured soybean root cells is related to the growth factor additions to the MS medium. Most extensive invasion is found after transfer of bacterially populated plant cells to MS medium containing no exogenous growth supplements (Fig. 3*a*). With the addition of whole CM (Fig. 3*b*) and the combination of CM and 2,4-D (Fig. 3*c*) the number of cells inhabited by the *Rhizobia* is markedly reduced. Assays for nitrogenase activity under each of the tested culture conditions (Tables 1 and 2) show this activity to vary directly with the degree of bacterial invasion (Table 1). The tissue without rhizobial involvement shows little acetylene–ethylene reducing activity (Table 1) in these assays[11,12]. Table 2 shows that although there is wide variation in the response of individual Acme soybean root cell cultures to *R. japonicum* strain 61A76, there is a definite symbiotic relationship capable of fixing N_2 established in liquid systems, as measured by the acetylene–ethylene technique. Attempts to establish an active symbiotic relationship using Kent variety soybean root cells in liquid culture have been less successful. Even with Acme variety soybeans, the use of a different strain of *R. japonicum* (ATCC 10324) resulted in a lower over-all nitrogenase activity although electron microscopy showed bacterial penetration of the cultured cells. Cotyledonary tissue has shown no propensity to establish a symbiotic association in the conditions of this study.

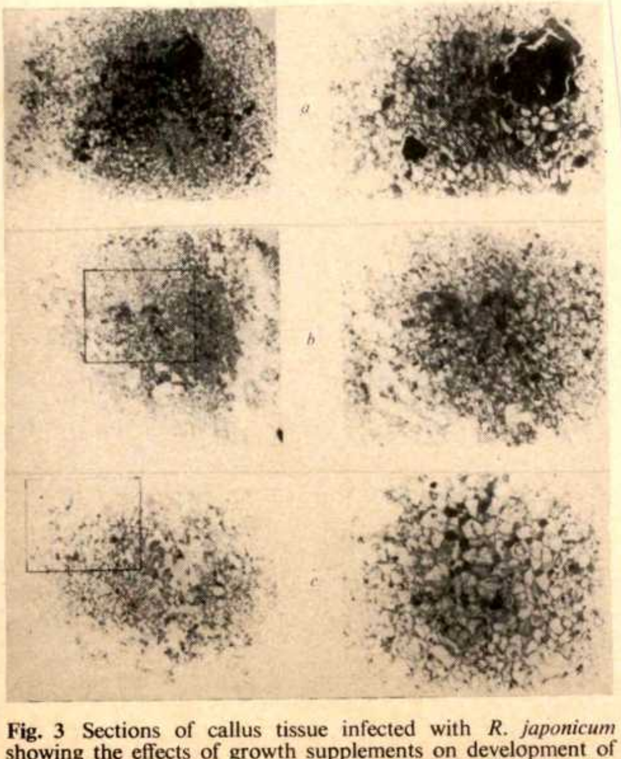


Fig. 3 Sections of callus tissue infected with *R. japonicum* showing the effects of growth supplements on development of the symbiotic association. See text for details of treatments. Left, × 350; right, × 700.

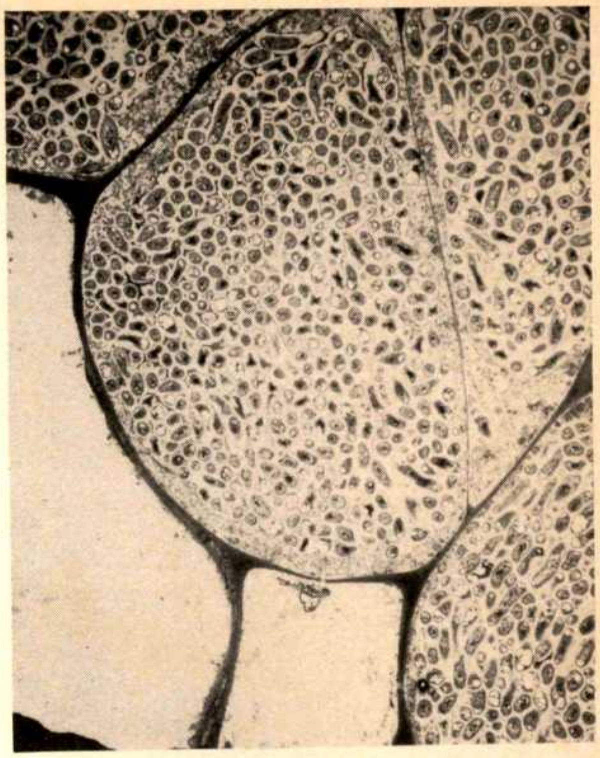


Fig. 4 Electron micrograph of cultured soybean callus infected with *R. japonicum* (× 4,160).

Table 1 Acetylene to Ethylene Reduction by Soybean Root Cell Cultures inoculated with *Rhizobium japonicum* Strain 61A76

	nmol C_2H_4/g fresh weight × 24 h	
	Light	Dark
MS	72.8	318.6
MS+10% CM	36.6	129.0
MS+10% CM+2 p.p.m. 2,4-D	7.7	37.9
Uninoculated control MS+10% CM+2 p.p.m. 2,4-D	0.4	2.1

The assay was carried out 21 days after initial bacterial inoculation and after transfer to semi-solid agar medium for 14 days. Incubation was under continuous light or dark conditions following initial establishment of symbiosis.

Table 2 Acetylene to Ethylene Reduction by Two Separate Soybean Root Cell Cultures containing *R. japonicum* Strain 61A76 in Symbiotic Relationship

	nmol C_2H_4/g dry weight × 24 h	
	1	2
Tissue +61A76 bacteria	638	88.0
Uninoculated control	307	25.7

Assays were made 3–5 days after addition of the microorganisms to liquid culture.

Electron Microscopic Examination of Symbiotic Cultures

Examination of *Rhizobium*-plant cell cultures with the electron microscope confirmed that *Rhizobia* have entered the cultured plant cells and displaced the normal cytoplasmic components (Fig. 4). In some cases the *Rhizobia* were enclosed within a vesicle which is morphologically analogous to the vesicles found in nodules obtained from whole soybean root systems (Fig. 5). It was further observed that the *Rhizobia* within the cultured root cells contained an inclusion which was similar in appearance to an inclusion of whole soybean nodule bacterioids designated as a polymer of β-hydroxybutyrate. This inclusion became more prominent with increasing time of incubation of the synthetic symbiotic system (up to 15–20 days after transfer). Microscopic examinations of uninoculated tissue showed that there were no microorganismal contaminants present. The media used for maintenance of the plant cell cultures were also found to be devoid of microorganisms and N_2-fixing activity; the bacterial inoculum contained only *Rhizobia* and exhibited no N_2-fixing activity. Thus it is clear that free-living N_2-fixing bacteria are not responsible for our observations.

Functionality of the Symbiotic Relationship

The functional system we have described provides a new tool for the elucidation of the mechanisms controlling nodulation and subsequent N_2-fixation in symbiotic associations.

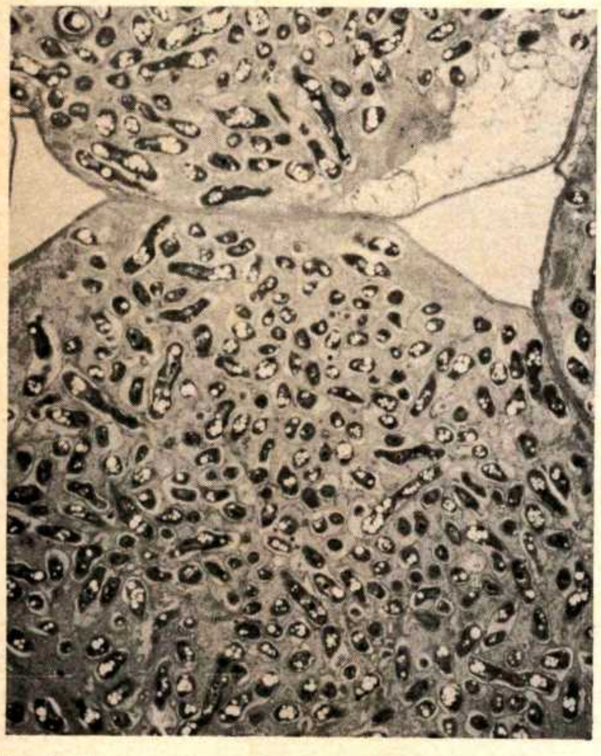


Fig. 5 Electron micrograph of a soybean root nodule infected with *R. japonicum* showing accumulation of β-hydroxybutyrate (×4,160).

The morphology of symbiosis *in vitro* is analogous to that of intact soybean root nodules[13,14]. *Rhizobia* within the cytoplasm of the cultured cells, as in root nodules, are at times enclosed within a vesicle. The intracellular bacteria (bacterioids?) are shown to contain an inclusion which has been tentatively identified as poly-β-hydroxybutyrate[15]. A structure similar in appearance to the infection thread of nodulating systems *in vivo* has been observed in the early stages of bacterial penetration of the cultured plant cells in the liquid milieu. This apparent mode of rhizobial entry into the cultured cells and the subsequent multiplication of the intracellular microorganisms to displace completely the cell cytoplasm are again analogous to processes which are known to occur *in vivo*.

It has further been found that following initial bacterial penetration of the cultured cells, depletion of exogenous supplies of plant growth factors (cytokinins and auxins) in the environment leads to increased cellular penetration by the microorganisms and greater nitrogenase induction. This suggests that the symbiosis, once initiated, is self-sufficient and that any exogenous additions tend to suppress its normal development and function. This agrees with the proposed roles of plant growth regulators in the establishment and maintenance of nodulated symbiotic systems *in vivo*[16,17]. Thus it may be possible to control the process of effective nodulation with factors which alter the levels of endogenous plant growth regulators such as auxins during the critical phases of invasion.

The reduction of acetylene to ethylene as a measure of nitrogenase activity shows that the N_2-fixing potential of the symbiosis increases with the proportion of cellular invasion by microorganisms, and the addition of plant growth factors depresses nitrogenase activity as well as the development of the symbiotic morphology. It should therefore be possible to increase the overall N_2-fixing activity of the system by regulating the degree of cell penetration and subsequent nitrogenase induction through chemical means.

Light does not seem to stimulate the symbiosis *in vitro* once it has been established; but cells grown in the light resisted rhizobial challenge, and no working symbiosis could be established in these cultures. It is possible that cells grown in the light develop a natural defence mechanism against invasion by microorganisms *in vitro*, perhaps through the phytochrome system[18].

The acetylene to ethylene reducing activities (and nitrogenase activity by analogy) achieved so far in the *in vitro* symbiotic system are of the order of 1% of those found in soybean nodules. Microscopic examinations of the symbiotic cell masses in culture showed that only 1–10% of the total cell population was infected with intracellular microorganisms. The activity per cell may therefore be comparable with that found in whole root nodules where more than 90% of the cells appear to contain bacteria. It is also pertinent (Fig. 3) that infection occurs only in the subsurface cells of the callus where physiological conditions might be expected to simulate more closely the cortex of intact roots. The initial infection of the tissue culture occurs through a modification of a peripheral cell of the callus mass and not through a root hair cell as in intact roots. This suggests that symbiosis *in vivo* might also be expected to occur through penetration of epidermal cells other than root hairs in suitable conditions.

We thank Mrs Mary Ann Mattson and Mr M. L. Van Kavelaar for technical assistance.

Received January 25, 1971.

[1] Lewis, K. N., and McCoy, E., *Bot. Gaz.*, **95**, 316 (1933).
[2] McGonagle, M. P., *Nature*, **153**, 528 (1944).
[3] Raggio, N., Raggio, M., and Burris, R. H., *Biochim. Biophys. Acta*, **32**, 274 (1959).
[4] Raggio, M., Raggio, N., and Torrey, J. G., *Amer. J. Bot.*, **44**, 325 (1957).
[5] Veliky, I., and LaRue, T. A., *Naturwissenschaften*, **54**, 96 (1967).
[6] Murashige, T., and Skoog, F., *Physiol. Plant.*, **15**, 473 (1962).
[7] Steward, F. C., and Shantz, E. M., *Chemistry and Mode of Action of Growth Substances* (edit. by Wain, R. L., and Wightman, F.), 312 (Academic Press, New York, 1956).
[8] Caplin, S. M., and Steward, F. C., *Nature*, **163**, 920 (1949).
[9] Hardy, R. W. F., Holsten, R. D., Jackson, E. K., and Burns, R. C., *Plant Physiol.*, **43**, 1185 (1968).
[10] Millonig, G., *J. Appl. Phys.*, **32**, 1637 (1961).
[11] Gamborg, O. L., and LaRue, T. A. G., *Nature*, **200**, 604 (1968).
[12] Stewart, E. P., and Freebairn, H. T., *Plant Physiol.*, **42**, Suppl., S-30 (1967).
[13] Goodchild, D. J., and Bergersen, F. J., *J. Bact.*, **92**, 204 (1966).
[14] Holsten, R. D., Hebert, R. R., Jackson, E. K., Burns, R. C., and Hardy, R. W. F., *Bact. Proc.*, 149 (1969).
[15] Klucas, R. V., and Evans, H. J., *Plant Physiol.*, **43**, 1458 (1968).
[16] Kefford, N. P., Brockwell, J., and Zwar J. A., *Austral. J. Biol. Sci.*, **13**, 456 (1960).
[17] Thimann, K., *Proc. US Nat. Acad. Sci.*, **22**, 511 (1936).
[18] Lie, T. A., *Plant and Soil*, **30**, 391 (1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Search for Isolated Radio Pulses from the Galactic Centre at 151.5 MHz

WE have extended our previous work[1] and searched specifically for pulses from the galactic centre (GC), which might be correlated with the events reported by Weber[2,3]. To discriminate against local interference we looked for pulses received simultaneously by widely spaced receivers; although our results are negative, they allow upper limits to be placed on the radio energy emitted in pulses from the GC.

Table 1 Galactic Centre Data

	Two-fold		Three-fold		Four-fold		Five-fold	
	No. of events	Hours	No. of events	Hours	No. of events	Hours	No. of events	Hours
All stations	187	636	4	368	0	209	0	78
CGH	85	512	1	117				
DGM	41	269	0	111				

Four groups have participated, with receivers at Cambridge, Dublin, Glasgow and Harwell, and a receiver in Malta (at 15.5° E, 36° N) has been operated by the Dublin group. The Malta site is 2,450 km from Cambridge, the nearest of the other sites. Each of the stations was equipped with two directional antennas connected as a phase-switched interferometer of spacing 5λ east–west. The antennas were near-vertical ladder arrays of 6 to 8 half-wave dipoles over reflecting screens, providing beams extending about 15° in elevation and directed towards the GC at transit. Our latitudes (51° to 56° N for the UK and Dublin sites) are far from ideal because the GC is visible for only about 6 h and rises to a maximum elevation of 9°. All stations operated at frequencies close to 151 MHz with bandwidths between 0.2 and 1.0 MHz. Cambridge and Glasgow had each a pair of receivers tuned to frequencies 1 MHz apart to measure dispersion and discriminate against interference. The outputs, with integration times between 0.3 and 3.5 s, were recorded on paper charts at speeds between 6 and 24 inch h^{-1}. The observing period was from May 1 to August 16, 1970, that is, when the GC was in transit during the night—and the analysis was restricted to times when the Sun was below the local horizons.

We found many more pulses satisfying the criterion that the amplitude should be at least 3 times peak to peak noise, than in the earlier observations[1]. Most of these could be attributed to increased interference arising from the high sensitivity of the antennas at low elevations, and also to the fact that the observations were carried out in the summer when atmospheric disturbances are more prevalent.

As before, the records of the individual sites were scanned for pulses which arrived in "coincidence" (that is, within any 2 min period) at two or more of the five sites. Some two-fold and three-fold coincidences were found, but there were no four-fold or five-fold coincidences. The number of coincident events is shown in Table 1 together with the length of time for which such events were possible. The data in Table 1 are restricted to times when the galactic centre was in the beam of the aerials. Data, however, were taken all through the night at some stations, and an analysis of these is shown in Table 2, together with the results of a "random" test in which the times of events were shifted by an arbitrary time relative to each other. From this test we conclude that the real two-fold rates are not significantly greater than the random expectation and we set a three-fold coincident rate, that is the difference between the genuine and the background rate, as 1 in approximately 200 h.

Table 2 Random Test, All Data

	Two-fold			Three-fold		
	Observed events	'Expected' events	Hours	Observed events	'Expected' events	Hours
All stations	242	270	1,114	6	3	586

We studied all the three-fold events in detail and Table 3 shows the date, time and type of each of the six events. All the three-fold events, except for that of July 28, 1970, were made up of pulses observed at three stations while at least one other station had an interference-free chart without any observable pulse. Also three of the events appeared only on one of the two frequency channels at Glasgow and/or Cambridge. The event of July 28 is exceptional because although it appears on both the frequency channels at Cambridge and Glasgow, it is not possible to draw any conclusions from the Dublin or Malta charts. An upper limit of 830 cm^{-3} pc can be placed on the dispersion measure of the source for this event. We conclude therefore that not only were no four-fold coincident radio pulses observed from the galactic centre in 209 h of observation but also that, with the possible exception of the July 28 event, no three-fold events of astronomical origin were observed in 368 h of observation. Weber has divided his events into four classes[4], roughly dependent on the energy of the event and the characteristic noise near the time of the event (see Table in ref. 4). If this classification is taken as some measure of the gravitational energy in the events, our upper limits on the rate of pulses suggest that there are no radio pulses associated with the two lower energy classes of event, which comprise more than 80% of the total. A consideration

Table 3 The Three-fold Events

Date (1970)	Time (UT) (h)	(m)	Class*	Comments
June 11	03	06	CDM$\overline{\mathrm{H}}$	Possible interference on D
July 2	20	40	DGH$\overline{\mathrm{M}}$	Galactic centre below horizon
July 11	02	14	DGM$\overline{\mathrm{CH}}$	G pulse on one channel only
July 23	21	29	GHM$\overline{\mathrm{C}}$	G pulse on one channel only
July 23	23	04	CGH$\overline{\mathrm{M}}$	C and G pulses on one channel only
July 28	20	09	CGH	Both channels on C and G†

* A bar indicates cases in which the traces were clean but there was no evidence for a pulse.

† This event had exceptionally large pulses which were recorded at the three stations at the same times within the limits of error, about 10 s. The mean time, 20 h 09 m 25 s UT, was very close to that of sunset. It was also within a few hours of the outburst[8–11] of Nova Scuti (1970) which happened to be in a sidelobe of our antennas at 20 h 09 m. A further occurrence which could conceivably be related was a transient disturbance in the ionosphere at 19 h 58 m 02 s on the same day, only 11 min earlier. This appeared on a record made at Glasgow of phase fluctuations in a 50 kHz standard frequency transmission from Czechoslovakia. A sudden phase advance of 1 μs in the received signal was followed by a rapid return to the normal phase within about 2 s. The fast recovery makes this disturbance very unusual; no others like it were found throughout the period of the GC observations.

of the sensitivity of our equipment places an upper limit to the radio energy in our bandwidth of 2.5×10^{-17} J m^{-2} per pulse or, on the assumption that the source has a spectrum similar to that of other non-thermal sources, of about 2.5×10^{-14} J m^{-2} per pulse in the radio band. This may be compared with Weber's[2] estimated 10 J m^{-2} per pulse of gravitational energy in a bandwidth of 0.017 Hz.

Several factors may complicate such a comparison. (i) The plasma surrounding the source may have a plasma frequency greater than 151.1 MHz, so that the source is completely screened, or the emitted radio spectrum may be quite different from that of "normal" sources. (ii) The time scale of the radio emission may be extended by comparison with that of the gravitational pulses, perhaps because of the emission mechanism or a large dispersion measure. For pulse lengths $T>1$ s our limiting flux density will be increased by a factor T. The time scale for radio emission from a collapsing object, for example, is likely to be quite different from that of the gravitational pulses. The first of these must originate in the outer regions of the object whereas the second will be associated with the core regions. The recently proposed LeBlanc–Wilson model[5] of stellar collapse, however, implies a time scale of the order of a few tenths of a second for the emission of most of the radiated energy; (iii) Weber's source or sources may not have been in our beams during the period of observations, because the beam width of his antennas is greater than ours.

Because of these possibilities we emphasize that our findings do not invalidate Weber's results, but simply show that there are no radio pulses from the galactic centre at rates comparable with his rate of detection of gravitational events. When the actual times of gravitational events are published, a detailed comparison with radio observations will be possible.

Partridge[6] has described an unsuccessful search for microwave pulses associated with Weber's events, and Bahcall and Davis[7] have set an upper limit on the neutrino energy emitted.

We thank Mr W. McKellar and Dr J. White for the construction and maintenance of the antennas at AERE and UCD respectively, Mr A. McKellar for construction of much of the equipment at Glasgow, and Miss R. Kavanagh for the analysis of charts at UCD and for handling the computer program. We also thank Lady Weipers, Supervisor of Cochno Veterinary Field Station, Glasgow University, for providing an observing site and facilities. R. F. H. acknowledges financial assistance from a CSIRO Fellowship.

W. N. CHARMAN
J. H. FRUIN
J. V. JELLEY

Nuclear Physics Division,
Atomic Energy Research Establishment,
Harwell,
Berkshire

R. F. HAYNES
E. R. HODGSON
P. F. SCOTT
J. R. SHAKESHAFT

Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
University of Cambridge

G. A. BAIRD
T. J. DELANEY
B. G. LAWLESS

Physics Department,
University College,
Dublin

R. W. P. DREVER
W. P. S. MEIKLE

Department of Natural Philosophy,
University of Glasgow

Received May 18, 1971.

[1] Charman, W. N., Jelley, J. V., Fruin, J. H., Hodgson, E. R., Scott, P. F., Shakeshaft, J. R., Baird, G. A., Delaney, T., Lawless, B. G., Drever, R. W. P., Meikle, W. P. S., Porter, R. A., and Spencer, R. E., *Nature*, **228**, 346 (1970).
[2] Weber, J., *Phys. Rev. Lett.*, **22**, 1320 (1969).
[3] Weber, J., *Phys. Rev. Lett.*, **25**, 180 (1970).
[4] Weber, J., *Phys. Rev. Lett.*, **24**, 276 (1970).
[5] LeBlanc, J. M., and Wilson, J. R., *Astrophys. J.*, **161**, 541 (1970).
[6] Partridge, R. B., *Phys. Rev. Lett.*, **26**, 912 (1971).
[7] Bahcall, J. N., and Davis, R., *Phys. Rev. Lett.*, **26**, 662 (1971).
[8] Alcock, G. E. D., *Intern. Astron. Union Circ.* No. 2269 (1970).
[9] Locher, K., *Intern. Astron. Union Circ.* No. 2270 (1970).
[10] Allen, D. A., *Intern. Astron. Union Circ.* No. 2270 (1970).
[11] Ishiguro, M., *Intern. Astron. Union Circ.* No. 2281 (1970).

Rapid Optical Variability of the Source PKS 1514–24

THERE are two cases in which radio sources have been identified with optical objects previously known as variable stars: the Seyfert galaxy 3C120 with the star BW Tau (ref. 1) and VRO 42 22 01 with BL Lac (ref. 2). As a systematic search for possible new concidences, 807 variable stars were selected from the *General Catalog of Variable Stars*[3] and its first supplement[4] using the following criteria: (i) type given as irregular or unknown; (ii) no spectral type given; and (iii) galactic latitude $|b_{\mathrm{I}}|>10°$. This list was checked for coincidences against *A Master List of Radio Sources*[5] and the fourth instalment of the Ohio survey[6]. Only two coincidences were found.

The source ON 231 lies close to the star W Com (variable from magnitude 13 to 15.5, type unknown). The position of W Com was measured on the Palomar Sky Survey, and a more accurate radio position was kindly provided by M. Davis. As Table 1 shows, the discrepancy is about two standard errors in both coordinates.

The image of W Com on the Palomar Sky Survey is starlike, but a faint nebulosity seems to extend toward the radio position. A more accurate radio position is needed to ascertain the identification.

The source PKS 1514–24 has been identified by Bolton *et al.*[7] as an E galaxy. This object had been discovered previously as

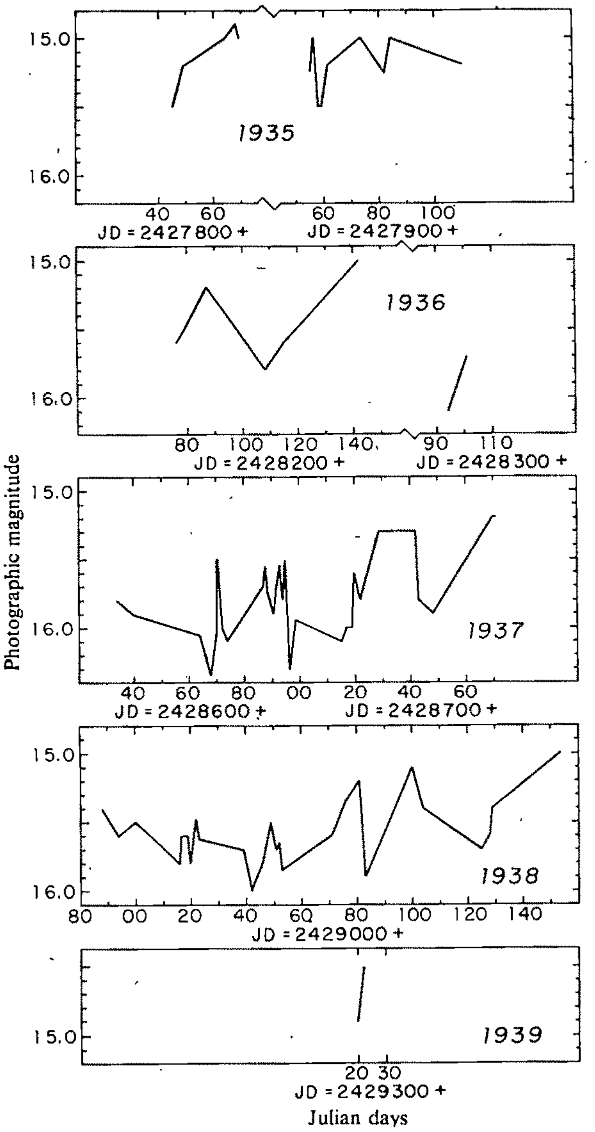


Fig. 1 Light curve of the variable star AP Lib.

the variable star AP Lib by Ashbrook[8]. The position of the star measured on the Palomar Sky Survey agrees with the first optical position given by Bolton *et al.*[7] and with the various radio positions. A second optical position given later by Bolton[9] is clearly in error.

Table 1 Positions of W Com and ON 231

	R.A. (1950.0)	Dec. (1950.0)
W Com	12 h 19 m 01.2±1 s	+28° 30′ 26″ ±10″
ON 231	12 h 18 m 58.2±2 s	+28° 29′ 24″ ±30″

The measurements by Ashbrook cover the period from February 1935 to March 1939, and the resultant light curve is shown in Fig. 1. The magnitude usually lies between 15 and 16, with limiting values of 14.5 and 16.4. There are periods of very fast and chaotic variations; variations up to 0.8 magnitude on a time scale of a few days are common. For some nights, several plates a few hours apart are available and yield variations of 0.7 magnitude in 5 hours on June 8, 1937, and 0.6 magnitude in 3 and 5 hours on May 3 and 4, 1938. In addition to these measurements, visual inspection of fifty-three patrol plates at Harvard College Observatory covering the interval from February 1939 to July 1951 shows high activity in July 1941, July 1944, and May 1945. The maximum brightness seems to reach $m_{\rm pg} \simeq 14$ in 1944.

The very fast variations of AP Lib resemble those of the peculiar object BL Lac, which are unique in that they are more rapid than those of any QSO or Seyfert galaxy. The amplitude of the variations of AP Lib is somewhat smaller[10,11] than those of BL Lac, but the observations are not as many or as closely spaced as in the case of BL Lac. VRO 42 22 01 has no measured low frequency component, but a spectrum which always increases toward high frequencies. The flux of PKS 1514–24 is constant (about 2 flux units) from 1,410 to 5,009 MHz (refs. 12 and 13). There is a strong discrepancy between the 408 MHz flux measured at Parkes (5.3 flux units, ref. 12) and at Molonglo (1.9 flux units, ref. 14). The Molonglo flux is likely to be more reliable[15], thus giving for PKS 1514–24 a flat spectrum over the observed frequency range. On the other hand, the disagreement about the 408 MHz flux could be an indication of radio variability.

Assuming that the extinction at the galactic latitude of AP Lib ($b_{\rm II} = 28°$) is 0.6 magnitude, and the mean flux density of VRO 42 22 01 is 6 flux units at 2,695 MHz, then the ratio of optical to radio (at 2,695 MHz) luminosities is about two times greater for BL Lac.

Photometric and spectrographic studies, as well as a search for radio variability, are needed to establish the properties of AP Lib and its possible similarity to BL Lac.

I thank Martha Ashbrook for making data available to me and Martha Liller for help in examining the Harvard patrol plates. NRAO is operated by Associated Universities, Inc., under contract with the National Science Foundation.

F. Biraud *

National Radio Astronomy Observatory,
Green Bank,
West Virginia

Received June 16, 1971.

---

* On leave from Département de Radioastronomie, Observatoire de Meudon, France.

[1] Penston, M. V., *Inf. Bull. Var. Stars*, 255 (1968).
[2] Schmitt, J. L., *Nature*, **218**, 663 (1968).
[3] Kukarkin, B. V., Parenago, P. O., Efremov, Y. I., and Kholopov, P. N., *General Catalog of Variable Stars* (second ed.) (Moscow, 1958).
[4] Kukarkin, B. V., Efromov, Y. I., and Kholopov, P. N., *First Supplement to the Second Edition of the General Catalog of Variable Stars* (Moscow, 1960).
[5] Dixon, R. S., *Astrophys. J. Suppl. Series*, **20** (1970).
[6] Ehman, J. R., Dixon, R. S., and Kraus, J. D., *Astron. J.*, **75**, 351 (1970).
[7] Bolton, J. G., Clarke, M. E., and Ekers, R. D., *Aust. J. Phys.*, **18**, 627 (1965).
[8] Ashbrook, M. D., *Harvard Annals*, **109**, No. 7 (1942).
[9] Bolton, J. G., *Pub. Astron. Soc. Pacific*, **80**, 5 (1968).
[10] Bertaud, C., Dumortier, P., Véron, P., Wlérick, G., Adam, G., Bigay, J., Garnier, G., and Duruy, M., *Astron. Astrophys.*, **3**, 436 (1969).
[11] Dupuy, D., Schmitt, J., McClure, R., van den Bergh, S., and Racine, R., *Astrophys. J. Lett.*, **156**, L135 (1969).
[12] The Parkes Survey of Radio Sources, *Aust. J. Phys., Astrophys. Suppl.*, 7 (1969).
[13] Shimmins, A. J., Manchester, R. N., and Harris, B. J., *Aust. J. Phys., Astrophys. Suppl.*, 8 (1969).
[14] Hoskins, D. G., and Murdoch, H. S., *Aust. J. Phys., Astrophys. Suppl.*, 15 (1970).
[15] Murdoch, H. S., and Large, M. I., *Mon. Not. Roy. Astron. Soc.*, **141**, 377 (1968).

Interplanetary Lyman–Alpha: Theory and Experiment

DISCUSSIONS of the results of the Vela 4 scattered Lyman-α experiment[1] by Bowers[2] and Blum and Fahr[3] contain errors and misinterpretation which we believe should be pointed out promptly. The use by both authors of "Vela 7" to refer to the Vela Launch 4 spacecraft also leads to confusion because the experimental data have been published as Vela 4 data.

Bowers asserts that Chambers *et al.*[1] imply that the source of the Lyman–α radiation observed is a shock front which is luminous at Lyman–α. Actually ref. 1 clearly states that the observed radiation is assumed to be solar Lyman–α, resonantly scattered from neutron hydrogen, and that the asymmetry in the observed radiation is related to asymmetry in the distribution of the scattering hydrogen. Although Blum and Fahr do not mention Lyman–α radiation in the publication[4] referred to by Bowers, their further published work[5] also attributes the observed Lyman–α to scattering of radiation originating at the Sun.

Bowers plots the region of observation of the Vela 4 experiment on his galactic line-of-sight plot (partly reproduced in Fig. 1), then indicates the location of the maximum in the observed Lyman–α radiation and notes that the maximum falls on the Milky Way. The path shown by Bowers, however, does not apply to the Vela 4 Lyman–α observations. The detectors in question were mounted on the centre column of the spacecraft and aligned to look outward along the spin axis. The satellites were Earth-oriented by maintaining the direction of the spin axis towards the centre of the Earth, and the observed region of the sky is mapped by the orbit of the satellite. The correct observed region is approximately 15° on either side of the orbit as shown in Fig. 1. Also, the conveniently localized maximum flux region shown by Bowers has no basis in fact. The broad maximum of the Vela 4 data, an example of which is shown in Fig. 2, occupies at least one third of the orbit. There is no obvious indication of crossing the Milky Way in any of the Vela data except, in some cases, a minor peak of amplitude about 15 Rayleigh near $\alpha = 105°$. The peak is consistent with ultraviolet measurements of εCMa by Stecher[6]. Further evidence of the lack of enhanced emission in the direction of the Galaxy is provided by the Mariner 6 data[7] and the OGO 5 measurements of Thomas and Krassa[8].

In attempting to relate theoretical calculations of the intensity of scattered Lyman–α radiation observed from the Vela orbit with experimentally obtained plots, Blum and Fahr[3] have treated the Vela 4 points as mean values to fit the smooth curve shown in Fig. 3; actually the plotted points are lower step points. The Vela 4 Lyman–α instrument output is telemetered in rather coarse digital form so that the observed signals lie in an interval between possible output steps. The lower step has been arbitrarily used because the experimental error in the Lyman–α experiment is large compared with the interval between steps. Much of the error, however, originates in the determination of the flux used for laboratory calibration so that the principal part of the error is proportional to the flux observed. Thus the shape of the plotted data is more significant than its absolute value, and a curve passing between a pair of points more accurately represents the data. If the curve of Fig. 3 were made to fit the data in the manner just described, the shape would be somewhat different from that depicted by Blum and Fahr and the correlation between theoretical predictions and measurements may not be as good as it appeared.

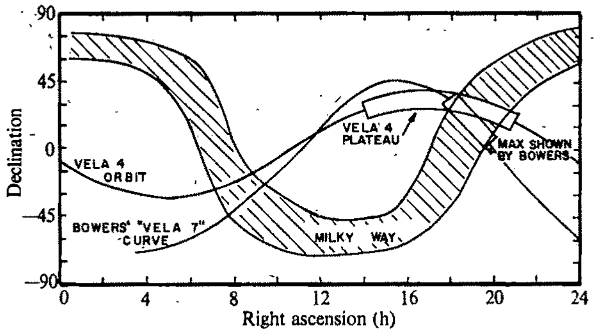


Fig. 1 The "Vela 7" portion of Bowers's Fig. 1 (ref. 2) with the Vela 4 orbit and plateau shaped maximum region added.

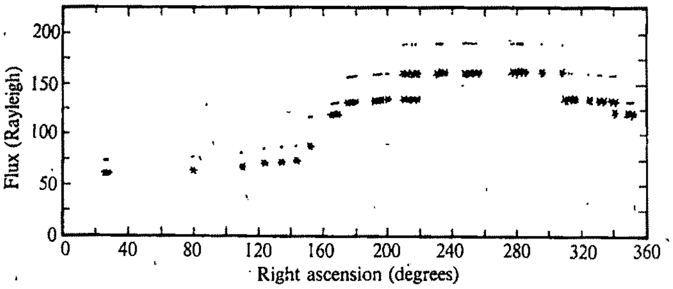


Fig. 2 Scattered Lyman–α data for the June 6, 1967, to June 10, 1967, orbit of spacecraft 4A. The lower step (★) to upper step (●) interval is a band in which the observed flux lies.

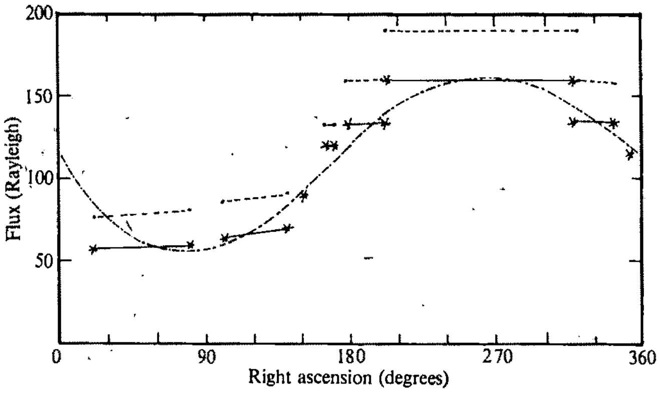


Fig. 3 The curve fit to the original Vela 4 points (★) by Blum and Fahr[3] with upper step points added (●).

We thank Ralph C. Bohlin for helpful comments during the course of this work which was carried out under the auspices of the US Atomic Energy Commission.

PAUL E. FEHLAU
W. H. CHAMBERS
JACK C. FULLER
WALTER E. KUNZ

University of California,
Los Alamos Scientific Laboratory, Los Alamos,
New Mexico 87544

Received May 12, 1971.

[1] Chambers, W. H., Fehlau, P. E., Fuller, J. C., and Kunz, W. E., *Nature*, **225**, 713 (1970).
[2] Bowers, B., *Nature*, **228**, 1182 (1970).
[3] Blum, P. W., and Fahr, H. J., *Astrophys. Lett.*, **5**, 127 (1970).
[4] Blum, P. W., and Fahr, H. J., *Nature*, **223**, 937 (1969).
[5] Fahr, H. J., *Nature*, **226**, 435 (1970).
[6] Stecher, T. P., *Astrophys. J.*, **159**, 543 (1970).
[7] Barth, C. A., *Astrophys. J.*, **161**, L181 (1970).
[8] Thomas, G. E., and Krassa, R. F., *Astron. Astrophys.* (in the press).

Re-evaluation of the Palaeogeographic Argument for an Expanding Earth

EGYED[1] has inferred that the Earth was expanding at a mean rate of 0.5 mm yr^{-1} during Phanerozoic time. The evidence on which he based this conclusion was taken from two series of palaeogeographic maps showing the distribution of land and sea since the early Cambrian, by Strakhov and H. and G. Termier respectively. With the aid of a planimeter Egyed estimated the percentage of continental areas covered by the sea for successive geological periods and was able to demonstrate clearly a secular decline since the early Palaeozoic with the more or less regular downward trend having superimposed on it a series of shorter phase oscillations.

As the volume of ocean water is hardly likely to have declined

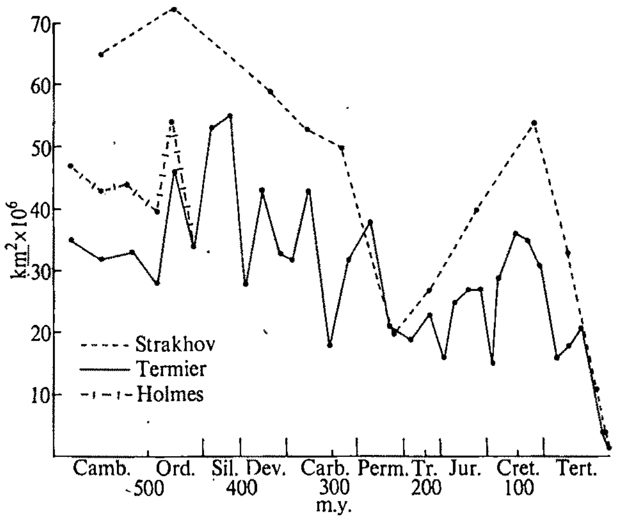


Fig. 1 Areas of continents covered by sea in Phanerozoic time.

during Phanerozoic time, and might indeed have been augmented by a small percentage, Egyed argued that the facts could only be explained by a gradual increase in the Earth's volume, so that ocean water drained more or less progressively from the continents into the growing oceans.

The essential palaeogeographic facts are not in dispute. As plotted in Fig. 1, the two groups of data, though differing appreciably, are in accord as regards the decline through time of areas of the continents inundated by seawater, the only notable (but temporary) reversal of this general trend being in the late Cretaceous. Of the two sets of data, that of the Termiers, based as it is on more numerous time intervals, gives a more accurate idea of the oscillatory nature of the change. The modification of the Cambro-Ordovician data proposed by Holmes[2] is accepted here. Apart from the areas of metamorphic rocks in Africa and elsewhere that were probably marine sediments originally, missed by the Termiers, it should be borne in mind that the older the rocks the greater is the likelihood of their having been removed by subsequent erosion or deeply buried beneath younger sediments beyond the limits of intensive drilling. For these reasons the amount of the secular decline is more likely than not to be underestimated in most palaeogeographic maps.

Both Egyed and those who have accepted his argument[2–4] have failed to consider an alternative explanation of the facts, that the average thickness of the continents has increased slightly through the course of the Phanerozoic. The isostatic uplift consequent on such increase would inevitably have the effect of reducing the area of marine inundation. There are grounds for believing that changes of this sort might, in fact, have taken place.

In the first place, many ancient shields have persisted for millions of years as positive areas and sediment sources. Maintenance of relief following removal of sediment would naturally result from isostatic adjustment, but obviously this could not persist indefinitely because the shields would achieve isostatic stability with low relief and relatively thin crust. In fact such shields often have crustal thicknesses greater than the average[5], even though metamorphic rocks of granulite facies may be exposed at the surface. Extensive tracts of the Archaean shields consist of the so called Greenstone Belts, which are believed to signify appreciably thinner crusts in early Pre-Cambrian times, perhaps only 12–15 km. This is based on various data, including the chemistry of the metabasalts and comparisons with present day island arcs[6]. The implication is that the shields have undergone a progressive "sialic underplating" since the early Pre-Cambrian as a result of slow differentiation in the underlying mantle.

In the second place, geochemical and other data suggest that the andesites of island arc complexes and the associated granitic batholiths in regions such as western North America originate primarily by differentiation of basaltic magmas arising from descending slabs of ocean floor along subduction zones, with only limited contamination by continental crustal materials[7,8]. At the rate of thickening implied by volcanicity since the Miocene, the whole of the Japanese crust could have been generated in about 3×10^9 yr[7]. This process provides a ready mechanism for creating continents which is consistent with the tenets of plate tectonics. Many former geosynclinal regions emerged from the sea following major orogenies and became incorporated into cratons bordering the ancient shields and so something more than lateral accretion is involved. Sutton[9] has incorporated such facts into his notion of chelogenic cycles, whereby tectonically mobile belts characterized by flysch-type marine sedimentation and vulcanicity are transformed into stable shield areas or cratons, on which only continental or shallow marine sediments were laid down, the latter often only at the margins. The conversion of the Caledonian geosyncline of the North Atlantic region into the "Old Red Sandstone Continent" in Devonian times is a good example, because it persisted as a positive area until well into the Mesozoic, when it was disrupted by the drifting apart of North America and Europe.

An approximate estimate of the amount and rate of thickening of the continental crust can be obtained by considering estimates of the changes in relative position of sea level in the past 600 million yr or so. Egyed[1] estimated that sea level in Cambrian times was about 500 m higher than today, if Strakhov's data were to be relied upon, or 275 m higher in the middle Ordovician, using the Termiers' data. Kuenen[10] was able to show from the world hypsographic curve that under present conditions a eustatic rise of 100 m would flood about 1/4 to 1/5 of the continents and a rise of 200 m nearly 1/3. He considered that the present continents have abnormally high relief and so thought that at most periods in the past a 100 m rise would flood more than 1/3 of the continental area. The palaeogeographic maps available to Kuenen seldom showed more than 1/3 of the continents submerged, and he concluded that the biggest transgressions did not involve eustatic rises of more than 200 m.

These figures suggest that the Termiers' data might be more accurate than those of Strakhov. An independent check on the maximum area covered by the Jurassic seas[11] also gives a figure (24%) much closer to that of the Termiers (27%) than Strakhov (39.5%).

If we take 300 m as a figure of the right order of magnitude for the amount of secular lowering of sea level since the early Palaeozoic, this would be accounted for by an average thickening of the continental crust of perhaps twice this amount. Such a figure implies an increase of only about 1 mm in 10^3 yr, with no more than about 2% of the present average thickness of the continental crust being added in the last demi-eon. Modest, almost negligible, change of this kind could be accounted for readily by the processes previously discussed. With such a plausible alternative interpretation being available the simple palaeogeographic argument for an expanding Earth cannot reasonably be sustained.

The shorter phased eustatic oscillations superimposed on the gradual lowering of sea level, given crude and oversimplified expression in Fig. 1, are to be accounted for in other ways. Whereas glacially controlled eustatic changes dominated Pleistocene palaeogeography it is doubtful whether the melting and freezing of polar ice played more than a minor role further back in time. Thus even the well documented late Palaeozoic Ice Age of the southern continents had no clearly perceptible large scale effect. On the contrary, the apparent disappearance of the ice sheets at the close of the Palaeozoic coincided with a pronounced marine regression rather than a transgression. The dominant control was much more likely to have been variations in the cubic capacity of the ocean basins due to the uplift and subsidence of mid-oceanic ridges and trench systems[12]. Evidence is now accumulating to suggest that uplift and subsidence of mid-oceanic ridges correlate respectively with increases and decreases of seafloor spreading rates[13]. The more fundamental control is presumably temporal variations in heat flow from the mantle.

The two most notable departures from a more or less gradual lowering of relative sea level in the Phanerozoic are the pronounced regression of the late Permian and early Triassic, and the equally striking transgression of the late Cretaceous. This is brought out more clearly in the less accurate Strakhov curve of Fig. 1. In fact both curves probably over-estimate by a considerable margin the area of continent covered by the sea at the close of the Palaeozoic, which was closer to the minimal values of the Pleistocene than anything before or since. Both phenomena probably relate to significant plate movements. Valentine and Moores[14] have argued that the suturing of Palaeozoic continents to form "Pangaea II" would be a sufficient cause of regression in the late Permian. With regard to the late Cretaceous, a major transgression could have been produced by one, or both, of the following factors: an acceleration of spreading rate or an increase in the length of the oceanic ridge system as significant dispersal of both northern and southern sectors of Pangaea got under way. The probable timing of continental breakup[15] suggests the latter cause to be sufficient.

I thank Dr E. R. Oxburgh and Dr S. W. Richardson for their helpful comments.

A. HALLAM

Department of Geology and Mineralogy,
University of Oxford

Received June 3, 1971.

[1] Egyed, L., *Geofis. Bura Apl.*, **33**, 42 (1956).

[2] Holmes, A., *Principles of Physical Geology*, second ed. (Nelson, London, 1965).

[3] Carey, S. W., in *Continental Drift: a Symposium* (University of Tasmania, 1958).

[4] Creer, K. M., *Nature*, **205**, 539 (1965).

[5] Oxburgh, E. R., *Geol. Soc. Lond. Spec. Publ.*, No. 3, 251 (1969).

[6] Anhaeusser, C. R., Mason, R., Viljoen, M. J., and Viljoen, R. P., *Bull. Geol. Soc. Amer.*, **80**, 2175 (1969).

[7] Oxburgh, E. R., and Turcotte, D. L., *Bull. Geol. Soc. Amer.*, **81**, 1665 (1970).

[8] Dickinson, W. R., *Rev. Geophys. Space Phys.*, **8**, 813 (1970).

[9] Sutton, J., *Proc. Geol. Assoc. Lond.*, **78**, 493 (1967).

[10] Kuenen, Ph. H., *Marine Geol.* (Wiley, New York, 1950).

[11] Hallam, A., *Earth Sci. Rev.*, **5**, 45 (1969).

[12] Hallam, A., *Amer. J. Sci.*, **261**, 397 (1963).

[13] Van Andel, T., and Heath, G. R., *Mar. Geophys. Res.*, **1**, 5 (1970).

[14] Valentine, J. W., and Moores, E. M., *Nature*, **228**, 657 (1970).

[15] Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).

Laser Radar Reflexions and Downward Infrared Flux Enhancement near Small Cumulus Clouds

WE wish to report some interesting simultaneous enhancements of backscattered laser pulses and the downward infrared flux in regions of clear air surrounding small cumulus clouds. The observations were made at Adelaide, South Australia, in March 1970, and at Aspendale, Victoria, in February 1971, but at Aspendale only a radiometer was operated.

We used a ruby laser (0.694 µm) with a pulse length of 0.5 µs, and the transmitter and receiver beamwidths were 1.0 mrad and 5.7 mrad respectively[1]. The field of view of the radiometer (10–12 µm) was 6 mrad and it had a minimum detectable radiance of 0.002 mW cm^{-2} sr^{-1} with an output bandwidth of 0.1 Hz (C. M. R. Platt, private communication). Both instruments pointed vertically upwards and were 100 m apart at Adelaide.

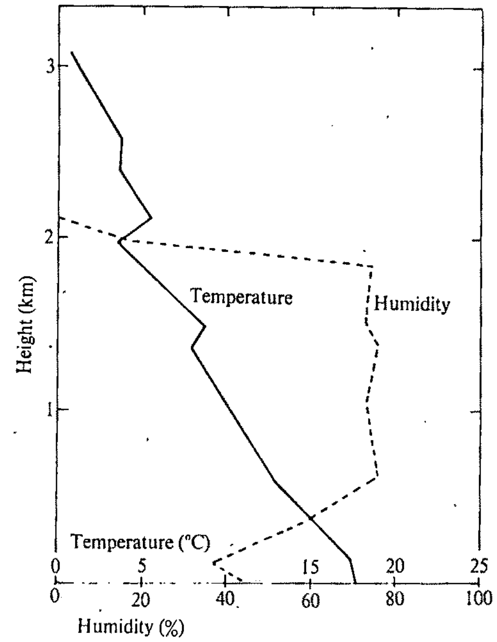


Fig. 1 Temperature and humidity profiles.

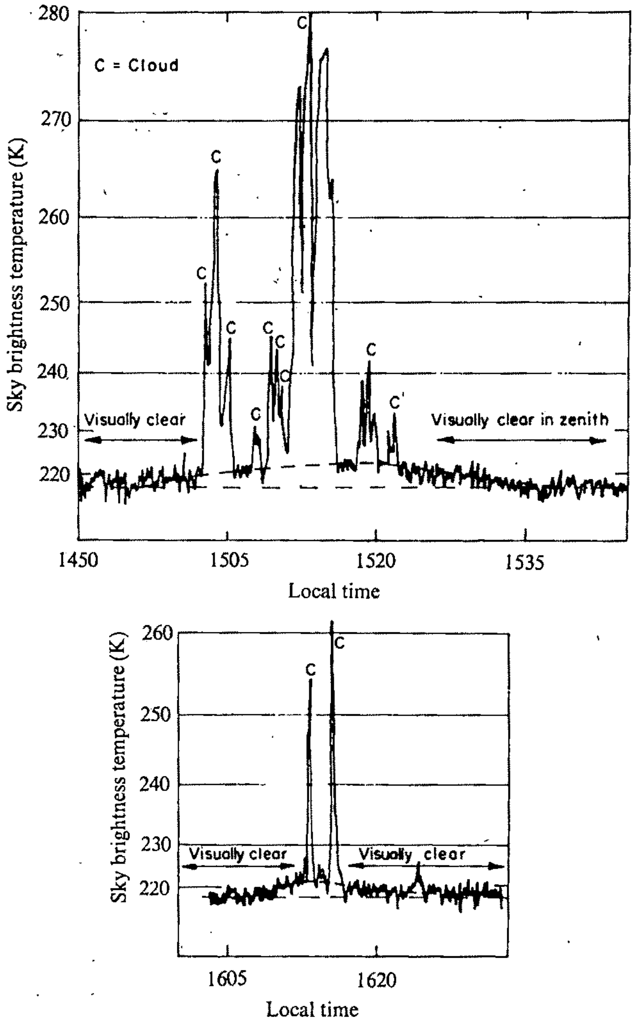


Fig. 2 Zenith sky brightness temperature (10 µm–12 µm).

In clear skies, backscattered radiation at the 0.694 µm wavelength of the laser is due to Rayleigh scattering from molecules and Mie scattering from aerosols, and the backscattered intensity is, of course, greatly increased when low cloud is present. On the other hand, atmospheric water vapour is responsible for the downward radiant flux at 10–12 μm when the sky is clear, although there is a small contribution from aerosols.

The weather at Adelaide was controlled by a cool southerly maritime airstream that was moist under an inversion at 2 km but dry aloft (Fig. 1). Small cumulus clouds were forming in groups and drifting over the site, but there was no rain. Conditions at Aspendale were similar.

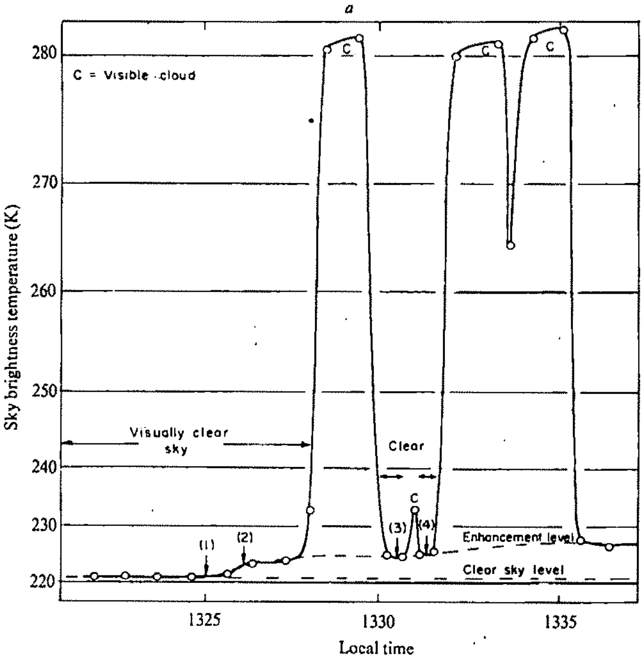
Fig. 2 shows the infrared enhancements that occurred around groups of cumulus. The arrival of visible cloud edges (*c*) is marked by sharp increases in downward radiance, but the interesting point is the slow increase in radiance that occurs outside the visible clouds. The increased radiance persists between clouds, but reverts to the original level after the passage of a group of clouds.

Fig. 3 compares the radiometer measurements with simultaneous laser radar (lidar) observations. Aerosols must be responsible for most of the observed variation in the backscattering coefficient I′(*h*) which is normalized to unity at 2 km. This is because between 0.4 and 2 km the molecular backscatter would only vary by the factor 1.14 (because of the pressure change).

The enhancement of I′(*h*) is very marked, particularly in the region of the cloud base which was estimated from the measured radiance and radiosonde data and is correct to 200 m, and it is clear that the enhancement continues in the gaps between clouds. Fig. 3 also shows reasonable agreement between the onset of the lidar enhancement and the increase in radiance. In this figure, in contrast to Fig. 2, the clouds have fairly constant radiances, indicating that they must have been optically thick.

The same pattern was observed four times on March 19, 1970, as four different cloud groups passed overhead. Fig. 4 shows another example.

The most likely explanation is that hygroscopic aerosols are taking up water and swelling under conditions of increased humidity as the air below the condensation level is lifted, and cools. That must be why the maximum enhancement of backscatter occurs near the level of the cloud base, the condensation level. Particles at 95% humidity have about twice the radius of those in the dry state, and towards 100% humidity the increases are from three to ten depending on the nature of the



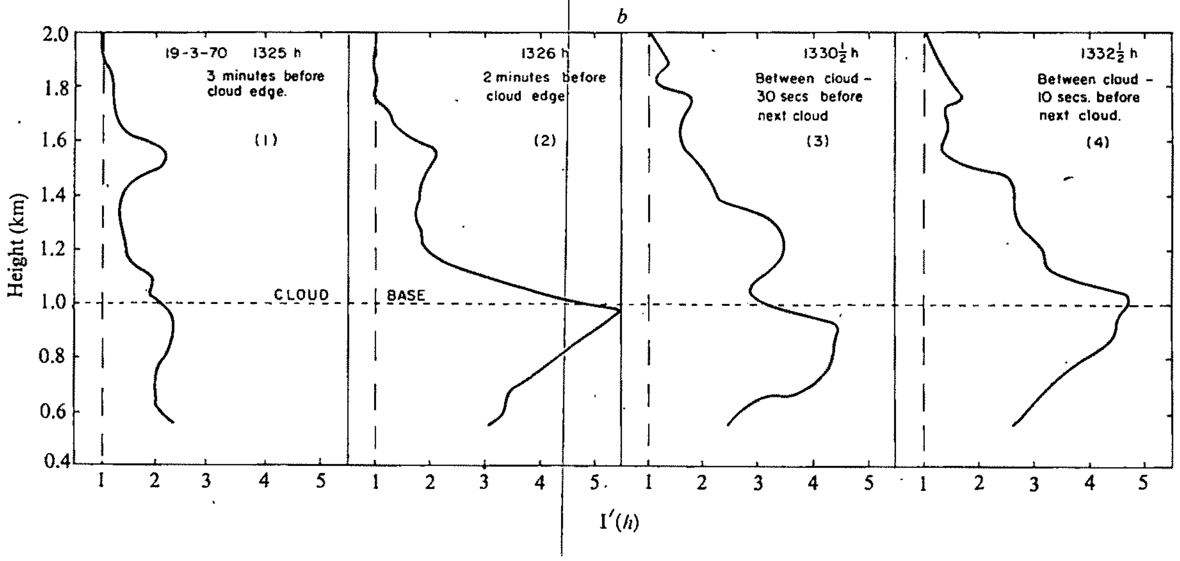


Fig. 3 *a*, Zenith sky brightness temperature (10 μm–12 μm). *b*, Lidar intensity–height profiles (normalized at 2 km).

particles[2,3]. And because the increased volume is composed of liquid water, which is highly absorbing in the infrared, the emissivity of the particles is increased. Clearly the increase in particle size and change in refractive index affect the lidar observations. Table 1 shows the relative increases in backscattered intensity predicted by Mackinnon[4] for the case when half the aerosols in both continental and maritime distributions are hygroscopic. These results can be compared directly with experiment as the morning humidity at Adelaide was 75% at 1 km, and the increase in aerosol backscatter measured by us agrees approximately with that predicted by Mackinnon if the humidity approaches 100%.

Table 1 Humidity Effects on Backscattered Lidar Power from Aerosols, Hygroscopic Aerosol Radius and Infrared Emissivity at (10–12 μm)

Relative humidity H (%)	Ratio of aerosol backscattered power at H to that at 75% humidity[4] C	M	Ratio of aerosol radius at H to that at 75% humidity C	M	Increase in emissivity E_λ C	M
96	1	2	1.7	2	0.0025	0.002
97	1.3	4				
98	1.5	4.5				
99	3	5	2	3.5	0.003	0.005
100	~5	~10	3	10	0.01	0.035

Mean observed emissivity=0.02.

C, Continental distribution; M, maritime distribution.

The corresponding change in infrared emissivity for a homogeneous layer of aerosols has been computed using similar continental and maritime distributions to those used by Mackinnon. For an optically thin layer, and neglecting scattering, the emissivity E_λ is given by $(1-\tau_\lambda)$, where τ_λ, the optical thickness, has been computed from Mie's theory. Computed values of E_λ for a 300 m layer are shown in Table 1 and compared with the mean observed value. It can be seen that the theoretical emissivity approaches the measured emissivity for either distribution if the relative humidity approaches 100%. The lidar results seem to favour the continental distribution although the actual value of $I'(h)$ at the condensation level is greater than that shown because of the low laser pulse resolution. At a coastal site such as Adelaide one would expect the aerosols to be a mixture of continental and maritime types, and the theoretical computation certainly gives order of magnitude agreement with observation.

It should be pointed out that the infrared emissivity will also increase near the clouds if the specific humidity of the air (humidity mixing ratio) is increased, due either to entrainment of moist air or the evaporation of water droplets near the cloud edges through mixing with the surrounding air. For example, the emissivity of a layer 300 m thick would increase by 0.01 if the specific humidity changed from 5.7 g kg^{-1} (early morning value at the cloud base) to the saturated value of 7.3 g kg^{-1} at the cloud base temperature. In all probability the infrared enhancement is due to both swelling of aerosols and increased water vapour, but the lidar enhancement can only be due to an increase in aerosol size in this model. But there is also the possibility of an increase in aerosol density near the clouds because of updrafts. Even if the aerosols remained dry, the density increase might account for the observed increase in lidar backscatter intensity, but the infrared emissivity increase would be considerably less than that observed. (This was verified by combined observations of aerosols in dry conditions.) Thus it seems that the aerosols must also swell to account for the large emissivity change.

Table 2 Average Extent of Lidar and Radiometric Enhancement around Small Cumulus Cloud Groups

Date	Windward (km)	Leeward (km)
March 19, 1970	1.3 (4)	—
February 10, 1971	3.4 (3)	5.8 (2)

Figures in brackets give number of observations.

Table 2 summarizes the spatial extent of the observed enhancement. The phenomenon carries with it the implication that in

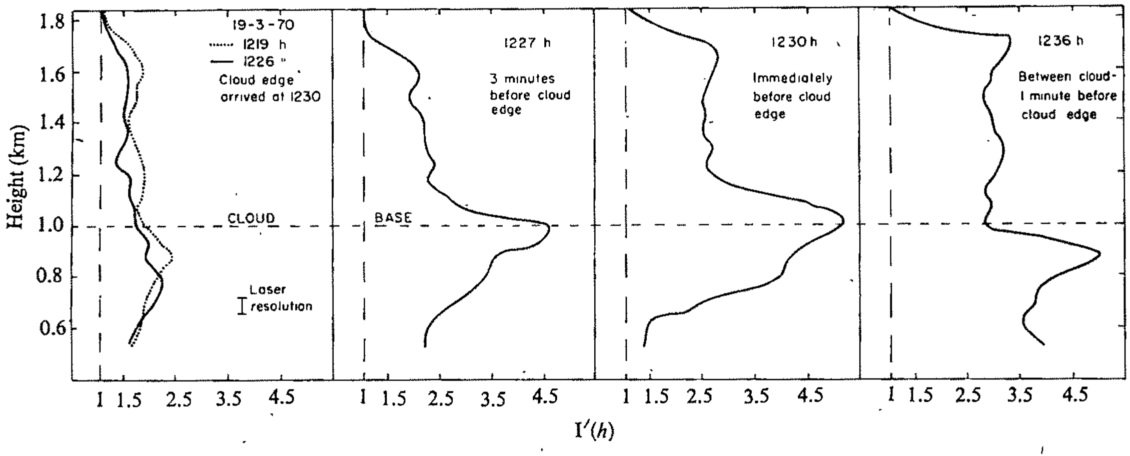


Fig. 4 Lidar intensity–height profiles (normalized at 2 km).

some cases there is a broad region of uplift in and surrounding groups of small cumulus clouds. So the clouds seem to be fed from below over a broad region.

On a few occasions, however, the effect was absent, or much reduced. This may be because when cumulus clouds are decaying the updraughts are replaced by downdraughts[5].

It is interesting that Wilkins *et al.*[6], using a wavelength difference technique, observed haloes around cumulus clouds in visible and near infrared photographs taken from Apollo 9 (SO-65). The haloes were also evident in a photodensitometer profile across a cloud bank image, and extended 1 km from the cloud edge. It seems likely that these authors and ourselves are observing the same phenomenon.

We thank Dr W. G. Elford for cooperation in making these measurements possible.

C. M. R. Platt

CSIRO Division of Meteorological Physics,
Aspendale, Victoria 3195

D. J. Gambling*

Department of Physics,
University of Adelaide,
Adelaide 5000

Received April 16, 1971.

* Present address: Weapons Research Establishment, Salisbury 5108, South Australia.

[1] Bartusek, K., Gambling, D. J., and Elford, W. G., *J. Atmos. Terr. Phys.*, **32**, 1535 (1970).
[2] Wright, H. L., *Quart. J. Roy. Meteorol. Soc.*, **65**, 411 (1939).
[3] Kasten, F., *Tellus*, **21**, 631 (1969).
[4] Mackinnon, D. J., *J. Atmos. Sci.*, **26**, 500 (1969).
[5] Borovikov, A. M., *et al.*, *Cloud Physics* (edit. by Khrgian, A. Kh.). Translated from Russian by Israel Program For Scientific Translations (Jerusalem, 1963).
[6] Wilkins, E. M., Eden, D. D., and Chivian, J. S., *Remote Sensing of Environment*, **1**, 221 (1970).

Effect of Time on the Mechanical Behaviour of Failed Rock

When a rock specimen is compressed from an initial intact state to complete collapse, the failure locus can be traced out on a force–displacement diagram as shown by the solid line in Fig. 1*a*. Until recently, this curve was followed in experiments only to the point where the compressive strength was reached; the rock then disintegrated violently. This catastrophic failure is caused by the elastic unloading of the testing machine after the peak of the force-displacement curve and is not an intrinsic property of the rock. The elastic strain energy released by conventional "soft" testing machines exceeds the amount of energy required to break the rock and the excess energy is liberated in the form of kinetic energy. Uncontrollable failure can be avoided by the use of "stiff" testing machines which store very little strain energy[1–6], or by closed loop servocontrolled testing machines which can reduce the load faster than the specimen breaks[7–9].

When the force–displacement curve is scaled by the cross-sectional area and length of the specimen, a complete stress–strain curve is obtained. For the design of structures in rock it is important to establish whether this curve is a material property or varies with the experimental conditions. In particular, the effect of time on the complete stress–strain curve must be established. It is known that the part of the stress–strain curve of rock before the peak is time dependent[10–15] and it is likely that the section after the peak is even more sensitive to time because the specimen is in a continual state of progressive collapse.

All research on the time dependent behaviour of rocks before the peak of the stress–strain curve has been carried out with reference to strain rate and creep. The only research on the effects of time in the region of the curve after the peak[15] was concerned with the effect of strain rate on the complete stress–strain curve for sandstone. Bieniawski[15] found that the descending side of the stress–strain curve became steeper as the strain rate increased. He also attempted to study the creep of failed rock but could not achieve a constant stress condition because the rock deformed along the elastic unloading characteristic of his testing machine. In soft or stiff mechanical testing machines, only a limited range of boundary conditions can be achieved precisely. This is presumably the reason why the effects of strain rate and creep have been studied exclusively. With the advent of closed loop servocontrolled testing machines, it is now possible to achieve almost any required boundary conditions and all aspects of the effect of time on the complete stress–strain curve for rock can be studied.

In a servocontrolled testing machine the specimen is loaded by a hydraulic actuator and the actuator pressure is adjusted by means of a servovalve. A feedback signal (t) representing some experimental condition of the specimen is electronically compared with a programme signal (p) representing the required condition. If a difference exists, an error signal operates the servovalve and the pressure on the rock is adjusted until t coincides with p. The mode of operation of the closed loop is shown in Fig. 1*b*. The comparison of t and p can be made 10,000 times per second and the response time of the system can be as low as 0.002 s. If a constant stress rate is

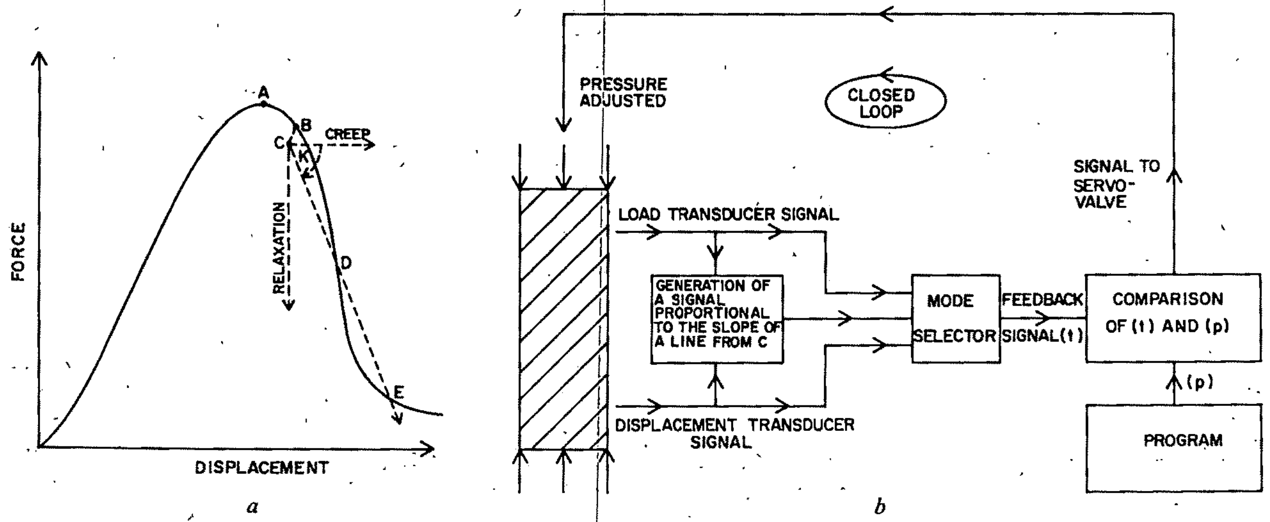


Fig. 1 *a*, Typical complete force–displacement curve for rock; *b*, closed loop servocontrolled system.

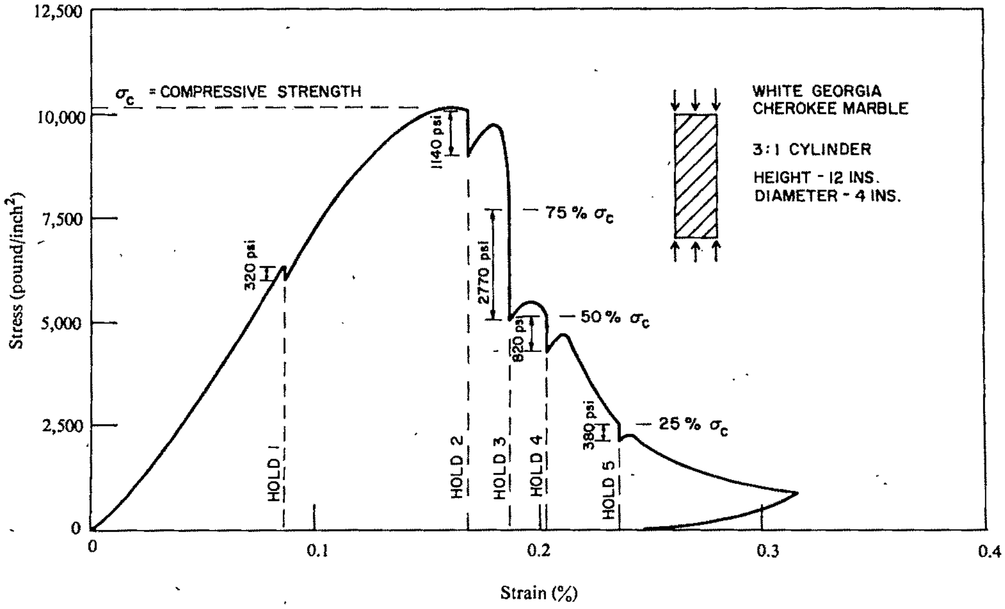


Fig. 2 Stress relaxation in failed marble.

required, the feedback signal is generated directly by a load cell and p linearly increases with time. The rock will fail very violently at the peak of the stress–strain curve in these conditions because the machine attempts to increase the stress as the rock is breaking. When a constant strain rate is required, the feedback signal is generated by a displacement transducer and again compared with a linearly increasing programme signal. As the specimen displacement is linearly increased by the operation of the closed loop, the associated stress rises to a maximum and then slowly falls as the complete stress–strain curve is traced out. To study creep, the programme is stopped at any required stress level with the feedback signal corresponding to the load. A constant stress level is then achieved by the operation of the closed loop. To study relaxation, the programme is stopped at any required strain value and the feedback signal then corresponds to the specimen displacement.

An example of stress relaxation is given in Fig. 2 which is a complete stress–strain curve for white Georgia Cherokee marble loaded in uniaxial compression. The specimen was strained at room temperature at a constant rate of $1.5 \times 10^{-6}\ s^{-1}$ except for the five points on the displacement axis at which the displacement was held constant for one hour. The first displacement hold was made during the apparently linear ascending portion of the force–displacement curve. The remaining four holds were made just after the peak and at 75%, 50% and 25% of the compressive strength. The amount of stress relaxation along each of these constant displacement lines is shown in Fig. 2. When the strain is increased after a hold, the curve seems to rise to a continuation of the original curve, indicating that the descending part of the complete stress–strain curve is not affected by this type of variation in the strain increase. The same result was obtained by Bieniawski[15] for time deformation of sandstone along the 1,110 MN/m unloading characteristic of his stiff testing machine. The maximum amount of stress relaxation occurred at the displacement corresponding to 75% of the maximum stress. This suggests that stress relaxation will be a maximum where the stress–strain curve is steepest, that is, when the relaxation line is closest to the failure locus.

Both creep and stress relaxation are phenomena associated with very specialized applied boundary conditions. In practice, for example, a mine pillar left for a period of time, the rock is subjected to neither a constant load nor a constant deformation but to a condition dictated by the unloading of the surrounding rock. To study time-dependent deformation along the line of arbitrary slope $-k$, both the load and displacement transducer signals must be used to generate the feedback signal. If the experimental force–displacement coordinates are above the line *CE* in Fig. 1*a*, the error signal should cause the servovalve to reduce the pressure. Conversely, if the coordinates are below *CE*, the pressure should be increased. The operation of the closed loop with a slope feedback thus allows a study of time deformation along any such line. If a specimen is strained to a point *B* on the complete stress–strain curve and then unloaded slightly to a point *C* as shown in Fig. 1*a*, the closed loop operation can be modified to study any type of time dependent behaviour. A constant stress condition would result in creep back to the failure locus. A constant displacement condition would result in stress relaxation to a safer condition (provided the stress–strain curve increased monotonically). If the machine was programmed to follow the line *CDE*, the specimen could deform to the failure locus at *B*, drop quickly to *E* and then stabilize at some lower stress value.

The ideas and data presented in this communication were obtained during research for the Advanced Research Projects Agency (principal investigator, Professor C. Fairhurst).

JOHN A. HUDSON

Department of Civil and Mineral Engineering,
University of Minnesota,
Minneapolis, Minnesota 55455

Received May 14, 1971.

[1] Barnard, P. R., *Mag. Concrete Res.*, **16**, 203 (1964).
[2] Hughes, B. P., and Chapman, G. P., *RILEM Bull.*, **30**, 95 (1966).
[3] Cook, N. G. W., and Hojem, J. P. M., *S. Afric. Mech. Eng.*, **16**, 89 (1966).
[4] Bieniawski, Z. T., *Intern. J. Rock Mech. Min. Sci.*, **4**, 407 (1967).
[5] Wawersik, W. R., and Fairhurst, C., *Intern. J. Rock Mech. Min. Sci.*, **7**, 561 (1970).
[6] Wawersik, W. R., and Brace, W. F., *Rock Mechanics* (in the press).
[7] Rummel, F., and Fairhurst, C., *Rock Mechanics*, **2**, 189 (1970).
[8] Hudson, J. A., Brown, E. T., and Fairhurst, C., *Rock Mechanics* (in the press).
[9] Brown, E. T., Hudson, J. A., Hardy, M. P., and Fairhurst, C., *Rock Mechanics* (in the press).
[10] Hardy, H. R., *Quart. J. Colorado School of Mines*, **54**, 3, 134 (1959).
[11] Murrell, S. A. F., *Bull. Inst. Mining and Metallurgy*, **71**, 7, 353 (1962).
[12] Wiid, B. L., *The Time Dependent Behaviour of Rock. The Strength and Deformation Characteristics*, MEG 514 (CSIR, Pretoria, 1966).
[13] Scholz, C. H., *J. Geophys. Res.*, **73**, 3295 (1968).
[14] Jaeger, J. C., and Cook, N. G. W., *Fundamentals of Rock Mechanics* (Methuen, London, 1969).
[15] Bieniawski, Z. T., *Rock Mechanics*, **2**, 123 (1970).

Optical and Acoustic Detection of Ball Lightning

THE recent controversy about ball lightning[1-4] stimulates me to report an observation of such an event, which occurred in September 1963.

This event occurred at approximately 11 a.m. in the entrance hall of the Plöckenhaus, a restaurant below the Plöckenpass in the Carnic Alps (Austria). Students from a field trip of the Geology Department of the Universität Heidelberg were staying inside this restaurant because of a thunderstorm with heavy rain. Some of the students were sitting in the guest room and some were standing in the entrance hall. The outside doors of the house were open. The door between the guest room and the entrance hall was, as I remember, closed. The floor of the entrance hall consists of heavy limestone plates. Also in the entrance hall was a St Bernard dog, lying on the floor.

Suddenly, through the open outside doors, a whitish yellow ball appeared just above the floor. It was slightly larger than a tennis ball, and its speed was about that of a walking person. The light ball moved about 2 m in the direction of the dog and finally exploded with a loud bang, like a gun shot. The dog jumped away as it saw the moving light ball. All the people in the entrance hall (about five) saw the ball and heard the bang. People from the guest room came out to ask what happened, because they had heard the bang. I do not remember feeling any radiation heat from the bang, nor smelling anything after the explosion. We, the bystanders, explained this event as a "Kugelblitz" (ball lightning).

The optical observation by several people, the reaction of the dog, and the acoustic perception of the people in the adjacent room are hard to explain by the optical illusion hypothesis for ball lightning.

GÜNTHER A. WAGNER

Department of Geology,
University of Pennsylvania,
Philadelphia 19104

Received May 24, 1971.

[1] Argyle, E., *Nature*, **230**, 179 (1971).
[2] Jennison, R. C., *Nature*, **230**, 576 (1971).
[3] Charman, W. N., *Nature*, **230**, 576 (1971).
[4] Davies, P. C. W., *Nature*, **230**, 576 (1971).

On Fireballs

THE recent discussions on fireballs prompt me to put on record that a few years ago I saw one when staying at a villa near Malcesine on the east shore of Lake Garda in Italy.

A bad thunderstorm had been raging and, because I still harbour a childish phobia about looking at lightning, I had not been looking out of the window. But as the storm slackened and was passing away, I did look out and saw a brilliant ball of white fire "sitting" still on one of the pylons carrying power lines along the east side of Garda, only a few hundred yards from our villa. It sat on one of the horizontal cable bearing cross-beams, and its diameter was less than the vertical distance between the cross-beams. There could be no question of my, or my friends, seeing an after-image of lightning, particularly as I never look at lightning myself.

We looked at the fireball for some time, but as it did nothing but sit still, we lost interest; when we looked out later, it had disappeared.

CHARLES H. GIBBS-SMITH

Residence C,
Victoria and Albert Museum,
London SW7

Received June 7, 1971.

BIOLOGICAL SCIENCES

Crystal Structure of the Visual Chromophores, 11-*cis* and all-*trans* Retinal

THE visual chromophores, 11-*cis* and all-*trans* retinal (Fig. 1), are present in both rod and cone cells of vertebrate retinas and in the corresponding organs of insects and crustacea[1]. The isomerization of the 11-*cis* isomer to the *trans* form may be the only step in the visual process which is initiated by light; several succeeding changes in the visual pigment are known to occur in the dark[2,3]. In spite of their importance in vision, the conformation of the chromophores have not been determined. Their three dimensional configurations in the crystalline state are presented in this communication.

Theoretical chemists have attempted to explain the stability and predict the structure of 11-*cis* retinal. Recent calculations predict a very slight difference in energy for the two isomers[4-6], which is consistent with thermodynamic measurements[7]. However, the structural models proposed differ from the conformation found in the crystal*. In these calculations, bond distances and angles characteristic of *trans*-carotenoids were assumed, and various conformational possibilities were explored by varying torsional angles about bonds. The discussion below indicates that several small but significant distortions in bond angles may be critical in the folding process.

The collection of X-ray diffraction data requires a relatively stable crystal. Fortunately, crystals of the *cis* isomer are more stable than its solutions, which easily isomerize at room temperature. Preliminary attempts to collect data indicated that a special environment was needed. The four-circle goniostat of an automatic diffractometer was enclosed in a light-tight container maintained at 16–17° C and continuously purged with dry nitrogen. In this manner, it was possible to collect 2,345 integrated intensities from one freshly synthesized crystal over about 4.5 days. The data were corrected to compensate for a 20–25% downward drift in monitored intensities. A total of 2,995 independent reflexions was also collected from the *trans* isomer.

(The 11-*cis* isomer formed twinned crystals in space group $P2_1/c$ with $a=7.540$, $b=10.666$, $c=22.102$A, $\beta=95.19°$, and four molecules/cell. The *trans* isomer crystallized in $P2_1/n$, with $a=14.961$, $b=8.279$, $c=15.316$A, $\beta=104.87°$, and four molecules/cell. Both isomers were crystallized from petroleum ether (Baker Analyzed Reagent, boiling point 36–37.5° C). Crystallization was initiated at 5° C and completed at −20° C. The *cis* isomer was synthesized when needed from commercially available *trans* retinal, using the methods of refs. 8 and 9.) The unit cell parameters found for the *trans* isomer agreed with those reported by Kuwabara *et al.*[10].

Approximate positions for the carbon and oxygen atoms of the two isomers were established by the symbolic addition procedure[11]. Refinement of the atomic positions and their apparent anisotropic thermal parameters was accomplished with standard least squares refinement programmes. For both isomers, the hydrogen atoms of the chain and most of the methyl hydrogens were located in difference maps. Based on cell data the agreement factors, that is the crystallographic R-factors, are at present 12.6% (11-*cis*) and 9.9% (*trans*). Further refinement and full hydrogen determinations seem feasible, and the final results, along with more crystallographic detail, will be published later.

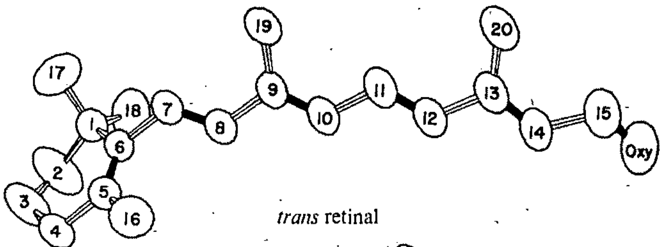
The suggested three dimensional structures of the two retinals are presented in Fig. 1. The all-*trans* retinal chain is planar,

---

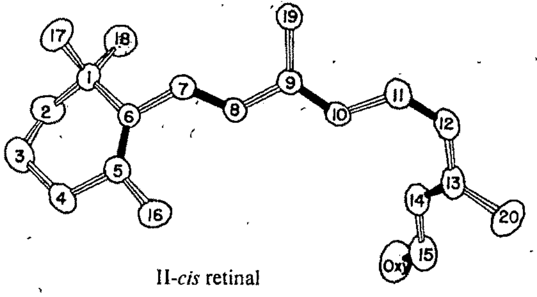
* Since our manuscript was prepared an article has appeared[16] which does theoretically propose a ground-state configuration for 11-*cis* retinal, closely resembling that reported here. The torsional angles of the chain are predicted by Honig and Karplus, and these parameters effectively define the shape of the molecule. Their torsional angles are all within 11° of our experimental values.

with the six-membered ring inclined to the plane of the chain. The inclination is given by the torsional angle of the 5-6-7-8 segment which measures 59° (the torsion angle of a planar *trans* conformation is 180°, and of a planar *cis* conformation is 0°; torsional angles are defined in ref. 12). This arrangement is typical of carotenoids, and the *trans* isomer does not differ significantly in any details from previously reported *trans* carotenoids[13].

The *cis* isomer is similar to the *trans* at the ring-chain junction; the ring torsion measured at atoms 5, 6, 7, and 8 is 40°. The chain segment from atoms 6 to 13 is essentially planar; this is contrary to the theoretical predictions mentioned earlier. The proposed models were nonplanar in this region, with torsions at the 10–11 or the 11–12 bonds ranging from 10 to 50°. The X-ray analysis indicates rotations from planarity of 8°, 4°, 0°, and 3° at the 8–9, 9–10, 10–11, and 11–12 bonds, respectively. The planar environment at the 10–11 bond corroborates a prediction of Patel[14], based on NMR observations. There is, however, an unexpectedly large torsion at the 12–13 bond, so that the atoms from C(12) to the terminal oxygen define a distinctly separate planar segment. The torsion at bond 12–13 is 39° from an s-*cis* conformation; therefore, the planar segments attached to this bond are rotated 141° from the *trans* conformation. As a result, the C(13)=C(14) and the C(15)=O double bonds are no longer parallel to the conjugated double bonds of the other segment. The best planar representation (structural formula) for this configuration is illustrated in Fig. 2, which also presents the bond distances of the two isomers. The corresponding bond distances of the chain are similar for the two isomers, even near the bend in the chain.



trans retinal

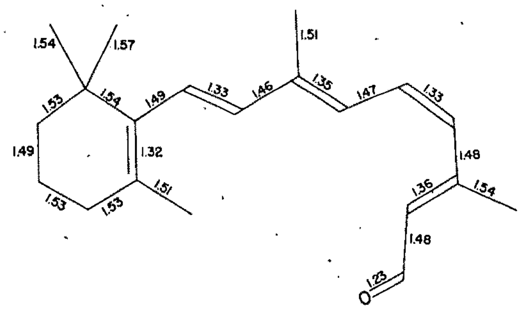


11-*cis* retinal

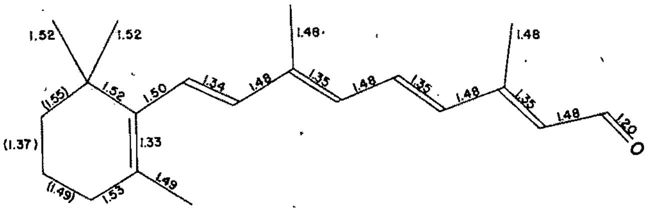
Fig. 1 Structures of the two retinal isomers. The ellipsoids drawn at each atom position indicate the relative magnitudes of the apparent thermal vibration amplitudes of the atoms. The solid black bonds represent the double bonds of the molecules.

Distortion of the bond angles of the *cis* retinal chain was noted at three points. The bond angles at atoms 11, 12, and 13 are 127.3, 130.3, and 120.6° in the *cis* isomer; the respective angles in the *trans* isomer are 121.6, 124.5, and 116.6°. The *cis* retinal has two short intramolecular approaches: atom 10 is 3.18 A from atom 13, and 3.23 A from atom 14. These atoms would approach more closely, and presumably would repel one another strongly, if the *cis* isomer were constrained to the bond angles characteristic of the *trans* isomer. Such a constraint is normally assumed in theoretical molecular folding calculations.

The problem of the conformation of the 11-*cis* retinal in solution remains. Large changes with temperature in the ultraviolet spectrum suggest an equilibrium involving more than one form of retinal. Torsions about the 10–11 and/or 12–13 bonds were discussed previously[15] to explain the spectral data. At that time, torsions about the 10–11 bond were preferred. One of us (W. S.) has since examined additional spectroscopic evidence, and now considers two conformations, differing by a large torsion about the 12–13 bond, to be the most probable forms in solution, with the s-*trans* form prevailing at lower temperatures (77 K).

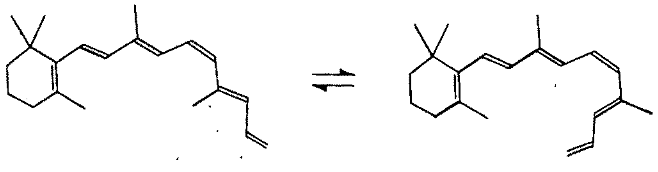


11-*cis* retinal



trans retinal

Fig. 2 Bond distances for the two retinal isomers. The planar chain of the *trans* isomer is drawn to scale and displays a slight curvature; this has been noted in other reports on carotenoid structures[13]. The bond distances in the ring of *trans*-retinal, which are enclosed in parentheses, represent the average values of a disorder. A ring puckering disorder was noted in canthaxanthin[13] in the C_2–C_3 region, and a similar disorder is indicated for the *trans* isomer.



s-*trans* s-*cis*

The labels s-*trans* and s-*cis* are approximate; in each case, twists away from these planar forms would be expected. A more detailed report on the spectroscopy of the retinals will be published elsewhere.

This research was supported in part by a grant from the US National Institutes of Health to D. G. McConnell of the Ohio State University. We gratefully acknowledge the assistance of Ch. N. Rafferty in preparing the 11-*cis* retinal crystals.

R. GILARDI
I. L. KARLE
J. KARLE

Laboratory for the Structure of Matter,
Naval Research Laboratory,
Washington, DC 20390

W. SPERLING

Institute for Research in Vision,
Ohio State University,
Columbus,
Ohio 43210

Received February 15; revised April 9, 1971.

[1] Wald, G., *Nature*, **219**, 800 (1968).
[2] Hubbard, R., and Kropf, A., *Proc. US Nat. Acad. Sci.*, **44**, 130 (1958).
[3] Kropf, A., and Hubbard, R., *Ann. NY Acad. Sci.*, **74**, 266 (1958).
[4] Langlet, J., Pullman, B., and Berthod, H., *J. Chim. Physiol.*, **67**, 480 (1969).
[5] Nash, H. A., *J. Theoret. Biol.*, **22**, 314 (1969).
[6] Wiesenfeld, J. R., and Abrahamson, E. W., *Photochem. Photobiol.*, **8**, 487 (1968).
[7] Hubbard, R., *J. Biol. Chem.*, **241**, 1814 (1966).
[8] Dieterle, J. M., and Robeson, C. D., *Science*, **120**, 219 (1954).
[9] Oroshnik, W., Brown, P. K., Hubbard, R., and Wald, G., *Proc. US Nat. Acad. Sci.*, **42**, 578 (1956).
[10] Kuwabara, M., Hamanaka, T., and Mitsui, T., *J. Physiol. Soc. Japan*, **25**, 920 (1968).
[11] Karle, J., and Karle, I. L., *Acta Cryst.*, **21**, 849 (1966).
[12] Klyne, W., and Prelog, V., *Experientia*, **16**, 521 (1960).
[13] Bart, J. C. J., and MacGillavry, C. H., *Acta Cryst.*, **B24**, 1587 (1968).
[14] Patel, D. J., *Nature*, **221**, 825 (1969).
[15] Sperling, W., and Rafferty, C. N., *Nature*, **224**, 591 (1969).
[16] Honig, B., and Karplus, M., *Nature*, **229**, 558 (1971).

Optical Activity of LSD–DNA Mixtures

REPORTS on the fluorescence-sensitive binding of d-LSD (d-lysergic acid diethylamide), l-LSD and their 2-bromo derivatives to calf thymus DNA[1], and on changes induced in DNA circular dichroism by LDS-25 (ref. 2), have led to the belief that it is the intercalation of the drug in DNA that causes the chromosomal damage[3-6] and abnormalities in human offspring[7] that have been associated with LSD. Our studies of circular dichroism, however, have failed to show that LSD has any effect on DNA conformation.

To begin with, the circular dichroism (CD) of d-LSD, l-LSD and d-2-bromo-LSD at high concentrations does not conform with Beer's law: at concentrations greater than 0.02 mg/ml., for example, the CD bands in the region 260–290 nm are suppressed; at 0.1 mg/ml., the same bands reappear with inversion of the 220 nm band. These variations have not been explained but it is unlikely that at such high concentrations they are biologically relevant. As a consequence of this unexplained behaviour of LSD, we restricted our *in vitro* studies to solutions of DNA and LSD in which the LSD concentrations were less than 0.01 mg/ml., concentrations at which the results were reproducible and at which the Beer's law dependence of their spectra is verifiable.

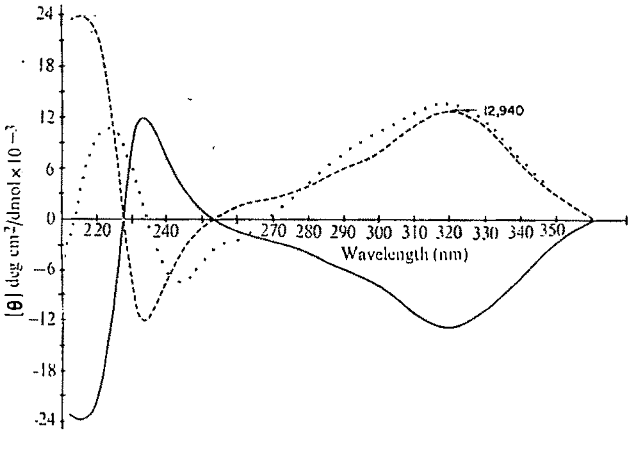


Fig. 1 The circular dichroism of l-LSD (——), d-LSD (- - -) and d-2-bromo-LSD (. . .). CD was measured on a Cary 60 spectropolarimeter with circular dichroism attachment, standardized with an aqueous solution of d-10-camphorsulphionic acid (K and K Laboratories, Plainview, New York), giving $E_L - E_R = 2.20 \pm 0.05$ at 290 nm. Solutions at *p*H 7.0 and 25° C in 0.02 M K_2HPO_4 gave the same spectra with both 0.01 M Tris (hydroxymethyl)aminomethane and 0.15 M NaCl. d-LSD, l-LSD and d-2-bromo-LSD are supplied in an L(+) tartrate buffer at 0.12 mM. Measurements were made at four different concentrations of LSD (maximum 0.01 mg/ml.) to verify conformity with Beer's law (see text). The LSD : tartrate ratio is constant for all of these studies[2,6]. Over the spectral region 215–370 nm L(+) tartrate does not contribute asymmetrically to the optical activity of d-LSD and l-LSD in the conditions studied. In all studies reported here, the l-LSD used was found to be the optical mirror of the d-LSD, as shown in the figure.

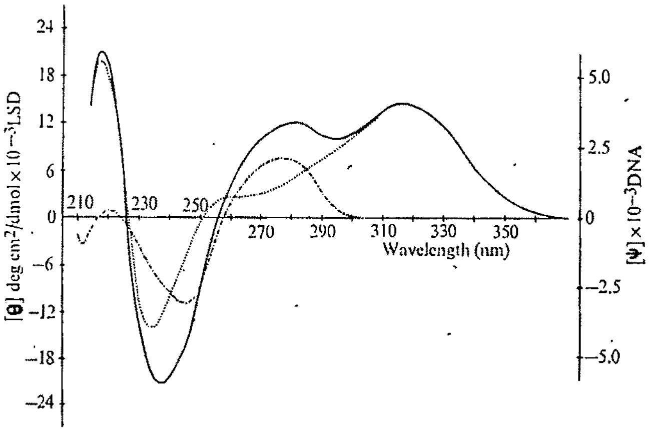


Fig. 2 The circular dichroism spectra of d-LSD (. . . .), DNA (.-.-.-) and their combination (——). The combination is a simple summation of the component spectra. The summed curves are the same in 10–50 mM Tris, 20–50 mM K_2HPO_4 and 100–150 mM NaCl at *p*H 6.5, 7.0 and 8.0. These are the conditions used by Wagner. DNA solutions were measured at concentrations of 20, 30, 60, 75, and 120 μg/ml.; the spectra with added LSD were always the sum of the component spectra. These particular curves were copied from spectra measured on a DNA solution at 20 μg/ml. d-LSD at 3.3×10^{-5} M. Spectra were measured at room temperature. Calf thymus DNA was purchased from Sigma Chemical Co., St Louis, Mo. Other DNA was supplied by Professors Antero So and Sheldon Greer whose DNA (*B. subtilis*) was shown, apart from physical measurements, to possess full biological activity; the biological activity and integrity of the DNA were determined by assaying the capacity of low concentrations of DNA to transform two genetic markers (*his* 31 and *ind* 168) that are linked to the extent of 65%.

In previous work on this subject, the purity of the LSD sample with respect to isomeric forms was not determined. Some have used a racemic mixture (Wagner, private communication). To ensure that our comparisons between d-LSD and l-LSD are valid, solutions were used which gave mirror-image CD spectra of the particular isomer that was used in each case (Fig. 1). Solutions of each isomer are then pure or at worst slightly cross-contaminated to the same degree by the other enantiomer, because they also give characteristic rotations at the sodium D line.

Studying the circular dichroism of DNA in the presence of d-LSD (the psychotomimetic congener) we found no significant changes; the changes were less than 1% of those reported by Wagner[2] in the same conditions; the interaction of d-LSD and DNA, shown by the fluorescence quenching experiments of Yielding and Sterglanz, did not cause any conformational change in either the d-LSD or DNA. In the spectral region 400–200 nm, the circular dichroism of the d-LSD–DNA complex results simply from a combination of the individual d-LSD and DNA spectra (Fig. 2). The calculated LSD+DNA and observed spectra are identical. The DNA was considered to be "native" when they gave CD spectra of native DNA, as defined by Brahms and Mommaerts[8]. The absence of any new optically active contribution to the DNA circular dichroism, when d-LSD was present in the DNA solution, was verified in three different solutions, 0.15 M NaCl, 0.01 M Tris and 0.02 M K_2HPO_4, each at *p*H 6.5, 7.0 and 8.0. Additional annealing of the DNA to close the small open stranded regions and to correct mismatched base-pairing by warming to 50, 60 or 67° C and slow cooling produced no changes in the ability of d-LSD to "intercalate" with DNA, that is, it produced no changes in optical activity other than those associated with the LSD and DNA themselves. This was true whether the DNA was heated in the presence or absence of any of the LSD forms. Furthermore, DNA from *Bacillus subtilis*, *Escherichia coli*, bovine spleen, sheep bone marrow and phage T4 gave the same negative results, showing the absence of any interaction for a wide range of $(A+T)/(C+G)$ ratios.

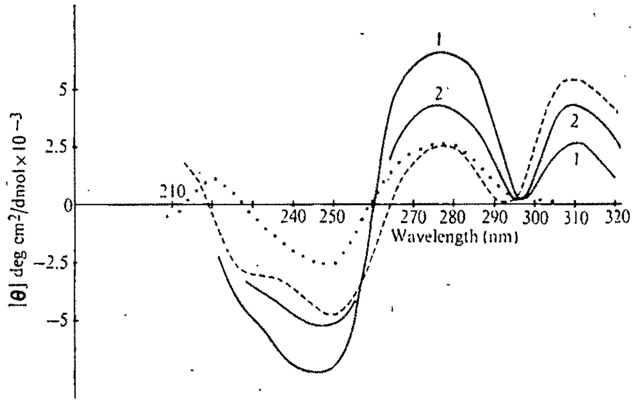


Fig. 3 The CD spectra of ethidium bromide–DNA mixtures. . . ., Curve represents pure calf thymus DNA. The differences in intensities between curves 1, 2 and the broken line relate to the age of the DNA solution. Curve 1 describes the freshest DNA solution; curve 2 was obtained from solutions that had been stored at 4° C for a week, and the dashed curve represents a much older solution of DNA, about 4 weeks old. One can obtain the dashed curve on a freshly prepared solution of old DNA which has been stored as a cold dry powder. The dashed curve is closest to the one published by Wagner[2]. Several samples of calf thymus DNA (Sigma) gave even higher ellipticity values at 272 nm with ethidium bromide, and it may be that they were the most "native" of the more than thirty preparations tested, but the curves reported here are the three which proved to be characteristic of three reproducibly different states of DNA. In spite of the variation in ethidium bromide-induced changes, the DNA alone always gave the same CD spectrum. In all cases the concentration of DNA is 30 μg/ml. and the concentration of ethidium bromide is 3.3×10^{-5} M. The more intense spectra were most consistent for solutions in 10 mM Tris, as compared with phosphate and NaCl solutions.

To eliminate artefacts arising from nicking of the DNA during handling as a complicating factor, experiments were done in two ways: in the first, solutions of fresh DNA were gently poured; and in the second, DNA solutions were extruded through hypodermic syringes with small bore needles. But, in spite of the different degrees of shearing, the results of the two were identical; in both cases, the circular dichroism of the LSD–DNA solution was the sum of the individual LSD and DNA spectra.

The fluorescence changes seen by Yielding and Sterglanz on the binding of LSD to DNA indicated to them that the sort of interaction which occurs is a "general property of this group of drugs", and that the "binding ratio was one drug molecule per base moiety in the DNA chain". It would seem, therefore, that while the drugs of this group may interact with DNA in such a way that their fluorescence emission is suppressed, circular dichroism indicates no conformational change, certainly none similar to that observed in DNA in the presence of the trypanocidal dyes, ethidium bromide or isometamidium chloride. In this respect we are in agreement with Yielding and Sterglanz: the behaviour of dn, ln and d-2-bromo-LSD in the presence of DNA is the same, regardless of the form of LSD.

The CD of ethidium bromide–DNA combinations is shown in Fig. 3. Since ethidium bromide is a good example of the planar compounds believed to intercalate with DNA[9–11], its effect on DNA circular dichroism serves as a useful basis for comparison in assessing the possible effects of LSD. We found, for example, that the character of the ethidium bromide-induced optical activity is influenced considerably by the age of the DNA solution (Fig. 3).

There is no similarity in behaviour between the LSD–DNA solutions (Fig. 2) and the ethidium bromide–DNA complexes (Fig. 3) and it is unlikely therefore that LSD forms this type of complex with DNA. For example, a significant reciprocal behaviour in ellipticity was found (Fig. 3) in the ethidium bromide–DNA interaction between the positive band at 272 nm, which arises from ethidium bromide. It was also observed by Aktipis and Martz[9–11] and it is in accord with Tinoco's[12] general theory of optical activity. It is evidence for a strong interaction between ethidium bromide and DNA which in no way resembles that between DNA and LSD in any of its forms.

We have also studied the CD spectra of combinations of LSD forms with whole tRNA and pure tryptophanyl-$tRNA_{trp}$ of *E. coli*. No additional optical activity was generated beyond the contributions of tRNA and LSD measured singly, and neither d-LSD nor l-LSD affected the capacity of $tRNA_{trp}$ to charge tryptophan.

Recently, it was reported[13] that LSD does not damage chromosomes of *E. coli* except at very high concentrations and high ultraviolet light intensity. Unfortunately, the isometric purity of the LSD used in this work was not determined and a maleate salt of LSD was used whereas others have generally used an L(+)tartrate salt. We emphasize the importance of measuring the relative concentrations of the dn and ln forms and of a uniformity in the preparations used. The conflicting results of studies of LSD-induced chromosomal damage[14] may be largely attributable to differences in isomer concentrations and buffering agents in the LSD preparations. Furthermore, some early LSD–DNA spectra seem to be inaccurate, and do not provide a firm basis for consideration of the mode of action of LSD.

We thank Professors Robert G. Heath and Robert J. Boucek for encouragement and assistance and Dr John A. Scigliano for supplying the LSD for these studies. The work was supported in part by grants from the National Institutes of Health, the US Public Health Service, the Florida Heart Association and the Programme Project Grant.

A. H. BRADY
ELISABETH M. BRADY
F. C. BOUCEK

Department of Medicine,
University of Miami School of Medicine,
and the Laboratories for Cardiovascular Research,
Howard Hughes Medical Institute,
Miami, Florida

Received August 31; revised December 4, 1970.

[1] Yielding, K. L., and Sterglanz, H., *Proc. Soc. Exp. Biol. Med.*, **128**, 1096 (1968).
[2] Wagner, T., *Nature*, **222**, 1170 (1969).
[3] Alexander, G. J., Miles, B. E., Gold, G. M., and Alexander, R. B., *Science*, **157**, 461 (1967).
[4] Irwin, S., and Egozcue, J., *Science*, **157**, 312 (1967).
[5] Cohen, M. M., and Mukerjee, A. B., *Nature*, **219**, 1972 (1968).
[6] Cohen, M. M., Hirschhorn, K., and Frosch, W. A., *New Engl. J. Med.*, **277**, 1043 (1967).
[7] Hsu, L. Y., Strauss, L., and Hirschhorn, K., *J. Amer. Med. Assoc.*, **211**, 987 (1970).
[8] Brahms, J., and Mommaerts, W. F. H. M., *J. Mol. Biol.*, **10**, 73 (1964).
[9] Waring, M. J., *J. Mol. Biol.*, **13**, 269 (1965).
[10] LePecq, J. B., and Paoletti, C., *J. Mol. Biol.*, **27**, 87 (1967).
[11] Aktipis, S., and Martz, W. M., *Biochem. Biophys. Res. Commun.*, **39**, 307 (1970).
[12] Tinoco, jun., I., *Adv. Chem. Phys.*, **4**, 113 (1962).
[13] Papirmeister, B., and Wolpert, J. S., *Biochem. Biophys. Res. Commun.*, **39**, 307 (1970).
[14] Smart, R. G., and Bateman, K., *Canad. Med. Assoc. J.*, **99**, 805 (1968).

LSD-25 Does Not Intercalate in DNA

APART from its hallucinogenic effect, lysergic acid diethylamide (LSD-25) is believed to cause chromosome abnormalities[1-3], which have been attributed to a direct interaction of the LSD-25 and DNA[4]. The apparent resemblance of the circular dichroism spectra of the DNA–ethidium-bromide complex and mixtures of LSD-25 and DNA has led to the suggestion that LSD-25 might be intercalated in the DNA[5].

A more specific and sensitive method for studying intercalation is provided by the interaction of drugs with twisted, circular, covalently closed duplex DNA[6-8]. By intercalating molecules into the DNA the duplex is unwound and, because the total number of duplex turns and supertwists is a topological invariant, this leads to the unwinding of the supertwists and therefore to the conversion of twisted circles into open circles[9]. This change in form can be followed by sedimentation analysis, or, more conveniently, in a viscometer[15]. We have used this method to verify Wagner's suggestion[5] that LSD-25 intercalates.

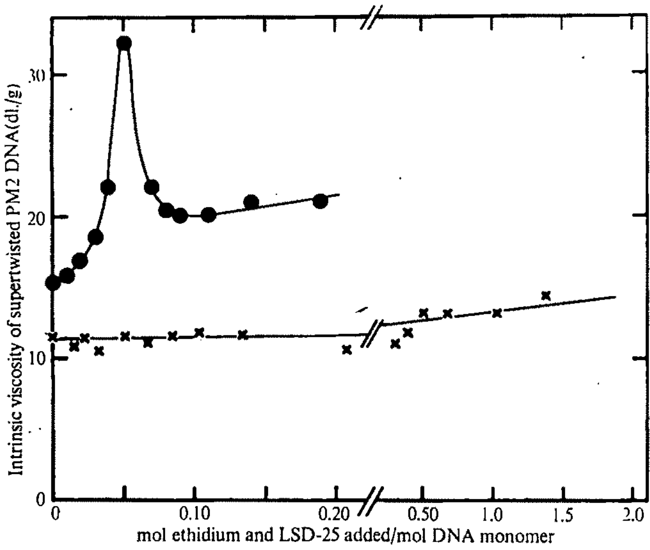


Fig. 1 The intrinsic viscosities of the DNA from phage PM2 as a function of the ethidium (●) and the LSD-25 (×) concentration present. Conditions were: temperature 20° C; DNA concentration, 27 μg ml.$^{-1}$ of 15 mM NaCl, 1.5 mM sodium citrate, *p*H 7.0 (●), and 20.3 μg ml.$^{-1}$ of 10 mM sodium phosphate, *p*H 7.0 (×). The intrinsic viscosities were determined in a rotating cylinder viscometer (Krannich GMbH, Göttingen) according to Zimm and Crothers[10]. Ethidium was a gift from Boots, and LSD-25 was purchased from Sandoz.

Fig. 1 shows the results of an experiment in which we compared the effect of LSD-25 and the intercalating dye ethidium on the closed circular duplex DNA of bacteriophage PM2 (refs. 11 and 12). Whereas the results with ethidium were as expected, no interaction between DNA and LSD-25 was detectable, in the range of concentrations in which strong interaction was found by others[4,5].

We therefore repeated previous experiments, in which interaction between LSD-25 and DNA was detected spectrophotometrically. In contrast to the results of Yielding and Sterglanz[4], the spectra of different mixtures of LSD-25 and DNA between 220 and 600 nm were the exact summations of the two components separately; in contrast to Wagner's results[5], we also found complete additivity for the circular dichroism spectra.

These results indicated that either LSD-25 does not interact directly with DNA, or that our LSD sample contained no LSD-25. The latter alternative was excluded by infrared spectroscopy and NMR studies. We therefore conclude that chromosome damage in the presence of LSD-25 is not a consequence of the intercalation of LSD-25 into DNA.

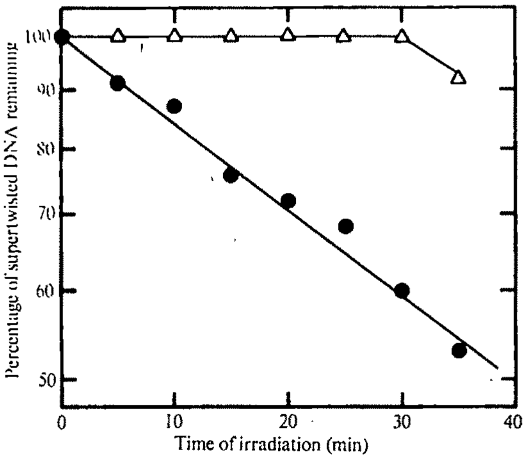


Fig. 2 Breakage of the supertwisted from of PM2 DNA by ultraviolet irradiation in the presence of LSD-25. Samples were irradiated with (●) and without LSD-25 (△) with a germicidal lamp. The radiation below 300 nm was cut off by a glass filter. Conditions were: DNA concentration, 63.5 μg ml.$^{-1}$; LSD-25 concentration, 310 μM; medium 15 mM NaCl + 1.5 mM sodium citrate, *p*H 7.0. The conversion of the supertwisted PM2 DNA was followed by band sedimentation of small samples through neutral 3 M CsCl in an MSE analytical ultracentrifuge. The percentage of supertwisted DNA was estimated from scannings at 260 nm, using a Dupont 310 curve resolver.

During these experiments, we noted that irradiation of DNA with ultraviolet light in the presence of LSD-25 led to phosphodiester bond breakage, even if most of the radiation below 300 nm was cut off with a glass filter. This phenomenon was illustrated by the experiment in which phosphodiester bond breakage in DNA was measured by the conversion of the closed circular duplex form of PM2 DNA to slower sedimenting material (Fig. 2). Electron microscopy confirmed that the irradiation led to the formation of open circles rather than linear molecules. There was no conversion when DNA was irradiated in the absence of LSD-25 or when DNA–LSD mixtures were kept in the dark. Although the conditions used in this experiment were very different from those prevailing *in vivo*, it should be realized that the molecular weight of PM2 DNA is only 6×10^6, whereas a human diploid nucleus contains 4×10^{12} daltons of DNA[13]. LSD-25-dependent photo-destruction of DNA could therefore contribute to chromosome damage in tissue culture experiments and possibly *in vivo*, and photo-sensibilization of an *Escherichia coli* mutant, deficient in repair, by high concentrations of LSD-25 has been reported[14].

We thank Dr B. F. van Gelder for discussions and Mr C. Kruk, Mrs F. Fase-Fowler, Mr W. de Jong, Mr G. Pauw and Miss J. E. de Boer for assistance. A Cary 60 instrument with a 6002 CD attachment was purchased from funds from the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

E. M. SMIT
P. BORST

*Laboratory of Biochemistry,
Department of Medical Enzymology,
University of Amsterdam*

Received March 5, 1971.

[1] Cohen, M. M., Marinello, I., and Bach, N., *Science*, **155**, 1417 (1967).
[2] Irwin, S., and Egozine, J., *Science*, **157**, 313 (1967).
[3] Bick, Y. A. E., *Nature*, **226**, 1165 (1970).
[4] Yielding, K. L., and Sterglanz, H., *Proc. Soc. Exp. Biol. Med.*, **128**, 1096 (1968).
[5] Wagner, T. E., *Nature*, **222**, 1170 (1969).
[6] Crawford, L. V., and Waring, M. J., *J. Mol. Biol.*, **25**, 23 (1967).
[7] Waring, M. J., *Nature*, **219**, 1320 (1968).

[8] Ruttenberg, G. J. C. M., Smit, E. M., Borst, P., and Van Bruggen, E. F. J., *Biochim. Biophys. Acta*, **157**, 429 (1968).
[9] Bauer, W., and Vinograd, J., *J. Mol. Biol.*, **33**, 141 (1968).
[10] Zimm, B. H., and Crothers, P. M., *Proc. US Nat. Acad. Sci.*, **48**, 905 (1961).
[11] Espejo, R. T., and Canelo, E. S., *Virology*, **34**, 738 (1968).
[12] Espejo, R. T., Canelo, E. S., and Sinsheimer, R. L., *Proc. US Nat. Acad. Sci.*, **63**, 1164 (1969).
[13] Vendrely, R., in *The Nucleic Acids* (edit. by Chargaff, E., and Davidson, J. N.), **2**, 155 (Academic Press, New York, 1955).
[14] Papirmeister, B., and Wolpert, J. S., *Biochem. Biophys. Res. Commun.*, **38**, 319 (1970).
[15] Smit, E. M., and Borst, P., *FEBS Lett.*, **14**, 125 (1971).

Induced Optical Activity of 2,4-Dinitrophenyl-lysine Specifically Bound to Mouse MOPC-315 Myeloma Protein

THE optical activity generated by the interaction of small ligands with proteins, visible as extrinsic optical rotatory dispersion (ORD) Cotton effects or circular dichroism (CD) bands, has been used extensively to study the binding of ligands to non-antibody proteins[1,2]. We describe here the use of circular dichroism to monitor the binding of DNP-lysine to a mouse IgA myeloma protein with high affinity for dinitrophenylated haptens[3-5].

The MOPC-315 mouse tumour line of Potter and Lieberman[6] was maintained in 5–8 month old BALB/c mice, and the IgA myeloma protein was purified from ascitic fluid by affinity chromatography[5]. DNP-lysine (ε-N-2,4-dinitrophenyl-L-lysine)-'Sepharose' was prepared by the method of Porath, Axen and Ernback[7]. We washed a 10 ml. DNP-'Sepharose' column with 200 ml. of 0.15 M NaCl–50 mM sodium borate buffer (*p*H 8.4), 10 ml. of 1% (w/v) bovine serum albumin, 200 ml. of 1 M acetic acid and 200 ml. of borate-buffered saline immediately before use. MOPC-315 ascitic fluid was dialysed against borate-buffered saline, and the protein was reduced at 25° C with 10 mM dithiothreitol for 1 h, and alkylated for 30 min at 4° C with a 10% molar excess of iodoacetamide. The reduced and alkylated protein was dialysed against borate-buffered saline and applied to a DNP-'Sepharose' column, which was washed with the same solvent, and the MOPC-315 myeloma protein was eluted with 1 M acetic acid. The recovered protein was immediately dialysed against borate-buffered saline (*p*H 8.4) at 4° C, and insoluble materials were removed by centrifugation. Examination of the purified protein by immunoelectrophoresis with rabbit anti-mouse serum and anti-MOPC-315 protein sera yielded only a single component.

Circular dichroism was measured at 25° C with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment, and standardized with d-camphor-10-sulphonic acid. Cell path lengths of from 1.0 cm to 0.1 mm, and protein concentrations between 0.8 and 4×10^{-3} g per 100 ml., were used to determine the CD spectrum (600 to 200 nm) of the MOPC-315 protein. The data are given in molar ellipticities, $[\theta]_\lambda$, in degrees cm^2 dmol^{-1}, where $[\theta]_\lambda = \frac{3{,}300}{lc}(A_L - A_R)_\lambda$, and l = the path length (cm), c = the molar concentration and $(A_L - A_R)_\lambda$ is the observed difference in absorption between left and right circularly polarized light at wavelength λ. The average residue weight, M_0, taken as 110 (rather than the molecular weight) for the MOPC-315 protein was used to obtain the protein $[\theta]_\lambda$ values. We determined optical density difference spectra with a JASCO ORD/UV-5 spectrophotometer. The quenching of MOPC-315 protein fluorescence by DNP-lysine (Sigma)[3] was measured with a Zeiss double M4 QIII monochromator spectrofluorometer with a Farnell stabilized EHT power supply and a Keithley 610 B electrometer, in a thermostatically controlled cell compartment at 25° C. The data were corrected for dilution and internal quenching as before[8]. Protein concentrations were determined from the optical density at 280 nm (corrected for light scattering), measured with a Zeiss PMQII spectrophotometer, with an extinction coefficient, $E_{1\ \mathrm{cm}}^{1\ \mathrm{mg\ ml.}^{-1}}$, of 1.44 for the MOPC-315 IgA protein[3,5]. The molar extinction coefficient, ε, at 362 nm for DNP-lysine was taken as 1.75×10^4 (ref. 5).

The CD spectrum of the MOPC-315 protein is shown in Fig. 1*A*. No circular dichroism was evident in the protein spectrum from 600 to 315 nm. DNP-lysine (up to 1.3×10^{-4} M) in free solution, and in the presence of immunoglobulins lacking DNP-specificity, also had no optical activity in this wavelength range (Fig. 1*B*). In contrast, binding of the DNP-lysine ligand by the MOPC-315 protein generated distinct circular dichroism bands (Fig. 1*B*). A broad positive band centred near 410–420 nm, a sharper positive band at 380 nm and a broad negative band extending from 360 nm to 270 nm and centred near 295 ± 15 nm were observed in the CD spectrum of the MOPC-315–DNP-lysine complex (Fig. 1*A* and *B*). The absorption spectra of DNP-lysine in free solution, and DNP-lysine specifically bound to the MOPC-315 protein, are also recorded in Fig. 1*B*. The redshift and hypochromicity[3] were evident after complexing the ligand with the myeloma protein, the absorption maximum being shifted from 362 nm for free DNP-lysine ($\varepsilon = 1.75 \times 10^4$) to 368 nm for bound DNP-lysine ($\varepsilon = 1.52 \times 10^4$).

Hapten-binding curves were constructed by serially adding DNP-lysine (with a Hamilton microsyringe) to a constant amount of MOPC-315 protein, and measuring the circular dichroism at 325 nm and 380 nm. The $(A_L - A_R)_{325\ \mathrm{nm}}$ data (uncorrected for dilution) have been plotted as a function of the total DNP-lysine concentration (Fig. 1*C*). The mean value for $(A_L - A_R)_{325\ \mathrm{nm}}$ at saturation (that is $(A_L - A_R)_{\max}$ at DNP-lysine concentrations greater than 8×10^{-5} M), corrected for dilution, was 5.32×10^{-4}. The $(A_L - A_R)_{325\ \mathrm{nm}}$ values were divided by the total DNP-lysine concentration to give the apparent value of $[\theta]_{325\ \mathrm{nm}}$, and these values are also plotted in Fig. 1*C* as a function of the total DNP-lysine concentration. The mean of the maximum values for $[\theta]_{325\ \mathrm{nm}}$ (apparent) (total DNP-lysine concentration less than 6×10^{-5} M) gave $[\theta]_{325\ \mathrm{nm}}$ as -2.10×10^4 degrees cm^2 dmol^{-1}. Similarly, $[\theta]_{380\ \mathrm{nm}}$ was calculated to be 1.68×10^4 degrees cm^2 dmol^{-1}. The molar concentration of antibody combining sites was determined from the ratio $((A_L - A_R)_{\max}/[\theta]_{325\ \mathrm{nm}})$ to be 1.71 mol of sites per 153,000 g of protein.

The intrinsic association constant is an additional parameter for evaluating $[\theta]_\lambda$. The association constant, K_A, obtained for the MOPC-315 protein from the fluorescence quenching data (Fig. 1*D*) was 0.6×10^7 mol^{-1}, consistent with the value reported by Eisen, Simms and Potter[3]. The association constant, $K_A = \frac{r}{(n-r)c'}$, where r is the number of moles of hapten bound per mol of bivalent antibody, $n = 2$, and c' is the free hapten concentration[3,8]. The value of r at each dilution may be obtained from the observed $(A_L - A_R)$ values, corrected for dilution, as the ratio $(2(A_L - A_R)$ observed$/(A_L - A_R)_{\max})$. At $r = 1$, $c' = \frac{1}{K_A}$, $c = [(\text{total DNP-lysine concentration}) - c']$. and $[\theta]_\lambda = \frac{3{,}300}{cl}[(A_L - A_R)_\lambda$ at $r = 1]$. The values for $[\theta]_{325\ \mathrm{nm}}$ and $[\theta]_{380\ \mathrm{nm}}$ calculated by this procedure were -2.13×10^4 and 1.66×10^4 degrees cm^2 dmol^{-1}, respectively, consistent with the values we obtained from the $[\theta]_\lambda$ (apparent) against total DNP-lysine concentration plots (Fig. 1*C*).

For lower affinity antibodies ($K_0 \sim 10^5$ mol^{-1}), circular dichroism is an alternative method to determine directly the association constant.

The optical activity of the inherently symmetric DNP-chromophore is generated by the interaction of the DNP group with asymmetrically placed vicinal groups in the antibody combining site of the MOPC-315 protein. The reciprocal relationship of the positive and negative CD bands, together with the large molar extinction coefficient of the DNP group, suggests that the optical activity of the DNP-lysine–MOPC-315 protein complex results (at least partly) from dynamic coupling of electronic transitions of the DNP group and a protein chromophore (for example, tryptophan or tyrosine) in the antibody combining site[2,9,10]. Little and Eisen[11] have studied the difference spectra resulting from the association of DNP ligands and TNP ligands with antibody, and suggested that a tryptophan residue is present in the combining sites of anti-DNP and anti-TNP antibodies, from several sources. Circular dichroism presents a further experimental parameter with which to explore this possibility. The magnitude of the rotational strength of reciprocal CD bands depends on the relative orientation of the transition dipole moment vectors of the interacting chromophores[2,10]. Circular dichroism bands which result principally from vicinal perturbations static in nature (for example, asymmetric distribution of charge on vicinal groups), are also sensitive to the relative orientation of the ligand and the amino-acid residues in the antibody combining site. Circular

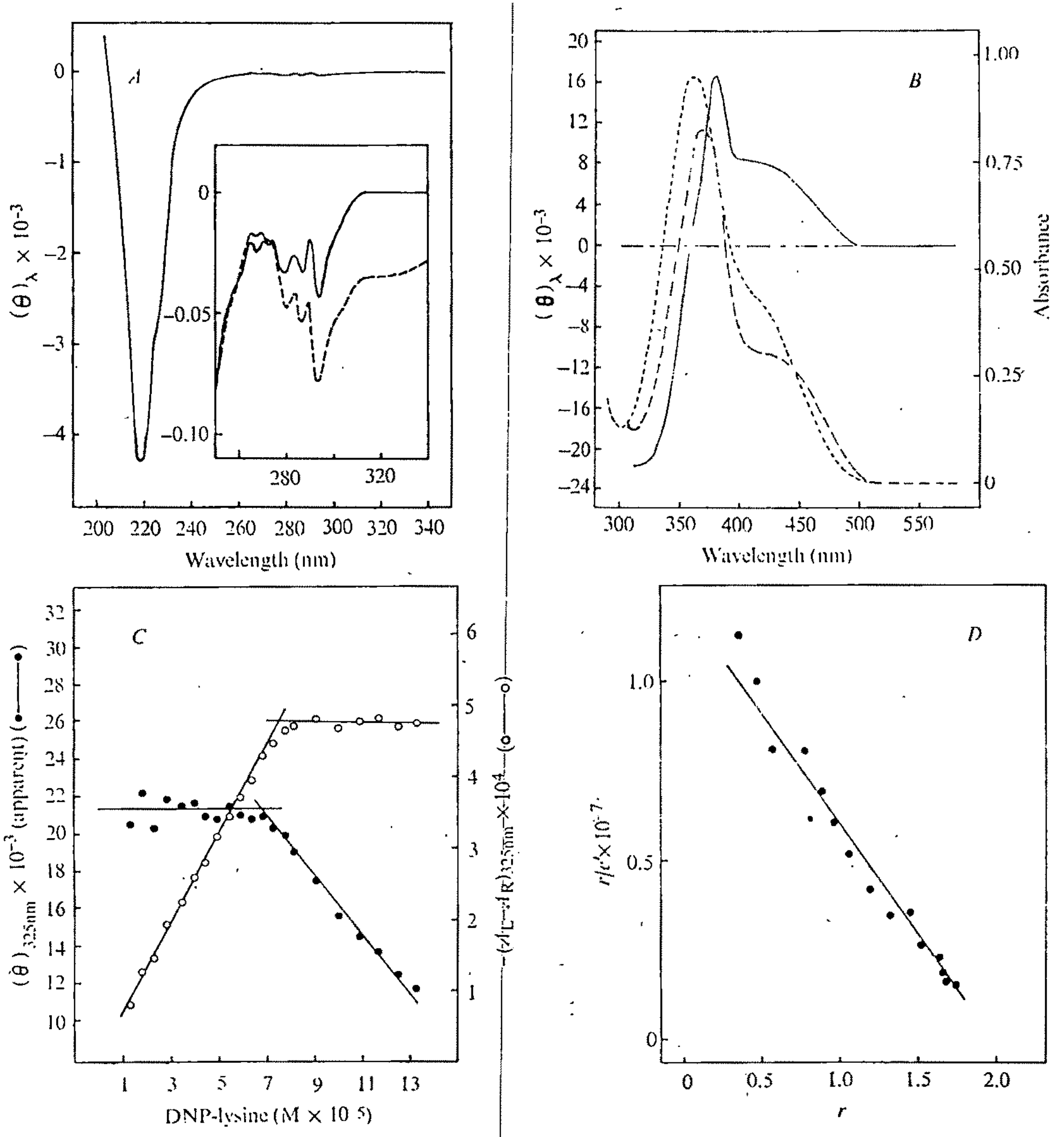


Fig. 1 *A*, Circular dichroism spectrum of MOPC-315 IgA myeloma protein in 0.1 M NaCl–10 mM sodium phosphate buffer (*p*H 7.2), or 0.1 M NaCl. The inset illustrates the aromatic region with an expanded $[\theta]_\lambda$ scale (———). The circular dichroism spectrum of the DNP-lysine–MOPC-315 protein complex in the protein aromatic amino-acid absorption region is also illustrated in the inset (– – –). DNP-lysine in free solution showed no circular dichroism from 600 to 220 nm. *B*, Circular dichroism spectrum of ε-N-2,4-dinitrophenyl-L-lysine in free solution in 0.15 M NaCl–50 mM sodium borate buffer (*p*H 8.4) (— — —), and bound to the MOPC-315 IgA myeloma protein (———). The $[\theta]_\lambda$ values below 315 nm have been corrected for the CD contribution from the protein alone. The absorption spectrum of DNP-lysine (- - -) and an equal molar concentration of DNP-lysine bound to the MOPC-315 protein (— — —) are also shown. The same concentration of MOPC-315 protein without DNP-lysine was used as a reference to obtain the difference spectrum of DNP-lysine bound to protein. The DNP-lysine–MOPC-315 difference spectrum was examined at $r=1.2$ where more than 99% of the total DNP-lysine present was specifically bound to protein. *C*, Hapten binding curves constructed by serially adding 10–20 µl. of 1.07 mM DNP-lysine to 14.8 mg of MOPC-315 protein in 2.0 ml. of 0.15 M NaCl–50 mM sodium borate buffer (*p*H 8.4). The $(A_L-A_R)_{325\,nm}$ data, uncorrected for dilution, have been plotted as a function of the total DNP-lysine concentration (○). The values of $[\theta]_{325\,nm}$ (apparent) $= \frac{3{,}300}{l}\,[(A_L-A_R)/(\text{total DNP-lysine concentration})]$ have also been plotted as a function of the DNP-lysine concentration (●). *D*, DNP-lysine hapten binding data for the MOPC-315 protein of *C*, obtained by fluorescence quenching. Data are plotted in terms of *r* and *c′*, where *r* is the number of moles of hapten bound per mol of bivalent antibody, and *c′* is the free DNP-lysine concentration. The molar concentration of antibody combining sites ($n=2$) was determined from the CD binding data (see text).

dichroism, therefore, is a powerful method for the study and comparison of the fine structure of the antibody combining site.

This research was supported in part by a grant from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service.

JOHN M. ROCKEY*
KEITH J. DORRINGTON†

MRC Molecular Pharmacology Unit,
Medical School, Hills Road, Cambridge

PAUL C. MONTGOMERY‡

National Institute for Medical Research,
Mill Hill, London NW7

Received September 3; revised November 27, 1970.

* Present address: Department of Ophthalmology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

† Present address: Department of Biochemistry, University of Toronto.

‡ Present address: Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

[1] Beychok, S., *Ann. Rev. Biochem.*, **37**, 437 (1968).
[2] Gratzer, W. B., and Cowburn, D. A., *Nature*, **222**, 426 (1969).
[3] Eisen, H. N., Simms, E. S., and Potter, M., *Biochemistry*, **7**, 4126 (1968).
[4] Underdown, B. J., Simms, E. S., and Eisen, H. N., *Fed. Proc.*, **29**, 437 (1970).
[5] Goetzl, E. J., and Metzger, H., *Biochemistry*, **9**, 1267 (1970).
[6] Potter, M., and Lieberman, R., *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 187 (1967).
[7] Porath, J., Axen, R., and Ernback, S., *Nature*, **215**, 1491 (1967).
[8] Rockey, J. H., *J. Exp. Med.*, **125**, 249 (1967).
[9] Tinoco, I., *J. Chim. Phys.*, **65**, 91 (1968).
[10] Urry, D. W., in *Spectroscopic Approaches to Biomolecular Conformation* (edit. by Urry, D. W.); 33 (Amer. Med. Assoc., 1970).
[11] Little, J. R., and Eisen, H. N., *Biochemistry*, **6**, 3119 (1967).

Localization of A Antigen Sites on Human Erythrocyte Ghosts

DETAILED immunochemical knowledge of the heterosaccharide antigen determinants of the ABO blood groups has been gained from the study of glycoproteins of mammalian secretions[1-3]. Less is known about the chemical composition and spatial distribution of the ABO antigen determinants in erythrocyte membranes. Because the erythrocytes envelope appears as a uniform unit membrane after positive staining[4], standard electron microscopy has failed to relate the ABO antigen sites to any discrete membrane structure[5].

We have demonstrated a new method for the study of the topochemistry of membrane surfaces[6] which utilizes freeze-etching, a technique involving fracture, which splits the membrane and exposes its inner, hydrophobic matrix[6,7] (Fig. 1, left), followed by etching, which exposes the membrane surface by lowering the ice table[6,8] (Fig. 1, right).

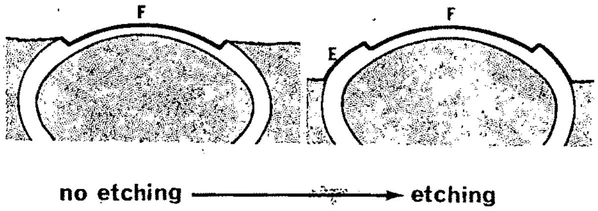


Fig. 1 Faces exposed by freeze-etching red blood cell ghosts: fracture splits the membrane (fracture face, *F*); subsequent etching lowers the ice table and exposes the membrane surface (etched surface, *E*). Because the membrane is split, there is a step or ridge between the fracture face and the etched surface.

In the erythrocyte membrane, the hydrophobic matrix exposed by the fracture process, is structurally differentiated into smooth areas and particles[9,10]. The particles average 85 Å in diameter (uncorrected for shadow thickness) and seem to protrude to the membrane surface[9]. Their chemical nature is unknown, although indirect evidence suggests that they are proteins[9,11-14]. We show here that the A antigen sites of the human erythrocyte ghost are associated with these membrane particles.

Human erythrocyte ghosts from blood group A_1, and controls from blood group O, were prepared[15] from fresh whole blood drawn in acid citrate dextrose buffer. Immune anti-A (Hyland Division, Travenol Laboratories, Los Angeles) was fractionated by chromatography on DEAE cellulose, and the IgG fractions (eluted with 0.01 M phosphate buffer, *p*H 8.0) were pooled and concentrated by ultrafiltration. The A antigen sites were labelled indirectly with ferritin conjugated to the IgG fraction of rabbit anti-human IgG[16]. A_1 or O ghosts (50 μl. of packed ghosts plus 50 μl. of 20 mM phosphate buffer, *p*H 7.5) were incubated for 75 min at room temperature, with 5 mg of the anti-A IgG fraction in a total volume of 8 ml. After washing three times with 100 volumes of the buffer, the cells were incubated at room temperature with 0.4 ml. of the conjugate for 150 min. The cells were centrifuged and resuspended in cold 20 mosmolar phosphate buffer containing 1 mM $MgCl_2$, sedimented, resuspended in a 1 : 10 dilution of the same buffer and then washed three times in distilled water. For freeze-etching, portions of the pellet were mounted on cardboard disks and quenched in chlorodifluoromethane ('Freon 22') cooled by liquid nitrogen. Specimens were fractured, etched for 1 min at −100° C and replicated in a Balzers apparatus[17].

Washing the ghosts in distilled water caused appreciable aggregation of almost all the membrane particles (Fig. 2)[9]. The patterns formed by these particle aggregates helped us to recognize their protrusions on the etched surface and enabled us to judge the location of the ferritin molecules relative to the membrane particles (Fig. 2*b* and *c*).

Extensive examination of the replicas showed that the ferritin-markers, and thus the anti-A antibodies, were consistently associated with the particle aggregates on the surfaces of A_1 erythrocyte ghosts (Fig. 2*b* and *c*). It could be argued, however, that the A antigen sites were not associated with the membrane particles, but that antigens and particles co-aggregated during washing. We thought this unlikely, because if the antigen sites were not associated with the membrane particles random co-aggregation should produce some particle-free antigen aggregates and, consequently, ferritin labelling on the smooth zones of the ghost membrane. We counted the ferritin molecules which corresponded to zones devoid of particles; in a survey of 121 of those smooth zones, only three ferritin molecules were found (there were 296 ferritin molecules on the remainder of the etched surfaces). Furthermore, in an analysis of the position of 1,500 ferritin molecules, only sixteen were not associated with any particle aggregates. Because no ferritin was observed on group O controls, we concluded that the binding of the ferritin was a specific antigen–antibody reaction which localized the A antigen sites on the membrane particles.

The number of marker molecules was much lower than the current estimates of the number of antigen sites of A_1 cells. Our experimental conditions were not chosen to optimize binding to a substantial proportion of the antigen sites, but to minimize agglutination which would make it impossible to expose the outer membrane surface by etching. Preliminary experiments to observe directly the anti-A antibody suggest that most membrane particles possess A antigen sites. This makes the agreement between the current estimates of the number of A antigen sites in A_1 cells ($8-11 \times 10^5$)[18] and the number of membrane particles in the human erythrocyte membrane (4,200 μm^{-2} or about 6.2×10^5 per erythrocyte)[9,11] interesting[19], but it may be coincidental.

Because the molecular species containing the A and B antigens in the human erythrocyte membrane are probably glycoprotein[20-22] and glycolipid[23,24], the correspondence between the antigen sites and the membrane particles implies

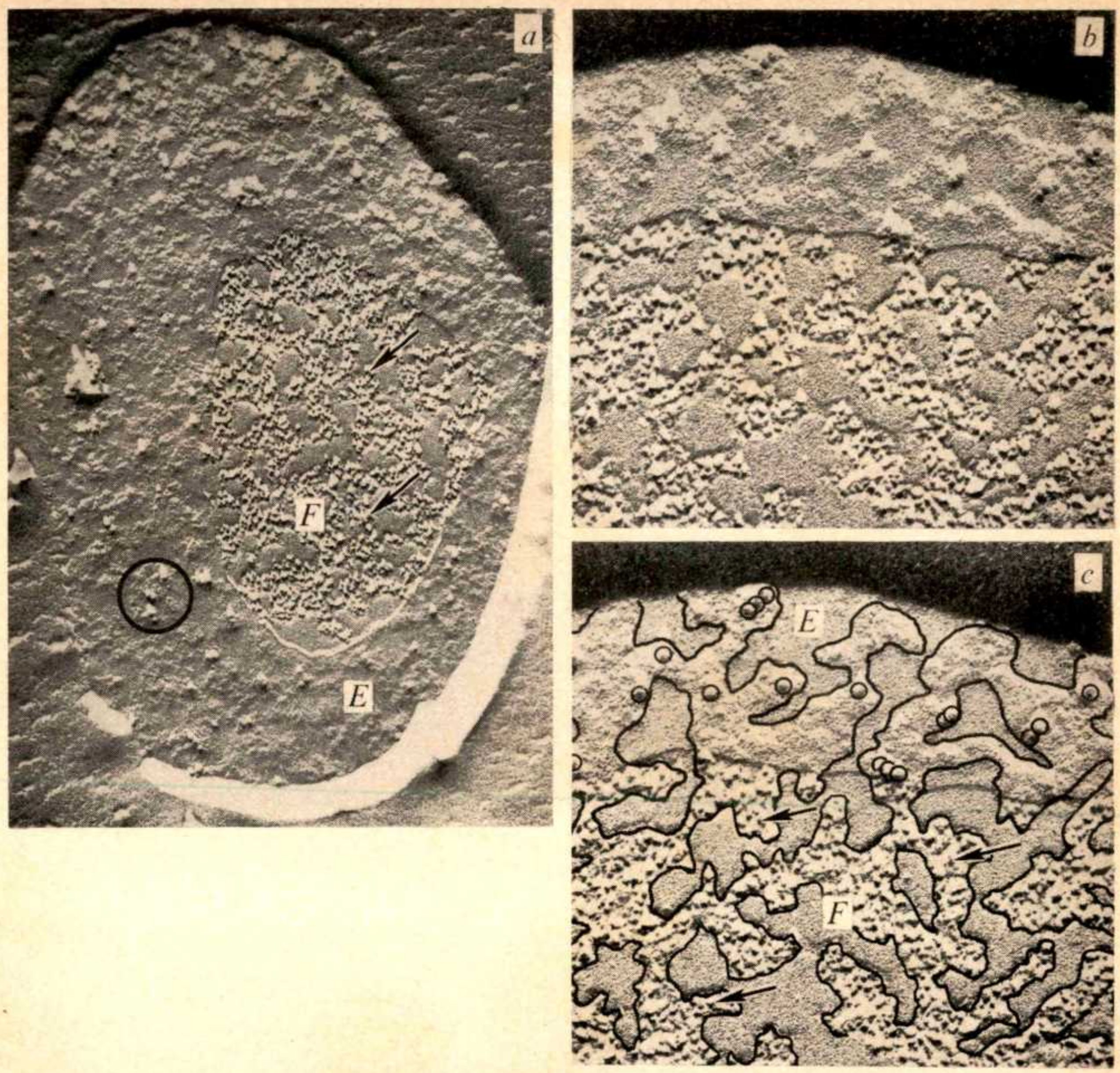


Fig. 2 Freeze-etched A_1 human erythrocyte ghosts labelled with ferritin conjugate indirectly bound to A antigen sites (*a*, 75,000; *b* and *c*, ×130,000). A ridge separates the fracture face (*F*) from the etched surface (*E*). Aggregates of membrane particles (arrows) are visible on the fracture faces and protrude to the etched surfaces where they appear as roughened zones (*b*, outlined and labelled in *c*); the outlines of the roughened zones on the etched surface are continuous with the outlines of the particle aggregates on the fracture face. Ferritin molecules (encircled, *a* and *c*), and therefore A antigen sites, are associated only with the protrusions of the membrane particles on the etched surface.

that the particles contain glycoprotein or glycolipid moieties. Although most of the membrane particles are preferentially associated with the inner half of the membrane[9,11], they must protrude to the outer surface where they carry the heterosaccharide ABO determinant. The particles probably have other functions; they seem to traverse the entire membrane, thus providing a structural pathway which may be important in transport phenomena. Extrapolating the present results to the native erythrocyte membrane should be viewed with caution, although preliminary experiments showed that there was no loss of haemagglutination titre in erythrocyte ghosts, either before or after washing in water.

The combination of immunochemical and freeze-etch techniques used here can be extended to study the relationship of membrane particles to other antigen systems, receptor sites, enzymes and transport functions in erythrocyte and other membranes.

We thank Drs Leon Wofsy, Anne Good, Hugh Fudenberg and Mrs Rose Bonhag for advice; Dr Herbert Perkins of the Irwin Memorial Blood Bank for blood samples; and Susan Whytock and Eleanor Crump for technical assistance. This research was supported in part by the US Atomic Energy Commission; P. P. S. was supported in part by Estação Agronómica Nacional, Oeiras, Portugal.

PEDRO PINTO DA SILVA
STEVEN D. DOUGLAS
DANIEL BRANTON

Department of Botany,
University of California,
Berkeley,
California 94720

Received August 3; revised August 26, 1970.

[1] Kabat, E. A., *Blood Group Substances* (Academic Press, New York, 1956).
[2] Race, R. R., and Sanger, R., *Blood Groups in Man* (Davis, Philadelphia, 1968).
[3] Watkins, W. M., *Science*, **152**, 172 (1966).
[4] Robertson, J. D., *Biochem. Symp.*, No. 16, 3 (1959).
[5] Lee, R. E., and Feldman, J. D., *J. Cell Biol.*, **23**, 396 (1964).
[6] Pinto da Silva, P., and Branton, D., *J. Cell Biol.*, **45**, 598 (1970).
[7] Branton, D., *Proc. US Nat. Acad. Sci.*, **55**, 1048 (1966).
[8] Tillack, T., and Marchesi, V. T., *J. Cell Biol.*, **45**, 649 (1970).
[9] Engstrom, L., *Structure in the Erythrocyte Membrane*, dissertation, Univ. California, Berkeley (1970).
[10] Weinstein, R. S., in *Red Cell Membrane Structure and Function* (edit. by Jamieson, G. A., and Greenwalt, T. J.), 36 (Lippincott, Philadelphia, 1969).
[11] Branton, D., *Ann. Rev. Plant. Physiol.*, **20**, 209 (1969).
[12] Deamer, D. W., Leonard, R., Tardieu, A., and Branton, D., *Biochim. Biophys. Acta*, **219**, 47 (1970).
[13] Tillack, T. W., Scott, R. E., and Marchesi, V. T., *Fed. Proc.*, **29**, 489 (1970).
[14] Marchesi, V. T., *Fed. Proc.*, **29**, 600 (1970).
[15] Dodge, J. T., Mitchell, C., and Hanahan, D., *Arch. Biochem. Biophys.*, **100**, 119 (1963).
[16] Shick, A. F., and Singer, S. J., *J. Biol. Chem.*, **236**, 2477 (1961).
[17] Moor, H., and Muhlethaler, K., *J. Cell Biol.*, **17**, 609 (1963).
[18] Economidou, J., Hughes-Jones, N. C., and Gardner, B., *Vox Sang.*, **12**, 321 (1967).
[19] Weinstein, R. S., Williams, R. A., and Someda, K. S., *Lab. Invest.*, **18**, 337 (1968).
[20] Whittemore, N. B., Trabold, N. C., Reed, C. F., and Weed, R. F., *Vox Sang.*, **17**, 289 (1969).
[21] Zahler, P., *Vox Sang.*, **15**, 81 (1968).
[22] Zahler, P., and Weibel, E., in *Protides of Biological Fluids* (edit. by Peeters, H.), **16**, 71 (Pergamon, Oxford, 1968).
[23] Hakomori, S., and Strycharz, G. D., *Biochemistry*, **7**, 1279 (1968).
[24] Koscielak, J., *Biochim. Biophys. Acta*, **78**, 313 (1963).

Treatment of Murine Leukaemia with Heterologous Antiserum or Cyclophosphamide

WE have compared chemotherapy and immunotherapy in AKR mice injected with a syngeneic tumour, strain BW5147 lymphatic leukaemia. Previous investigations disclosed significant therapeutic benefit from isologous or homologous antiserum against mouse leukaemia[1–4] or with antiserum raised against murine tumour viruses[5]. In contrast, toxicity has been a significant obstacle to the therapeutic use of heterologous antiserum[6,7]. Reports[8–12] of successful use of heterologous antiserum, however, suggested that further study was warranted, and an experiment was designed to compare quantitatively the therapeutic activity of antiserum with conventional chemotherapy.

Strain BW5147 leukaemia was invariably lethal for untreated AKR mice. Median survival time (MST) was 10.0±0.6 days after injection of 10^6 cells intraperitoneally in thirty-two passages utilizing 200 mice. Dispersed cell suspensions were prepared as described previously[13].

Cyclophosphamide ('Cytoxan') was selected for chemotherapy because of previous studies with L1210 leukaemia, in which a 10^{-3} (99.9%) reduction in tumour was achieved with 100 mg/kg[14]. Toxicity of cyclophosphamide in AKR mice was similar to toxicity in other mice[15], and LD_{50} in fifty AKR mice was 375 mg/kg.

Antitumour serum (ATuS) and antilymphocyte serum (ALS) were prepared by intravenous injections of tumour cells or normal AKR spleen and thymus cells in New Zealand rabbits. Serum was decomplemented and AKR red blood cell (RBC) absorption was performed until haemagglutination titre was negative. In four preliminary experiments, tumour-injected mice treated with RBC-absorbed ATuS had an increase in MST of 42–89% (ref. 16). MST was not prolonged by ALS or normal rabbit serum.

The effects of cyclophosphamide and RBC-absorbed ATuS were compared after injection of 10^6 BW5147 cells intraperitoneally. Single cyclophosphamide doses of 20, 40, 60, 80, 120 or 160 mg/kg were given. ATuS was administered in doses of 3.0 ml. once, 1.5 ml./day for 3 days, or 0.25 ml./day until death. Untreated control mice were injected intraperitoneally with serial ten-fold dilutions of tumour cells from 10^2 to 10^7; MST with 10^6 cells was 9.50 days.

Increased survival was observed in tumour-injected mice which were treated with either cyclophosphamide or ATuS, and was proportional to the total dose of drug or serum administered. The most effective ATuS regimen (0.25 ml./day) prolonged MST 53%, to 14.50 days. This was similar to the effect of 60 mg/kg of cyclophosphamide, or a 99.99% reduction in tumour inoculum, to 10^2 cells (Fig. 1).

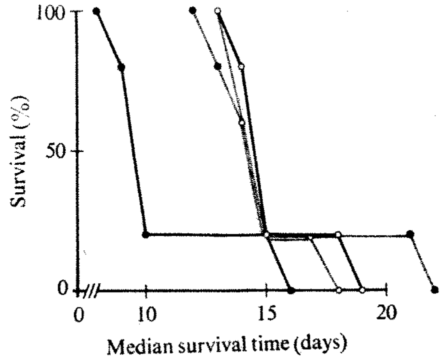


Fig. 1 Effect of rabbit antiserum, cyclophosphamide, or tumour dose reduction on median survival time.

	Tumour	Rx	MST	% prolonged
●——●	10^6	0	9.50	0
●---●	10^2	0	14.25	50
○——○	10^6	ATuS	14.50	53
○---○	10^6	Cy60	14.25	50

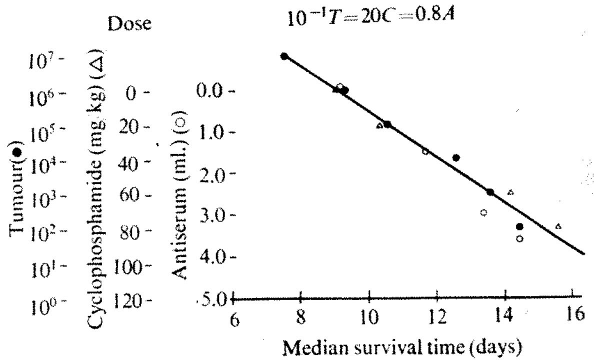


Fig. 2 Therapeutic equivalence of cyclophosphamide, rabbit antiserum and inoculum reduction (ATuS 5-69).

The dose related effects on survival are simultaneously displayed in Fig. 2, and are approximated as follows:

$$10^{-1}\ T=20\ C=0.83\ A$$

where T is the tumour dose (cells); C is the cyclophosphamide dose (mg/kg); A is the antiserum dose (total, ml.).

This experiment provides direct evidence for the hypothesis[17,18] that the effect of heterologous antiserum may compare favourably with the effect of chemotherapy in murine leukaemia. When produced and absorbed by methods similar to those used for ALS[19,20], heterologous antiserum seems to be of significant therapeutic benefit in passive immunotherapy of murine leukaemia.

GEORGE J. HILL, II

Department of Surgery,
University of Colorado Medical Center,
4200 E. 9th Avenue,
Denver, Colorado 80220

Received October 8, 1970; revised January 18, 1971.

[1] Gorer, P. A., *J. Pathol. Bact.*, **54**, 51 (1942).
[2] Werder, A. A., Kirschbaum, A., MacDowell, E. C., and Syverton, J. T., *Cancer Res.*, **12**, 886 (1952).
[3] Amos, D. B., and Day, E. D., *Ann. NY Acad. Sci.*, **64**, 851 (1956).
[4] Mihich, E., *Cancer Res.*, **29**, 848 (1969).
[5] Chirigos, M. A., March, R. W., Hook, W., and Hoemann, R., *Proc. Soc. Exp. Biol. Med.*, **125**, 35 (1967).
[6] Levi, E., Golden, R., Zerubavel, R., and Fisher, J., *Cancer Res.*, **26**, 659 (1966).
[7] Thompson, J., *Blood*, **10**, 1228 (1955).
[8] Nungester, W. J., and Fisher, H., *Cancer Res.*, **14**, 284 (1954).
[9] Mohos, S. C., and Kidd, J. G., *J. Exp. Med.*, **105**, 233 (1957).
[10] Alexander, P., Connell, D. I., and Mikulska, A. B., *Cancer Res.*, **26**, 1508 (1966).
[11] Guthrie, R. K., Ingerbrigtsen, N. A., Savage, N., Hinkle, D. C., and Farris, W. J., *Canad. J. Biochem.*, **41**, 305 (1963).
[12] Reif, A. E., and Kim, C. H., *Proc. Amer. Assoc. Cancer Res.*, **10**, 72 (1969).
[13] Parks, S. D., and Hill, G. J., *Current Topics in Surgical Research*, **2**, 247 (1970).
[14] Skipper, H. E., Schabel, jun., F. M., and Wilcox, W. S., *Cancer Chemother. Rep.*, **35**, 1 (1964).
[15] Lane, M., *J. Nat. Cancer Inst.*, **23**, 1347 (1959).
[16] Hill, G. J., Atkins, R. C., Parks, S. D., Littlejohn, K., and Eiseman, B., *Surg. Forum*, **20**, 122 (1969).
[17] Miller, D. G., Moldovanu, G., Kaplan, A., and Tocci, S., *Cancer*, **22**, 1192 (1968).
[18] Mardiney, jun., M. R., Block, J. R., and Serpick, A. A., *Cancer Chemother. Rep.*, **53**, 3 (1969).
[19] Monaco, A. P., Wood, M. L., Gray, J. G., and Russell, P. S., *J. Immunol.*, **96**, 229 (1966).
[20] Iwasaki, Y., Porter, K. A., Amend, J. R., Marchioro, T. L., Zuhlke, V., and Starzl, T. E., *Surg. Gynec. Obstet.*, **124**, 1 (1967).

Development of a Specific Anti-leukaemic Serum for the Treatment of Leukaemia in Clinics

LEUKAEMIC cells are antigenically indistinguishable from their normal counterparts and antibodies formed against them are therefore likely to exhibit cross-reactivity with normal cells. There is, however, a difference in the net surface charge[1]—the charge which regulates the ability of the antibodies to approach the target cells—which could be exploited to prepare antibodies specific to leukaemic cells. To this end, the net surface charge on the white blood cells of normal O group people was suitably altered by tagging the cells with chemicals such as 1-fluoro-2,4-dinitrobenzene (FDNB). These cells were then used as antigens for producing antibodies against human leukaemic cells in rabbits and horses. Here we give a brief account of the production of purified serum specific for leukaemic cells using immunized horse. A preliminary report on the production of specific anti-leukaemic serum from immunized rabbit has already been published[2].

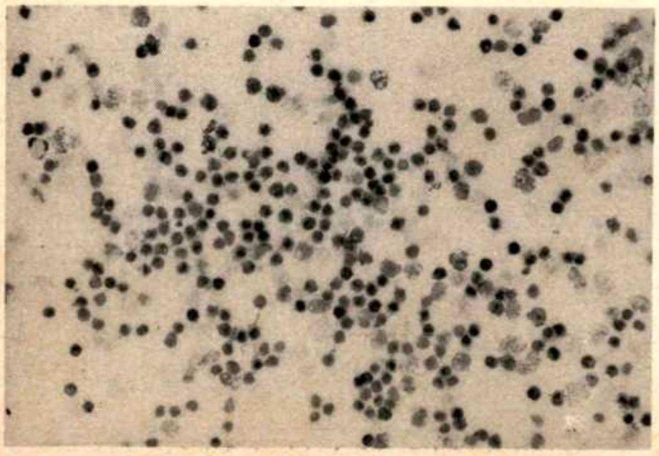


Fig. 1 Normal human WBCs treated with specific anti-leukaemic serum. No agglutination was seen.

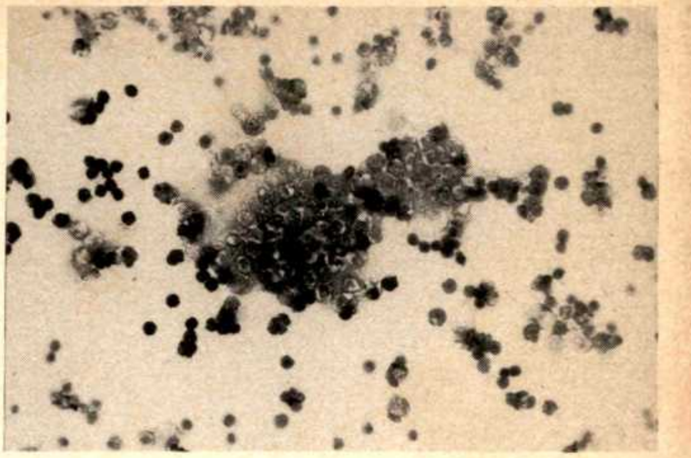


Fig. 2 Leukaemic cells treated with specific anti-leukaemic serum. Note the clumping and disintegration of the clumped up leukaemic cells.

Heparin, citrate or oxalate was added as anticoagulant to 300 ml. of blood from normal O group healthy people. Red blood cells were allowed to settle overnight with the help of dextran (5 ml. of 6% dextran to 100 ml. of blood). Supernatant and buffy coat were centrifuged to isolate the white blood cells. These were then alternately washed with normal saline and centrifuged at 600–800 r.p.m. at least five times to free the cells from plasma and anticoagulants. The few contaminating red blood cells were destroyed by a 30 s selective hypotonic shock[3,4] and the white blood cells were washed free of haemoglobin and stroma. This process was repeated until the white blood cells (WBCs) were completely uncontaminated. The viability of the WBCs was checked by the trypan blue dye-exclusion test of Ling[5]. They were then resuspended in normal sterile saline.

Saline suspension (1 ml.) containing 10^5 WBCs was mixed with 1 ml. of FDNB solution containing 10^9 molecules. The *p*H was adjusted to 7.2 and the mixture was shaken for 10 to 15 min at room temperature (22°–25° C). Untreated FDNB, if any, was removed from tagged cells by repeated washing and centrifuging (600–800 r.p.m.) with ten volumes of saline. FDNB tagged cells were resuspended in isotonic saline (10^7 cells/ml.). The tagged cells were again checked for viability by the dye-exclusion test.

Cell suspension (10 ml.) was injected subcutaneously to a horse each week for 22 weeks. The horse was then bled and the serum was prepared. The serum was heat inactivated and absorbed with pooled A, B and O human RBCs; the globulins were separated by ammonium sulphate and isoelectric precipitation[6]. The purified serum was tested for agglutination with leukaemic cells and normal WBCs (Figs. 1 and 2). Heparin was used as an anticoagulant[7] in the collection of blood samples. Non-specific aggregation of WBCs was prevented by minimizing the exposure of the WBCs to the anticoagulant (heparin) and by centrifugation at low speed (600–800 r.p.m.)[5] during the separation and washing of the cells. Titres were evaluated by the usual double dilution technique. Serial dilutions of the antisera were made with normal saline. Samples of 0.03 ml. containing 10^5 cells and 0.03 ml. of antisera at various dilutions were mixed in siliconized micro

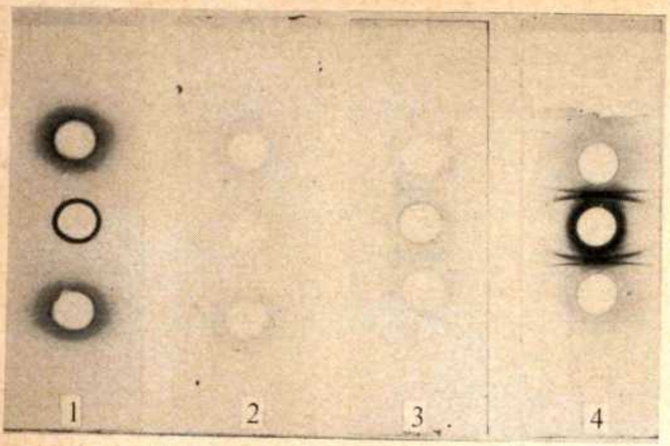


Fig. 3 Specificity of the anti-leukaemic serum demonstrated by the Ouchterlony gel diffusion method. Precipitation lines are obtained with leukaemic cells only. WBCs from normal subjects as well as plasma from normal or leukaemic persons did not reveal any cross-reactivity. Central well, anti-leukaemic serum. Top and bottom wells: 1, human plasma from normal persons; 2, human plasma from leukaemic patients; 3, membranous extracts of white blood cells from normal persons; 4, membranous extract of leukaemic cells. The extracts in wells 3 and 4 were from cells lysed by osmotic shock and the crude membranous material was extracted with mild alkaline solution (pH 7.4).

Table 1 Agglutination Titre with Purified Anti-leukaemic Serum against Leukaemic Cells separated from Leukaemic Patients

Patient No.	Blood group	Agglutination titre	Clinical diagnosis
Myeloid leukaemia cases:			
1	O	4,096	CML
2	A	8,192	CML
3	O	4,096	CML
4	B	4,096	CML
5	B	2,048	CML
6	O	2,048	CML
7	O	4,096	CML
8	AB	2,048	CML
9	A	2,048	CML
10	B	4,096	CML
11	O	4,096	AML
Lymphatic leukaemia cases:			
1	B	8,192	CLL
2	B	4,096	ALL
3	O	8,192	CLL
4	O	4,096	ALL
5	B	8,192	CLL

CML, Chronic myeloid leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphatic leukaemia; ALL, acute lymphatic leukaemia.

Table 2 Agglutination Titre with Purified Anti-leukaemic Serum against White Blood Cells from Normal People

No.	Blood group	Agglutination titre
1	B	0
2	O	0
3	A	0
4	B	0
5	O	4
6	A	0
7	B	0
8	O	0
9	B	0
10	B	0
11	O	4
12	O	0
13	B	2
14	AB	0
15	O	0

precipitin tubes and incubated at 37° C for 1 h. The tubes were centrifuged at 600–800 r.p.m. for 1 min and the degree of agglutination was determined. The maximum dilution of the serum which showed definite clumping (+1) was taken as the titre of the antisera. The specificity of the antiserum as well as the cross-reactivity, if any, with the plasma from normal human subjects and from leukaemic patients were studied by gel diffusion.

The agglutination titres are given in Table 1. The leukaemic cells were from a representative sample of sixteen leukaemic patients, eleven of whom had myeloid leukaemia, the other five having lymphatic leukaemia. Both types produced high titres compared with agglutination titres with normal WBCs (Table 2). The titres varied between 2,048 and 8,192 in both cases. Titres were negligible when normal WBCs were used.

The specificity of the anti-leukaemic serum was confirmed by clear-cut precipitin reactions with the leukaemic cells and by the lack of such precipitin reactions with normal WBCs (Fig. 3). Plasma proteins either from normal or leukaemic persons did not show any cross-reactivity. It is therefore clear that the anti-leukaemic serum is highly specific for leukaemic cells in general. A detailed report of purification, pharmacological studies and clinical trials will be published later.

M. B. SAHASRABUDHE
S. PREMA
K. R. MADYASTHA

Cancer Research Institute,
Parel,
Bombay-12

M. P. GOLLERKERI

Tata Memorial Hospital,
Parel,
Bombay-12

S. S. RAO

Haffkine Institute,
Parel,
Bombay-12

Received December 5, 1969; revised September 3, 1970.

[1] Ambrose, E. J., in *Cell Electrophoresis*, 79 (Churchill, London, 1965).
[2] Sahasrabudhe, M. B., *Indian J. Cancer*, **5**, 217 (1968).
[3] Astaldi, G., and Airo, R., *Blood*, **30**, 707 (1967).
[4] Fallon, H. J., Frei, E., Davidson, J. D., Trier, J. S., and Burk, D., *J. Lab. Clin. Med.*, **59**, 779 (1962).
[5] Ling, N. R., in *Lymphocyte Stimulation*, 38, 43 (North-Holland, Amsterdam, 1968).
[6] Wadsworth, A. B., in *Standard Methods of the Division of Laboratory and Research of New York State Department of Health*, third ed., 714 (1947).
[7] Thomson, A. E. R., and Mehrishi, J. N., *Europ. J. Cancer*, **5**, 195 (1969).

Induction of Specific Autoimmune Response to Leukaemic Cells in Human Leukaemia Patient by Chemically Tagged Normal "O" Group White Blood Cells

WHITE blood cells from normal healthy O group people, when suitably tagged with chemicals such as 1-fluoro-2,4-dinitrobenzene (FDNB), can be used as antigens to stimulate the production of antibodies to human leukaemic cells in rabbits and horse[1,2]. We have shown that chemically tagged normal O group white blood cells can be used successfully to induce a specific autoimmune response to leukaemic cells in human leukaemic patients.

The serum of a 26 year old male patient with acute lymphatic leukaemia who had been treated with 'Endoxan' (100 mg/day), 6-mercaptopurine (150 mg/day) and prednisolone (60 mg/day), and who was on a maintenance dose of prednisolone (5 mg/day) when the experiment was initiated, was tested for the presence of iso-antibodies against leukaemic cells from other patients. He was then immunized for 7 weeks with chemically tagged O group WBCs as described below.

Blood was periodically withdrawn to check the iso-antibody levels. Because the patient had a history of short remission periods, 6-mercaptopurine (150 mg/day) was administered in addition to the prednisolone during the immunization period. The patient was discharged from the hospital after seven weeks of the immunization schedule and a blood sample was obtained a month later in order to check the antibody levels.

O group WBCs from normal individuals were tagged with 1-fluoro-2,4-dinitrobenzene (FDNB)[2]. A sample (1 ml.) containing 10^7 FDNB-tagged cells was injected subcutaneously into the patient each week. The site of injection was varied. The patient experienced no discomfort or ill effects as a result of injections and the schedule was continued for 7 weeks. Blood (10 ml.) was withdrawn before the immunization schedule began to find out whether there were any inherent iso-antibodies present. This was repeated each week to check the production of iso-antibodies against leukaemic cells. The iso-antibody titre was determined by measuring the degree of agglutination with leukaemic cells from other patients, and also by studying the agar-gel precipitin reaction with leukaemic cells. Both procedures have been described before[2].

The immunological responses of patients with acute lymphatic leukaemia are believed to be comparatively poor. In addition, the patient was being given prednisolone, an immunosuppressive drug[3], as a maintenance dose. Yet the iso-antibody titre increased to 256 after 4 weeks (Fig. 1). The agar-gel diffusion test also revealed a single, clear precipitin line (Fig. 2). During treatment, when the patient was dosed with 6-mercaptopurine (150 mg/day), another potent immunosuppressive drug[3], the iso-antibody titre did not increase but remained at 128. The iso-antibody titre remained at that level even after the immunization schedule had been discontinued. This investigation suggests that it is possible to elicit a specific autoimmune response to leukaemic cells by using non-malignant antigens. Further data on use of active immunization in patients and their clinical response will be published later.

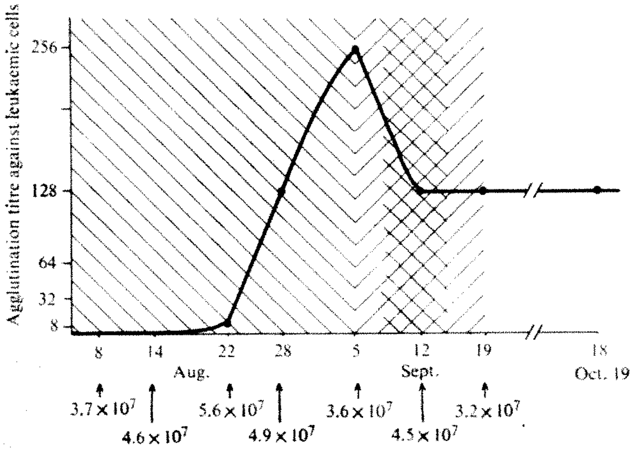


Fig. 1 Development of antibodies specific to leukaemic cells in human acute lymphatic leukaemia patient. Arrows indicate the schedule of immunization. ▧, Prednisolone administration; ▨, 6-mercaptopurine administration.

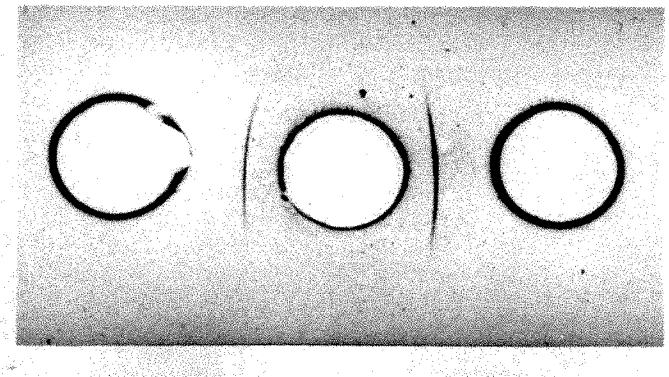


Fig. 2 Demonstration of specific precipitation reaction with the patient's serum against leukaemic cells by Ouchterlony gel diffusion method. Clear-cut precipitin lines were seen on either side of the central well. Central well: patient's serum after four immunizations; side wells: extracts of leukaemic cell membranes.

M. B. SAHASRABUDHE
K. R. MADYASTHA
S. PREMA

Cancer Research Institute,
Parel,
Bombay-12

R. D. LELE

Grant Medical College,
and J. J. Group of Hospitals,
Byculla,
Bombay-8

M. P. GOLLERKERI

Tata Memorial Hospital,
Parel,
Bombay-12

S. S. RAO

Haffkine Institute,
Parel,
Bombay-12

Received December 5, 1969; revised August 12, 1970.

[1] Sahasrabudhe, M. B., *Indian J. Cancer*, **5**, 217 (1968).
[2] Sahasrabudhe, M. B., Prema, S., Madyastha, K. R., Gollerkeri, M. P., and Rao, S. S., *Nature*, **232**, 197 (1971).
[3] Berenbaum, M. C., in *Immunity, Cancer and Chemotherapy* (edit. by Mihich, E.), 217 (Academic Press, 1967).

Synthesis of Nitrosopiperidine from Nitrate and Piperidine in the Gastro-intestinal Tract of the Rat

MANY nitrosamines are carcinogenic to a wide range of animal species[1-4]. The synthesis of nitrosamines from nitrite and secondary amines has been demonstrated *in vitro* with animal and human gastric juice[5,6] and *in vivo* in animals[6]; nitrosamine synthesis was *p*H-dependent[6-9]. The synthesis of the non-carcinogenic diphenylnitrosamine has been reported in the human stomach after oral administration of nitrate and diphenylamine[7]. Nitrosamines can also be synthesized from nitrate and secondary amines *in vitro* at near neutral *p*H in the presence of enteric enteria[10]. We have demonstrated *in vivo* synthesis of nitrosopiperidine from nitrite and piperidine in the stomach and small intestine of the rat[11]. We report here *in vivo* synthesis of the carcinogenic nitrosopiperidine from nitrate and piperidine in the rat stomach and small intestine, and also with rat gastric contents *in vitro*.

Male Sprague–Dawley rats weighing 200–275 g were used. Methods used for *in vitro* studies with the gastric contents and for *in vivo* studies using the stomach or small intestine have been described before[11]. The ranges of *p*H values for stomach and small intestine were 3.4–4.3 and 6.3–6.8, respectively. For *in vivo* studies, solutions of sodium nitrate and piperidine were incubated for 30 min in the isolated intestine and/or in the stomach of the same rat or of different rats (Table 1). After incubation, intestinal and stomach contents were removed and extracted with dichloromethane as described before[11]; the solvent extract was dried overnight with sodium sulphate using a mechanical shaker. Nitrosopiperidine in extracts was quantitatively determined by gas–liquid chromatography[11]. The identity of nitrosopiperidine was confirmed by thin layer chromatography[12] and by additional use of another column of 3% QF-1 (Dow Corning fluoroalkyl silicone) on silanized gas chrom p. Using these techniques, the recovery from saline of a standard sample of pure nitrosopiperidine was 35%.

Table 1 Formation of Nitrosopiperidine from Sodium Nitrate and Piperidine Hydrochloride

	Reactant concentrations (mg)			Nitrosopiperidine yield† (μg)			
				In different rats *in vivo*		In the same rat *in vivo*	
Replicate*	Piperidine HCl	$NaNO_3$	Gastric juice *in vitro*	Stomach	Small intestine	Stomach	Small intestine
1	1,250	25	25	44	54	39	34
2	1,250	25	27	40	20	30	40
3	1,250	25	24	41	8	38	8
4	1,250	25	13	8	2	32	1

* Each replicate is based on four rats.
† Estimated by gas–liquid chromatography.

Nitrosopiperidine was synthesized from nitrate and piperidine *in vivo* in the stomach and small intestine and from gastric juice *in vitro* (Table 1). Yields of nitrosopiperidine *in vivo* were generally somewhat higher in the stomach than in the small intestine, following incubation in the same or in different rats. Yields *in vitro* in the gastric juice were comparable with *in vivo* yields. These yields of nitrosopiperidine are underestimates in view of the relatively low recoveries of standard samples of nitrosopiperidine. Additionally, no effort was made to correct for nitrosopiperidine possibly absorbed during *in vivo* incubation.

The yields of nitrosopiperidine following *in vitro* and *in vivo* synthesis from nitrite and piperidine[11] were greater than for corresponding yields from comparable concentrations of nitrate and piperidine, as reported here. The synthesis of nitrosopiperidine even in small amounts, however, may have cumulative toxic effects, for nitrosopiperidine is a potent carcinogen[13]. Our results are in accord with human data on *in vivo* gastric synthesis of diphenylnitrosamine from nitrate and diphenylamine[7]; bacterial reduction of nitrate and nitrosamine synthesis in these studies was *p*H-dependent. The synthesis *in vivo* of the carcinogenic nitrosamine nitrosopiperidine from nitrate and piperidine is of practical interest in view of the common dietary occurrence of these precursors. Piperidine is a urinary constituent of normal human subjects, presumably formed by intestinal bacterial decarboxylation from dietary lysine[14]. Piperidine may also be ingested by man through some foods and spices; some bread flavouring agents contain piperidine[15]. Sodium nitrate and nitrite are used in the United States and elsewhere as preservatives and colour fixatives in cured meats and fish at permissible levels of 500 and 200 p.p.m. respectively[16]. Nitrite is also used alone at levels below 10 p.p.m. in smoked and cured tunafish. The use of nitrate or nitrite in certain types of cheese is permitted in some European countries. Nitrates are also widely distributed in many plants. Certain plants such as beets and spinach accumulate nitrates, especially when overfertilized[17] and in conditions of molybdenum deficiency[18–20]. Further, nitrate levels in drinking water, particularly in certain agricultural areas, may be high enough to induce acute methaemoglobinaemia in infants[21–25]. The transplacental transmission of nitrates and the development of methaemoglobinaemia in the foetuses of rats drinking water with high concentrations of nitrates has been recently demonstrated[26]. The implication of these findings to human cancer and the propriety of the continued use of nitrate and nitrite as food additives, and of drinking water with a high nitrate content, clearly merits critical consideration.

These studies were supported by grants from the US National Institutes of Health and National Air Pollution Control Administration.

B. S. Alam
I. B. Saporoschetz
S. S. Epstein

Laboratories of Environmental Toxicology and Carcinogenesis,
Children's Cancer Research Foundation, Inc.,
and the Department of Pathology,
Harvard Medical School,
Boston,
Massachusetts 02115

Received January 22, 1971.

[1] Magee, P. N., and Barnes, J. M., *Brit. J. Cancer*, **10**, 114 (1956).
[2] Magee, P. N. and Barnes, J. M., *Adv. Cancer Res.*, **10**, 163 (1967).
[3] Schmahl, D., and Osswald, H., *Experientia*, **23**, 497 (1967).
[4] Lijinsky, W., and Epstein, S. S., *Nature*, **225**, 21 (1970).
[5] Sander, V. J., *Arch. Hyg. Bakt.*, **151**, 22 (1967).
[6] Sen, N. P., Smith, D. C., and Schwinghamer, L., *Food Cosmet. Toxicol.*, **7**, 301 (1969).
[7] Sander, V. J., and Sief, F., *Arzneimittel-Forschung*, **19**, 1091 (1969).
[8] Sander, V. J., Schweinsberg, F., and Menz, H. P., *Z. Physiol. Chem.*, **349**, 1691 (1968).
[9] Mirvish, S. S., *J. Nat. Cancer Inst.*, **44**, 633 (1970).
[10] Sander, V. J., *Z. Physiol. Chem.*, **349**, 429 (1968).
[11] Alam, B. S., Saporoschetz, I. B., and Epstein, S. S., *Nature* (in the press).
[12] Sen, N. P., Smith, D. S., Schwinghamer, L., and Marleau, J. J., *J. Assoc. Off. Analyt. Chem.*, **52**, 47 (1969).
[13] Druckrey, H., Preussman, R., Ivankovics, S., and Schmahl, D., *Z. Krebsforsch.*, **69**, 103 (1967).
[14] Blau, K., *Biochem. J.*, **80**, 193 (1961).
[15] US Patent No. 3,336,140: CA 67,899,046 (1967).
[16] *Code of Federal Regulations*, 121 (1963–1964).
[17] Schupan, W., and Ernahr, Z., *Wissenschaften*, **6**, 207 (1965).
[18] Hewitt, E. S., and Jones, E. W., *J. Pomol. Hort. Sci.*, **23**, 254 (1947).
[19] Vlitos, A. J., in *Chemical and Biological Hazards in Food* (edit. by Ayers, J. C., Kraft, A. A., Snyder, H. F., and Walker, H. W.), 89 (Iowa State University Press, 1962).
[20] Brown, J. R., and Smith, G. E., *Res. Bull. University Missouri College of Agric.*, No. 920 (1967).
[21] Knotech, Z., and Schmidt, P., *Pediatrics*, **34**, 78 (1964).
[22] Steyn, D. G., Publ. No. 11, Univ. Pretoria, Union of South Africa (1960).
[23] Werner, V., and Thal, W., *German Med. Monthly*, **10**, 142 (1965).
[24] *Cyanosis of Infants Produced by High Concentration in Rural Wells* (*WHO/MCH*/13) (1949).
[25] Simon, C., Mazke, M., Kay, H., and Mrowitz, G., *Z. Kinderheilk*, **91**, 124 (1964).
[26] Nachman, G., and Shuval, H. I., in *Water Quality Research*, 89 (Ann Arbor–Humphrey Science publication, 1969).

Distribution and Concentration of Mercury in Autopsy Specimens of Human Brain

The dangers of mercury, a pollutant of natural bodies of water and a metal with a strong tendency to accumulate in the nervous tissues, have recently caused considerable concern. We have studied the distribution and concentration of mercury in the brain in order to determine the levels normally present.

Eight autopsy specimens were selected at random for the study. The subjects (Table 1) had lived, at least briefly, in an area where mercurial contamination of the regional inland waters and their fish has been demonstrated, but there was otherwise no known pathological exposure to the metal. The area includes Buffalo, New York, and the adjacent Great Lakes. Six brains were taken from the subjects 6–30 h after death; Nos. 1 and 2 were fixed in formalin before dissection. The tissues were analysed for mercury by neutron activation

Table 1 Content of Mercury in Various Regions of the Human Brain

Subject No.	1	2	3	4	5	6	7	8
Age and sex (yr, M/F)	70M	58M	73M	68F	79M	74M	33M	Full term stillborn M
Area of brain								
Frontal lobe cortex	——	0.25	0.12	0.03	0.11	1.69	0.06	0.04
Cerebellar cortex	0.71	0.72	0.67	0.08	0.48	1.85	0.10	0.04
Medulla	——	——	0.12	0.04	0.18	1.29	0.19	——
Pons	0.43	0.30	0.19	0.05	0.25	1.66	0.05	——
Mid brain	——	0.24	0.11	0.04	0.08	1.20	0.06	——
Corpus striatum	——	0.11	0.09	0.03	0.06	0.61	0.06	——
Thalamus	——	0.16	0.07	0.04	0.11	1.47	0.09	0.04
White matter	0.15	0.13	0.03	0.03	0.04	0.15	0.02	0.05
Angular and supramarginal Gyri	——	0.23	0.07	0.03	0.15	2.00	0.04	0.04

Values are expressed as p.p.m. of wet weight tissue.

analysis (presented by S. K. K. P., C. C. Thomas, jun., and J. A. Sondel at meeting of Amer. Chem. Soc., 1971). After neutron irradiation the tissues were wet ashed in a nitric, sulphuric and perchloric acid mixture with mercury carrier in reflux conditions. A preliminary sulphide precipitation was followed by isolation of the mercury by electrodeposition. The radioactivities from ^{197}Hg and ^{197m}Hg were measured by gamma-ray spectrometry to determine the levels of the metal in the samples. This method readily allowed the quantitation of mercury in the range 0.01 to 2.0 μg/g of wet tissue with a sample size of 1–3 g. The accuracy was better than 15% at mercury levels of 0.01 p.p.m. and exceeded 5% at concentrations of 2 p.p.m.

The relatively small number of patients precluded the definition of absolute normal values but some valid observations can be made. As observed in Table 1, patient No. 6 is remarkable in that he had very large amounts of mercury in all regions of the brain except in the white matter. This patient had been considered a chronic alcoholic with chronic brain syndrome. He had acquired syphilis in 1929 but it could not be determined whether he had been treated with mercurial preparations.

Generally, the highest concentrations of mercury were found in the cerebellum. This led us to consider whether this structure normally contains relatively high levels of mercury or whether this element is preferentially accumulated at this site. The former assumption would appear tenable, at least to some degree, for in those subjects whose cerebellar values were not maximal, either all the regions of the brain including the cerebellum had very high levels (No. 6) or the cerebellar concentration tended to be relatively prominent (No. 7). Further support for this thesis can be inferred from the data of subject No. 4. The brain of this patient consistently contained the lowest or nearly the lowest concentrations of mercury in all areas studied in comparison with other subjects. The concentration in the cerebellum nevertheless exceeded the other areas in this brain in all instances but one by a factor of at least two. Conversely, the high incidence of clinically demonstrable cerebellar malfunction and gross cellular destruction in this region in patients with documented mercurial poisoning supports the concept of localized preferential accumulation[1,4]. The significance of the mercurial concentration in the foetal cerebellum (0.04 p.p.m.) could not be readily evaluated because of incomplete sampling due to post-mortem autolysis.

The pons is also of interest since in patients Nos. 2, 3, 4 and 5 the pontine concentration of mercury was exceeded only by that of the cerebellar cortex.

Except for the one brain that had a larger concentration of mercury (No. 6), the sampled areas of the formalin-fixed brains (Nos. 1 and 2) contained a greater amount than the other specimens. An analysis of the fixative was not performed.

The white matter of the hemispheres consistently had the lowest or near lowest levels in all individuals. This low profile suggests a correlation between anatomical structure and accumulation of the metal. The white matter is primarily composed of nerve fibres and is devoid of the cell bodies of nerve cells. Thus, this metal seems to be predisposed to localization in the perykarya of nerve cells rather than in dendritic or axonic processes. The ability of mercury to enter enzymatic reactions as well as its presence in microsomal and mitochondrial fractions of mercury-loaded nervous tissue[5] are consistent with this concept.

All regions of the brain examined revealed the presence of mercury. Since the observed levels are within the limits of accuracy of the method used, mercury may well be present as a normal constituent of neuronal tissue. Further studies are necessary to confirm this possibility.

We thank Dr Walter Olszewski, Buffalo General Hospital, for supplying the human brains.

CHESTER A. GLOMSKI
HAROLD BRODY

Department of Anatomy,
State University of New York at Buffalo,
Buffalo, New York 14214

SIVASANKARA K. K. PILLAY

Nuclear Research Center

Received January 25; revised March 25, 1971.

[1] Takeuchi, T., in *Proc. Intern. Conf. Env. Contamination* (University of Michigan, Ann Arbor, in the press).
[2] Suzuki, T., in *Chemical Fallout* (edit. by Miller, M. W., and Berg, G. G.) (Thomas, Springfield, Illinois, 1969).
[3] Berglund, F., and Berlin, M., in *Chemical Fallout* (edit. by Miller, M. W., and Berg, G. G.) (Thomas, Springfield, Illinois, 1969).
[4] Hunter, D., and Russell, D. S., *J. Neurol. Neurosurg. Psychiat.*, **17**, 235 (1954).
[5] Yoshino, Y., Mozai, T., and Nakao, K., *J. Neurochem.*, **13**, 397 (1966).

Accelerated Elimination of Methyl Mercury from Animals

WE wish to suggest one principle that may be valuable in the treatment of methyl mercury poisoning—the notion that continuous oral administration of indigestible unabsorbable SH-compounds such as reduced hair powder may accelerate the elimination of methyl mercury from mice.

Organic mercury injected into animals is known to be[1,2] fairly well excreted into the bile for a considerable time and we have confirmed this result and have studied the chemical nature of the mercury excreted into bile after three rats (weighing about 200 g) were intramuscularly injected with 2 mg of methyl mercuric chloride each day for 5 days. The rats were killed 48 h after the last injection and the contents of the small intestine from three were washed out with 0.95 M phosphate buffer (*p*H 7.0) and pooled. The total mercury found in this material was 106.6 µg and about 85% of it was found in the high speed supernatant. The supernatant was used for the following studies. When a portion of it was treated with 1 M HCl for 1 day, extracted with benzene, and run on a gas chromatograph column containing 10% 1,4-butanediol succinate on 'Chromosorb W' and fitted with an electron capture detector, 83% of the mercury was found to be in the form of methyl mercury. It is not clear whether the rest of the mercury is organic or not. When the supernatant was directly extracted with benzene, 23% of the mercury was present as free methyl mercuric chloride. And when the supernatant was dialysed against forty volumes of 0.05 M phosphate buffer (*p*H 7.0) for 24 h, 80% of the mercury was found to be in the form of dialysable, low molecular derivatives. When 1 g of human hair powder treated with thioglycollate as described later was added to the supernatant, 82% of the mercury was bound to the hair powder and precipitated during 30 min incubation at 37° C. After repeating the experiment and obtaining a very similar result, our conclusion was that most of the mercury excreted in the bile was methyl mercury in the form of its chloride or low molecular weight compounds.

We thought that trapping these forms of mercury and protecting them from reabsorption from the intestine would decrease the accumulation of mercury in the animals. To test this idea we prepared human hair powder reduced with thioglycollate, a substance that has a good methyl mercury binding capacity because of the cysteine SH group and because it is resistant to trypsin digestion. About 20 g of clean human hair was cut into very small pieces with scissors and ground with a strong conical homogenizer in 400 ml. of dilute ammonia (*p*H 11) containing 9.3 g of thioglycollic acid. After adding another 5 g of thioglycollic acid the mixture was allowed to stand at room temperature for 2 h under nitrogen. The precipitates were collected by centrifugation, washed once with plenty of water and three times with acetone and with ether. The material was dried and stored in an evacuated desiccator. One gramme of this material could bind 60–70 mg of methyl mercuric chloride. It could also recover 80% of 100 µg of methyl mercuric chloride injected into mice. In this experiment, a whole mouse (20 g) was ashed with 20 ml. of concentrated HNO_3 and 20 ml. concentrated H_2SO_4 for 5 h and then reheated for 30 min before addition of 5 g of potassium permanganate. The mercury content of the ash and the recovery were measured by the dithizone method[3,4]. In a feeding experiment twenty-eight female mice (16–17 g) of the DD strain were poisoned by a single intramuscular injection of 200 µg of methyl mercuric chloride in saline solution and divided into two groups; one was fed on ordinary food (CREA II) and the other was fed the same diet plus 3.3% hair powder for 10 days. In another experiment thirty-four mice were poisoned with 400 µg of methyl mercuric chloride and the feeding was continued for 20 days. They were then killed. The results of mercury analysis of each animal are shown in Figs. 1 and 2.

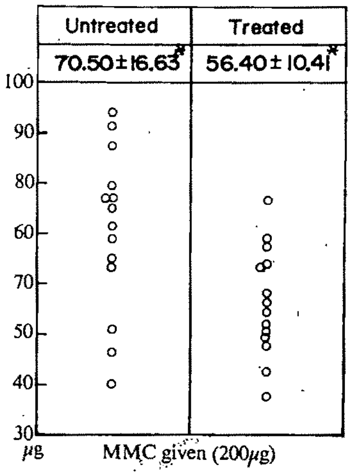


Fig. 1 Total mercury after 10 days. *Mean ± s.d. The difference was significant ($P < 0.005$).

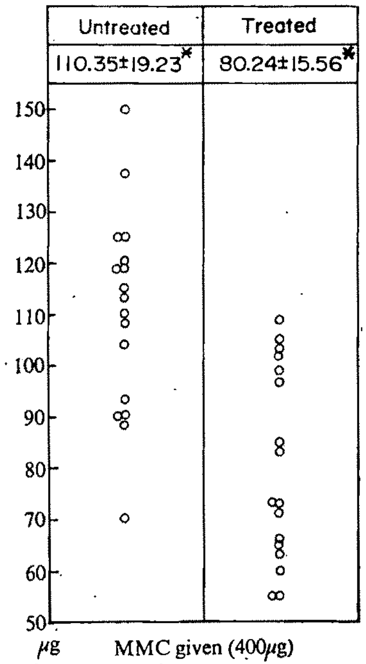


Fig. 2 Total mercury after 20 days. * Mean ± s.d. The difference was significant ($P < 0.005$).

The reduced human hair powder reduced the level of the residual methyl mercury in the mice poisoned with methyl mercuric chloride, indicating that methyl mercury excreted into intestine is eliminated by this procedure in the faeces. The results also suggest that reabsorption of methyl mercury excreted into bile is one of the reasons for the prolonged biological half life of methyl mercury in the animal and that any material which has methyl mercury binding capacity and is resistant to digestion and absorption from the intestine could be useful for eliminating methyl mercury from animals. Third, synthetic resins with SH groups may be effective for the same purpose. The procedure may be used in cases of accidental ingestion of organic mercury or it may be used prophylactically when food is consumed that is contaminated with methyl mercury.

We thank Dr Motoo Fujiki for assistance with gas chromatography.

HITOSHI TAKAHASHI
KIMIKO HIRAYAMA

Department of Biochemistry,
Toxicology Institute,
Kumamoto University Medical School, Kumamoto

Received March 10, 1971.

[1] Berlin, M., and Ullberg, S., *Arch. Env. Health*, **6**, 610 (1963).
[2] Friberg, L., *Amer. Med. Assoc. Arch. Indust. Health*, **20**, 50 (1959).
[3] Sandel, E. B., *Colorimetric Determination of Traces of Metals*, third ed., 621 (1959).
[4] Kai, F., *Kumamoto Igakukai Zattshi*, **37**, 19 (1963).

Two-stage Recovery of a Response in *Sepia*

A RESPONSE that has waned as a result of continuous stimulation without reinforcement may reappear when the stimulus is presented on another occasion: habituation, as defined by Thorpe[1], is "relatively permanent" but not complete. The extent of recovery of the response will clearly be a function of the length of time between presentations, but several workers, notably Hinde[2,3], have shown that the relation between recovery and rest interval is not a simple one. Recovery seems to occur in at least two stages, possibly reflecting the existence of separate underlying physical processes. Some recent experiments with the cuttlefish, described here, have revealed a two-stage recovery with much sharper separation than has been found previously.

Cuttlefish (*Sepia officinalis* L.) feed on prawns, which they capture by a rapid strike with the paired tentacles[4]. The strike is elicited entirely by visual cues so that a prawn presented behind glass is attacked in the usual way. Since the strike fails to seize the prawn, the cuttlefish attacks repeatedly but the rate of striking soon begins to diminish and a typical decremental response curve is obtained. We are here concerned only with the recovery of the response after it has waned: specifically, does the number of stikes in a second presentation period depend on the interval since the first presentation period?

A 'Perspex' cylinder containing seawater and two prawns was placed in a tank about 50 cm from a naive cuttlefish and was then left there for 20 min. During the first 5 min of this period the animal was observed and the number of strikes (*a*) it made was recorded. After a further 15 min, during which the animal was not observed, the tube was removed for an interval and was then replaced for a further 5 min. The number of strikes (*b*) in this second period was recorded and the ratio $\left(\frac{b}{a}\times 100\right)$ was calculated to give a measure of the percentage recovery of the response for that animal. Animals were tested after eleven different intervals varying from a few minutes to a day. At least twelve animals were tested after each interval; twenty were tested after the intervals 22 min, 30 min, 45 min and 60 min. Each cuttlefish was tested once and then discarded.

As would be expected the response (Fig. 1), which had waned almost completely during the 20 min initial presentation period, showed no recovery when the prawn was offered a few minutes afterwards. More surprising is that even 24 h later the response had only recovered to about 14% of its former value. These animals therefore showed considerable retention. They rarely attacked an animal that is their natural prey, though they attacked crabs presented at the end of the second presentation period: the waning of the response was evidently stimulus-specific and not simply the result of sensory adaptation. Moreover, because crabs were often caught with the tentacles (instead of with the arms alone[5]), it is unlikely that motor fatigue is an important factor in the waning of the response.

It is interesting that the recovery curve is not smooth: the response began to recover and then declined, until at 60 min it had almost disappeared. It then recovered again and by 90 min the response had apparently reached asymptote. That the "dip" in the curve between intervals of 22 min and 90 min is not the result of random fluctuation can be shown by χ^2 analysis of the frequencies of attacks during the first and second presentation, for all these rest intervals. In a 2×5 contingency table $\chi^2=34.89$ (d.f.$=4$, $P<0.001$). Furthermore, there is a highly significant difference not only between performance at 60 m and 90 m ($\chi^2=33.28$) but also between 60 m and 45 m ($\chi^2=13.78$); for both $P<0.001$ (d.f.$=1$). The experiment thus produces the apparently anomalous result that cuttlefish retain more about an event after 1 h than after 20 min.

How can this curve be explained? If it were classical "habituation" Hinde would claim that it results from the interaction of incremental and decremental processes with differing time courses, as, for example, in the recovery of the chaffinch mobbing response[3,6]. But on present evidence the possibility cannot be excluded that the behaviour is being influenced by negative reinforcement, by pain input from receptors in the tentacles. Several hard strikes of the tentacles on the 'Perspex' may be ample for learning in such fast-learning animals[7]. This experiment, then, may provide an example of a conventional "learning" situation. In such a situation Sanders and Barlow[8] have collected data for *Octopus* which show a tripartite retention curve, and their interpretation of this is that there are three memory systems: short, intermediate and long term. It is interesting that the transition from what they call "short-term" to "intermediate" memory occurs at a comparable time to the transition between the two stages of recovery shown here in *Sepia*, which could also be due to the operation of memories of differing time-course.

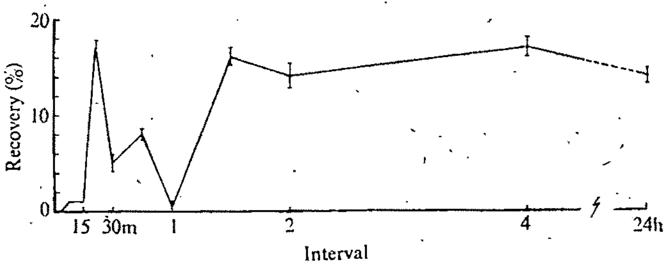


Fig. 1 Percentage recovery as a function of rest interval. Each point is the mean of at least twelve animals: bars indicate s.e.m. Means and s.e. were calculated after arcsin transformation of percentage scores.

This work was supported by the Science Research Council. I thank the staff of the Stazione Zoologica, Naples, for use of facilities there, and J. J. Barlow and G. D. Sanders for much helpful discussion.

J. B. MESSENGER

Department of Zoology,
The University,
Sheffield S10 2TN

Received February 4; revised March 26, 1971.

[1] Thorpe, W. H., *Learning and Instinct in Animals*, second ed. (Methuen, London, 1963).
[2] Hinde, R. A., *Proc. Roy. Soc.*, B, **142**, 306 (1954).
[3] Hinde, R. A., *Proc. Roy. Soc.*, B, **153**, 398 (1960).
[4] Messenger, J. B., *Anim. Behav.*, **16**, 342 (1968).
[5] Boycott, B. B., *New Biol.*, **25**, 98 (1958).
[6] Hinde, R. A., in *Short-term Changes in Neural Activity and Behaviour* (edit. by Horn, G., and Hinde, R. A.) (Cambridge University Press, 1970).
[7] Sanders, F. K., and Young, J. Z., *J. Neurophysiol.*, **3**, 501 (1940).
[8] Sanders, G. D., and Barlow, J. J., *Nature*, **232**, 203 (1971).

Variations in Retention Performance during Long Term Memory Formation

RETENTION performance as a function of time generally follows a typical monotonically decreasing curve[1-3], but there are a few reports showing an improvement with time[4-9]. Monotonic retention curves have been obtained with octopuses for short-term memory (STM)[10] and for long term memory (LTM)[11]. Here we present a preliminary report of one of a series of experiments using a rapid training procedure which enables us to study changes in retention performance over a period presumed to include the time courses of both STM and LTM.

Experimentally naive *Octopus vulgaris* (150–400 g) which had been in the laboratory for at least 5 days were used when they were attacking and eating crabs readily. These octopuses were trained not to attack a crab (*Carcinus*) in conditions similar to those in which Young[12] has shown that learning is stimulus specific. Training began by placing the crab, tied to a transparent rod, in the home tank at about 50 cm from the octopus. Just before the octopus completed its attack the crab was removed, the octopus was given a 4 V a.c. shock and the crab was immediately replaced. (An attack was judged to have occurred when it was necessary to remove the crab in

Pur Che istry Library

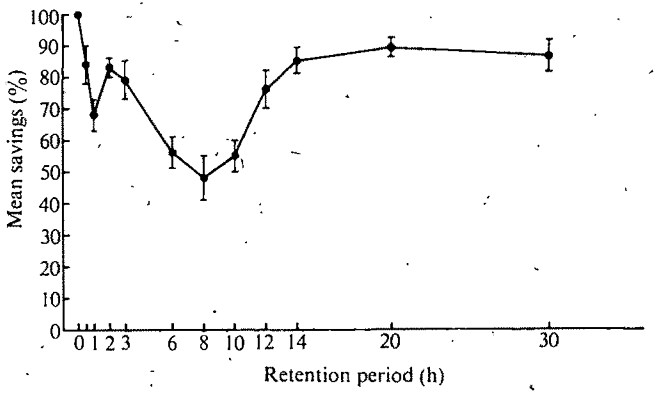


Fig. 1 Retention curve based on the mean percentage savings of octopuses trained and retrained using a 4 V shock to a criterion of no attack on a crab for a period of 1 min. Each point represents the mean scores of six or seven animals. The vertical bars mark one standard error above and below the mean.

order to prevent the octopus from touching it with its arms.) This sequence was repeated until a criterion time of 1 min passed during which there was no attack on the crab. The number of attacks and their latencies were recorded. Different groups of octopuses were retrained by the same procedure after retention intervals of 0.5–30 h. The groups were trained at different times throughout the day so that all retraining occurred during the same period (15.00–19.00 h). The differences between the attacks on training and retraining, expressed as a percentage of the training attacks (% savings), were used as a measure of retention performance. No octopus was retrained more than once.

The mean percentage savings (Fig. 1) decrease rapidly to 68% at 1 h but greater savings are made at 2 h (83%). From 2 h the savings fall steadily to 48% at 8 h only to increase again until at 20 h the octopuses show greater savings on retraining than at any previous time. Retention performance is still good at 30 h. The savings at 1 h are significantly lower than those at 0.5 h ($t=2.00$, $P<0.1$) and at 2 h ($t=2.50$, $P<0.05$). The savings at 8 h are significantly lower than those at 2 h ($t=4.52$, $P<0.01$) and at 14 h ($t=4.59$, $P<0.001$). The octopuses retrained at 1 h made nearly twice as many attacks as those retrained at 2 h while the difference in retraining attacks between 8 and 14 h was three-fold (Table 1). It seems, therefore, that in these conditions retention performance is not monotonically related to the length of the retention interval.

Table 1 Number of Attacks made at Various Intervals after Training

	Retention period (L)										
	0.5	1	2	3	6	8	10	12	14	20	30
No. per group	6	6	6	7	6	6	7	6	6	7	6
No. of training attacks: mean	13	15	15	16	15	15	18	18	16	16	14
s.d.	3.7	4.8	4.4	5.4	2.6	4.4	3.4	4.6	5.6	5.4	3.4
No. of retraining attacks: mean	1.8	4.7	2.7	3.1	6.3	7.7	8.0	4.5	2.3	2.0	2.0
s.d.	1.1	1.7	1.5	2.1	1.2	2.9	2.1	2.7	1.5	1.7	1.9

Several variables may have affected the tendency to attack. All octopuses were last fed with crabs during the afternoon of the day before they were trained. The time between feeding and retraining was therefore about 24 h for all except the 20 and 30 h groups. Thus the principal variations in the numbers of retraining attacks were all obtained at approximately the same hunger level. To carry out retraining during the same part of the day and at the same hunger level, training was given at different times of day. The numbers of training attacks may, therefore, have been influenced both by the hunger level and by possible variations in the daily activity pattern of the octopuses. The mean training attacks do show some variation (Table 1) but they are not correlated with the mean numbers of retraining attacks ($r=+0.42$, not significant at the 10% level). The differences in training performance would not account for the shape of the retention curve. Changes in activity during the day would not have influenced the retraining attacks as all retraining occurred during the same part of the day. The variation in sea water temperature (16–19° C) was probably not sufficient to have affected the results. We conclude that the variations in the retraining attacks are dependent on the length of the retention interval.

Although there may be other explanations of the shape of this curve we think it most likely that it reflects the state of the available memory. The results would then indicate that the memory for an event is maintained by three processes with different rise and fall times. These may be identified as STM, an intermediate memory (IM) and LTM. According to this hypothesis STM appears during training and declines rapidly, IM appears at about 2 h and declines less rapidly than STN, while LTM appears after approximately 12 h and declines slowly. The STM and IM control behaviour until LTM is available. A similar curvilinear function has been obtained by training *Octopus* with a 10 V and a 3 min criterion. Other combinations of these parameters tended to produce monocurves with different overall levels of retention performance. This suggests that the present results can be observed only within a limited set of training conditions. A detailed report of these experiments is in preparation.

Previous investigators have found dips in retention curves at 1 h using rats[4,5], at 30 min using cuttlefish[9] and at 2 min (ref. 6) and 30 min (ref. 8) using mice. None of these experiments included systematic tests between 2 and 24 h and so there are, to our knowledge, no reports of the second dip which we find with *Octopus* at 8 h. Evidence for multiple memory storage has depended to a large extent on demonstrations of the selective blocking of memory with electroshock, anaesthetics or antibiotics. On the basis of this evidence Booth[13] has estimated the time courses of successive physical mechanisms for holding retrievable information during memory consolidation. His expected rise and fall times for the three mechanisms are comparable with those obtained in the present experiment. In view of these observations it seems possible that a system of three memory processes may be universal.

This work was financed by a grant from the Science Research Council of Great Britian and, in part, by a fellowship from the Royal Society (G. D. S.). We thank Dr J. B. Messenger for showing us his results before publication.

G. D. SANDERS
J. J. BARLOW

Anatomy Department,
University College,
London WC1 E 6BT
and Stazione Zoologica, Naples

Received January 29, 1971.

[1] Sperling, G., *Psychol. Monog.*, **74**, 498 (1960).
[2] Peterson, L. R., and Peterson, M. J., *J. Exp. Psychol.*, **58**, 193 (1959).
[3] Ebbinghaus, H., *Ueber das Gedaechtnis: Untersuchungen zur Experimentellen Psychologie* (Dunker and Humbolt, Laizig, 1885).
[4] Kamin, L. J., *J. Comp. Physiol. Psychol.*, **50**, 457 (1957).
[5] Denny, M. R., and Ditchburn, R. E., *J. Comp. Physiol. Psychol.*, **55**, 1069 (1962).
[6] Irwin, S., Kalsner, S., and Curtis, A., *Fed. Proc.*, **23**, 102 (1962).
[7] McGaugh, J. L., *Science*, **153**, 1351 (1966).
[8] Zerbolio, D. J., *Psychon. Sci.*, **15**, 57 (1969).
[9] Messenger, J. B., *Nature*, **232**, 202 (1971).
[10] Wells, M. J., *J. Exp. Biol.*, **47**, 393 (1967).
[11] Sanders, G. D., *Brain Res.*, **20**, 59 (1970).
[12] Young, J. Z., *J. Exp. Biol.*, **52**, 385 (1970).
[13] Booth, D. A., *Molecular Mechanisms in Memory and Learning* (edit. by Ungar, G.), 29 (Plenum Press, New York, 1970).

BOOK REVIEWS

Communities of Sciences

Politics and the Community of Science. By Joseph Haberer. Pp. vi + 337. (Van Nostrand Reinhold: New York and London, February 1971.) £4.50.

Few would disagree with the statement of Dr Haberer that there is a crying need to throw "some light on the political nature of the community of science" and "the political behaviour of its members". Unfortunately, his book, which has been well received in the United States, strikes me as singularly unilluminating.

Professor Haberer is a political scientist who wishes to use history for the purpose of illustrating arguments about how scientists ought to act during social and professional crises. For the author, understanding the past on its own terms is clearly a secondary activity: "political analysis", he asserts on page 218, "does not require exhaustive historical reconstruction". We then must wonder why he devotes several hundred pages to case studies dating back to the seventeenth century. If Haberer is at all serious about the types of evidence he uses, his work must be judged in terms of not only the concision of his concepts but the accuracy of his examples as well.

The author's basic thesis is that scientific communities, when faced with the possibility of severe external sanctions, tend to practise "the politics of prudential acquiescence". According to him, such a tendency derives ultimately from two components of the scientific role, namely lust for power and intellectual arrogance. These norms, in turn, generate a community characterized both by a strong methodological ethic (truth-seeking) and by a weak institutional one. It is the latter quality, in Haberer's view, which generates political "sell-outs" on the part of scientists. Needless to say, this is hardly an attractive picture of scientific life, and we therefore turn with some interest to the data which ostensibly verify it.

From the last three and a half centuries of the history of modern science, the author has selected three incidents to sustain his narrative. The first involves the biographies of Bacon and Descartes (which also serve as his ideal-type projections of the 'Baconian' and 'Cartesian' scientist). Next comes the experience of leading German scientists during the period of Nazi rule. Finally, we are treated yet again to an exposition of the Oppenheimer affair.

Before analysing the specific events as Haberer discusses them, I think it best to detail a few sins of omission on his part. Surely a treatise on the political behaviour of all Western scientific communities from 1600 to the present which neglects the period 1650–1933, the accumulated experiences of British, French, Italian and Russian researchers, and the activities of all scientists save physicists cannot pretend to be representative, much less exhaustive. The failure to discuss at all the disparate ways in which science became a professional activity in different national settings during the nineteenth century is particularly astonishing, because this process lies at the heart of current battles over the social responsibility of scientists. (Equally astounding to British scientists of interwar vintage will be the author's assertion that "social responsibility" is purely a post-war concern.) I could only wish that Professor Haberer had bothered to explore, however haphazardly, post-Kuhnian developments in the history and sociology of science.

What about his examples? I just cannot see how the relevant scientific communities violated their professional values by refusing to support such court politicians as Bacon and Oppenheimer. Of course this statement sounds ludicrous when applied to Bacon, not only because he was not a scientist but also because there was no scientific community available to protest his downfall. As for the German scientists under Hitler, Haberer's lack of historical perspective leads him to condemn them on the grounds that they lacked the "civic courage" shown by such institutions as the church and the military. Leaving aside our feelings about the scientific community's relationship to Naziism, we should ask whether the author has made the right comparisons. I do not believe he has. The real question is: did the responses of German scientists differ significantly from those of social groups existing in similar institutional contexts, namely other German academics? To the best of my knowledge the answer is negative, which means that we should, in the first instance, look at the operation of the German university system rather than the universal norms of scientific life. Strangely enough, Fritz Ringer's account of *The Decline of the German Mandarin*, without directly discussing natural scientists, is a far more reliable guide to the actions of the scientific community than Haberer's book.

The author's handling of the Nazi episode really lays bare the poverty and amorphousness of his conceptual structure. What is the community of science? The attempt of Haberer to subsume all scientists and engineers working in academic, governmental, industrial and independent laboratories into a single, transcendent structure flies in the face of atomized, contemporary realities. How representative of the entire "community" are the actions and statements of leading scientists? Here the author assumes a fairly close correlation between leaders and led without ever supporting this notion either theoretically or empirically. What are the values of science which political action might threaten? Here Professor Haberer is at least of three minds. For most of the book, he talks about truth-seeking and independence of judgment. In the final chapter, however, we are told about the advancement of national or humanitarian goals. But he is also led to wonder "whether the community of science has any ultimate or intrinsic values at all—or if any are expressed in the institutional life of the enterprise". How then do we escape from such intellectual and moral bewilderment? At this point, the author comes dangerously close to adopting the "disestablishment of science" recently advocated by Bronowski—a utopian counsel of despair which, to my mind, is as politically irresponsible as it is impracticable.

Given the fact that *Politics and the Community of Science* is a pioneering effort, I realize that I may have been too harsh in my criticism. The reason for this is quite simple. I share the author's conviction—and not a few of his prejudices—that the political role of science in modern society may be the next generation's most critical problem. Apart from a serious and more general commitment to politics as such, we desperately need more hard thinking and judicious inquiry into this subject. I would not like to see such an effort guided by the methods of Professor Haberer.

P. G. Werskey

Cells in Charge

Cell Differentiation. Edited by A. Ole Schjeide and Jean de Vellis. Pp. xvi + 606. (Van Nostrand Reinhold: New York and London, February 1971.) £10.

Any biologist who has the stamina to read this book from cover to cover will emerge tired, but well informed about most of the approaches which are used to study differentiation at the cellular level. But the book describes the state of play, as it were, early in 1968; although the volume is published only this year, I could find no references to work reported in the literature after 1968, comparatively few references to work reported in that year, and citations to "recent reviews" proved to refer to articles published in 1967 or even 1966. But it is a mark that the book is well written and put together that comparatively little of what it says would now be regarded as misleading; it is merely a shame that the further experiments performed in the past year or so, which in some instances confirm suggestions made by the authors, are not presented to buttress these conclusions.

The first section of the book, entitled "General Concepts", comprises three chapters, the first of which, written by the two editors, should be compulsory reading for all cell biologists; this brief discussion is notable for the sense of perspective which it conveys of cell biology in general and of the succeeding chapters of this book in particular. But the two chapters which follow are perhaps a little too theoretical to fit well with the other, experimentally orientated chapters, and are unlikely to appeal to the graduate student level audience at which the book seems to be aimed.

The second and fourth sections are probably the most valuable. The second section, molecular biology, starts with a chapter which is very largely a review of the classical molecular biology of bacteria; as such this is not particularly valuable, for there are many other competent treatments of this subject. Although the editors no doubt felt that it would be best for this book to be complete in itself, I doubt the usefulness of yet another review of the evidence that DNA is the genetic material, to cite one example. A volume written at this level would do better to take for granted the classic studies of nucleic acid and protein synthesis and to turn directly to its topic. But, that said, the remainder of this section, which discusses the transcription of RNA, the cell cycle, nucleocytoplasmic interactions and membrane functions, is well written, reasonably thorough, and is bound to be useful for reference.

The succeeding section, on biochemistry and metabolism, which is concerned with topics such as the ageing of cells and changes in enzyme and other protein activities during differentiation, is perhaps too didactic to be more than a sidestream in cell differentiation.

The final section, which makes up half of the book, is concerned with particular systems which are being used to study differentiation. The first two chapters concern the differentiation of the cell as such, that is to say, the synthesis of structures such as polysomes and the endoplasmic reticulum. The chapters which then discuss the details of several individual systems are all useful as reference works describing the situation as it was early in 1968.

A notable omission from the book, which is perhaps not surprising in view of its age, is a discussion of cell hybridization and the uses to which this versatile technique may be put. If a second edition is prepared, the editors would do well to perform some judicious pruning, especially on the two more speculative chapters of the first section and of the review of molecular biology. A book of this nature is at its most useful when recently written, so it is to be hoped that such delays in production will not be repeated. And it is fair to add that a book as large and complex as is this one is only as useful as its index, which is, alas, so perfunctory as to be useless.

Benjamin Lewin

Introducing Microbes

Micro-organisms: Function, Form and Environment. Edited by Lilian E. Hawker and Alan H. Linton. Pp. xxii + 727. (Arnold: London, January 1971.) £6.

In an effort to provide an up to date undergraduate text in microbiology, Professor Lilian Hawker and Dr Alan Linton have enlisted the aid of twenty of their colleagues, each contributing one or more specialist chapters or sections. The aim has been to survey the whole field of microbiology so as to make it readily understandable by first year university students. Only a relatively small part of the book is devoted to strictly biochemical and genetical topics, an imbalance which has been made deliberately by the editors, who feel that this material is covered extensively in the existing textbooks. Discussions of growth and viability are followed by the two largest sections of the book, which deal with microbial structure, life cycles and classification and with microbial ecology. The final few chapters are devoted to selected aspects of economic microbiology.

The greatest difficulties confronting editors of a text produced in this way are those of achieving conformity of style, and balance and integrity of material. It is clear from the cross-referencing that the editors have been at pains to try to interrelate the contributions of the various authors but, nevertheless, a disappointment of the book for me was that the integration was not more fully realized. The treatment of growth physiology is the best to be found in any text at this level and, in particular, continuous cultivation is given much more than the usual nominal treatment. It is unfortunate therefore that reference to the application of steady state systems was not made by other contributors, who missed an ideal opportunity of emphasizing the dynamic nature of the chemistry, structure and functioning of microorganisms. In this context a rearrangement of chapters might have helped. For example, discussion of microbial nutrition and the effects of environmental parameters on growth could have been more effective after the reader had been introduced to chemostasis.

All the major groups of the Protista are covered comprehensively and the discussion has been based firmly on classical taxonomies. The latter approach does not make very compulsive reading but, at the same time, the student will be able to discover readily the breadth of his subject and find his way around the diverse groups of microorganisms. This section concludes with a useful account of numerical taxonomy but surely the reader deserves an assessment of the success of this rediscovered system of classifying, how it works out in practice and how widely it has been applied, without having to resort to the specialist primary literature. My only quibble with the otherwise interesting discussion of economic microbiology is that it tends to be too descriptive. The reader will find little or nothing on process design, applied genetics, new fermentation methods or cost analysis, all of which are of crucial importance to the industrial microbiologist.

The few reservations which I have about this book are offset by its general excellence, and certainly it will figure on my list of recommended first year texts. Microbiology is presented in the widest possible sense and the contributions from specialist authors have provided some fascinating and stimulating reading. The illustrations have been chosen with great care and are of the highest standard; they add much to the reader's comprehension and pleasure. Indeed, the whole production has been meticulous, and remarkably few printing or other errors have impinged on my eye. Specific bibliographies have, on the whole, been well selected and, most important in my view, the book is supplied with a good index. In conclusion this is a most welcome addition to teaching texts in microbiology and even if the price is high it is, alas, not now unusual for books of this kind.

Alan T. Bull

Bug Killers

Naturally Occurring Insecticides. By Martin Jacobson and D. G. Crosby. Pp. xii + 585. (Dekker: New York, January 1971.) $33.75.

The increasing apprehension over the use and persistence of synthetic insecticides on the one hand, and the build-up of insect resistance to many of these compounds on the other, has led scientists to search for other methods of chemical control of harmful insects. Fortunately there were already leads to be followed in existing biological control, and this book sets out to review the chemistry, biochemistry, toxicology and biology of the various compounds involved. The book is presented in three sections, depending on the source of the insecticides: botanical, insect-derived, and bacterial and fungal. Each chapter is written by a world authority on the subject although naturally there is considerable variation in the thoroughness of the treatment, and each has its own collection of references to the original literature.

The first section comprises chapters on the pyrethroids, rotenone and the rotenoids, nicotine and other tobacco alkaloids, the unsaturated isobutylamides and finally minor insecticides of plant origin. Each of these chapters contains detailed chemical treatment of the compounds involved as well as descriptions of application and effectiveness of treatment. The second section deals with arthropod venoms, and secretions and cantharidin, the juvenile hormones, and the ecdysones. Unfortunately the chapter on juvenile hormones, unlike most of the others, contains little chemical information on determination of structure and nothing on the numerous synthetic approaches to this important compound. In the final section the use of *Bacillus thuringiensis* as a microbial insecticide and a description of the chemistry and application of the destruxins and pericidins is given. The book is the first collection of such material and will be an important reference volume for the many scientists working in this large area. Unfortunately there are very few references to work published within the past two years which, therefore, excludes reference to much important work in this rapidly expanding field.

A. W. Johnson

High Speed Introduction

An Introduction to Ultracentrifugation. By T. J. Bowen and A. J. Rowe. Pp. xviii + 171. (Wiley-Interscience: London and New York, December 1970.) £2.50.

The ultracentrifuge is now so fundamental to the study of proteins and nucleic acids that an early introduction to the machine must form an essential part of the training of the research worker in these fields. And while there are several excellent reviews and monographs devoted to its use, an elementary but comprehensive text has so far been lacking. This book is intended to fill this gap; as the preface emphasizes, the treatment is purposely elementary, and the mathematical content approaches the minimal. Judgment on the book must accordingly be made within this frame of reference.

In the introductory chapter, the elementary theory of sedimentation velocity and its relation to molecular weight are outlined, and some of the early and now classical results are surveyed. The characteristics of the more important modern analytical instruments are then summarized; one wishes, perhaps, that the authors had been less guarded in their reference to the costs of maintenance and spare parts. Optical systems are described clearly: the diagrams illustrating the relation between the type of cell and the patterns it provides will be particularly useful to the beginner.

A chapter on the determination of sedimentation and diffusion coefficients, and a discussion of their significance, is followed by one devoted to the Lamm equation. Even allowing for the author's purpose, the treatment seems over-simplified: phrases such as "the velocity of the solute molecules" are to be found, and equilibrium is referred to as a "steady state". The treatment of diffusion is in terms of opposing osmotic and diffusion pressures (surely hypothetical) and the force-friction concept; to me, the phenomenological treatment of Gosting and others seems preferable, in its adherence to strictly operational concepts. Nevertheless, the essential significance of the transport coefficients is adequately described, and their relationship to experimental measurements established. The treatment of the continuity equation is detailed, but the attempt to avoid partial differentials leads to a rather cumbersome form for the important equations: once again, the explicit formulation of a flow would lead to a simpler result.

Sedimentation equilibrium is now firmly established as a principle of major importance in ultracentrifuge work, and the value of a book in this field must depend significantly on the clarity with which the essential concepts are described. It is sufficient of a comment that the equations of equilibrium are described here only in the context of zero flow: it is regrettable that the instructive comparison between equilibrium and the Archibald principle is thereby lost.

In the chapter on the determination of molecular weights, the most important of the many methods now in use are described. Due prominence is given to the high speed method of Yphantis but there is no guidance on the fundamental question of speed selection, nor is the simple expression stated for the time taken to attain equilibrium.

The chapter on the determination of molecular conformation provides a useful summary of procedures based on hydrodynamic models and this leads naturally to a discussion of the intrinsic viscosity and how it is determined. One might question the necessity for some of the detail in this section; Tanford's explicit equations relating the sedimentation coefficient and the intrinsic viscosity to the molecular weight of proteins as random coils could usefully have been included.

In the analysis of mixtures by sedimentation velocity, two complications are well recognized, if not always allowed for: these are the Johnston–Ogston effect and the more specific molecular associations associated with the name of Gilbert. Both these topics are discussed, and the essential precautions set out.

Dr Bowen's final chapter is concerned with preparative ultracentrifugation. It describes how preparative runs can be planned intelligently, and collects together much material that is widely scattered in the literature and in makers' manuals. The relative advantages of different types of rotor are assessed, and many quantitative comparisons are made: the section should be of particular value to people concerned with zonal separations.

The last two chapters, by Dr Rowe, are concerned with applications and some commonly encountered problems. Appropriately, emphasis is given to the problem of assessing the degree of homogeneity of substances examined in the analytical ultracentrifuge. As the author states, much of the evidence concerning homogeneity presented in the literature is not wholly convincing. The standard methods based on velocity measurements are described; in those based on sedimentation equilibrium, reliance is placed heavily on the very recent methods evolved by the author himself. It would have been helpful to have included the informative examples of Fujita and Williams. Some good examples of typical conformational changes in proteins and nucleic acids are quoted.

In the section on problems, some

typical questions are answered, and ways of overcoming difficulties suggested. There is much sense in this, although the occasionally flippant style may not be to every reader's taste. Some numerical problems are also included, with answers.

Although most of the errors are relatively trivial, some could mislead the inexperienced worker. Thus, on page 46, the suggested method for determining diffusion coefficients will not give a straight line plot; the defining equation for $\bar{v}$ (page 56) has an unnecessary subscript which obscures the significance of the differential; the initial concentration in the example of the Yphantis method (page 68) must be wrong by at least an order of magnitude. Most seriously, in the section on the Archibald method, the concentration at the meniscus is erroneously identified (equation 6.23) with the difference between the initial and plateau-region concentrations. Although this mistake is repeated in the example, the meniscus concentration is correct. Equations 6.26 and 6.27 are incorrect. In equation 7.20 the critical subscript to the partial differential is undefined. On pages 152 and 153, the symbol "Γ" appears where "ρ" is intended; once again, the critical subscript is omitted. Equation 10.12 is incorrect: the quantity ϕ' defined by Casassa and Eisenberg should replace $(\partial l/\partial c)$.

In spite of these errors and omissions, and some deficiencies in editing, I feel that this book's purpose has been achieved. The student who has read it will be in a position both to perform simple experiments and to understand the more rigorous approach of the primary sources, to which he will be led by generally excellent documentation. At its modest price, the book is good value.

J. M. CREETH

Electron Optics

Electron Optics. By O. Klemperer. Third edition in collaboration with M. E. Barnett. (Cambridge Monographs on Physics.) Pp. ix+506. (Cambridge University: London, January 1971.) £9.

THIS third edition is probably the best and most comprehensive introduction to electron optics currently available in English. It reflects the considerable developments that have taken place since the previous edition was written in 1953. In spite of the addition of a considerable amount of new work, the size of the book has increased by only seven per cent, the result of some vigorous pruning and rearrangement of material.

Descriptions of miscellaneous standard electron optical devices have been replaced by topics of a more fundamental character. The result is a work that reveals, with much greater clarity than before, the essentials of the subject. The authors have taken special pains to anticipate and alleviate the difficulties often experienced by students of electron optics. Thus, a single consistent set of practical units is used throughout. The authors perhaps carry discretion too far in not mentioning what the system is and also in expunging from this edition any mention of alternative systems including SI. This together with the indiscriminate use of the symbol μ (surely a printer's error?) both for the permeability of free space and that of ferromagnetic materials may temporarily confuse the unwary. Strangely enough, the word permeability does not occur in the index, probably the weakest part of the present volume. The general treatment of the subject, however, is highly praiseworthy. Important concepts such as vector potential are first defined mathematically and then interpreted physically.

Likewise, in order to direct attention to the essential steps in the argument, simple, even clumsy, mathematical symbols have been preferred to more elegant but elusive shorthand notation. The mathematical treatment is designed to appeal to experimental physicists rather than to mathematicians and is closely interwoven with experimental results and illustrative material.

All important topics are covered, some in greater detail than others. An extensive and well arranged bibliography directs the curious to the appropriate source material. The book can, therefore, be strongly recommended to students, young and old, of electron optics and indeed as a work of reference to all who are engaged in research and development in electron optics.

T. MULVEY

Introducing Information

Introduction to Information Science. Compiled and edited by Tefko Saracevic. Pp. xxiv+751. (Bowker: New York and London, 1970.) $27.50.

INFORMATION science is a name that might be applied to many areas of study related to information theory in the Shannon–Weaver sense, as well as to information processing by computer. In recent years, several university postgraduate schools have coupled the words with librarianship, to imply what has also been called documentation, but the content of this teaching is still principally the art and practice of organizing documentary records of all kinds, and it might seem presumptuous to claim that this could be called a science.

This book aims to demonstrate that beyond the pragmatic arts of information organization, there is emerging an academic discipline that is objectively concerned with an area of human activity—communication processes particularly as they are mediated by information systems. Information is generated from a myriad of sources, often in documentary form, and it is sought by a myriad of users. The two streams merge in a complex network of information systems. The study of the structure, objectives, functions, properties, behaviour and performance of information systems and of their individual components (human or inorganic) is the emergent field of "information science".

There is as yet no formal textbook of the subject. The book under review is a collection of sixty-six papers by leading investigators, published mostly during the past decade. The papers are almost exclusively theoretical or experimental, and do not include pragmatic descriptions of the development or operation of information systems. The claim that the book "introduces" information science is justified by skilful editorial organization and comment that aims to display the content of and interrelationships within the science. As structured by Saracevic, the field covered includes communication processes (interrelationships exhibited by the documentary record and the behaviour of information users), the structure and attributes of information systems and procedures, and the evaluation of systems. The final paper is an attempt to outline a general theory of communication, by W. Goffman.

Information science has become quantitative and experimental. There is a good deal of mathematical modelling in these papers, and the development and use of system performance measures. There is also much descriptive analysis of the structure of knowledge, of information use and so on. That a recognizably distinct discipline has emerged is clearly demonstrated, though it is also clear that it is still a very young science. Its object of study is a social relationship (communication) achieved through physical media (such as books or computers), and this complexity accounts for the peculiar difficulty of placing it in any traditional "system of the sciences".

The selections brought together by Saracevic do represent the most thoughtful and stimulating work that has been done in this new field, though it is of course easy to regret the absence of significant papers. The book should indeed serve to "introduce information science" to workers in related fields, and be a useful source for students. Unlike many books of "readings", it makes a real contribution to the development of its subject.

B. C. VICKERY

CORRESPONDENCE

Aid for Bengal

SIR,—I feel that the letter by K. C. Tripathi (*Nature*, **232**, 69; 1971) does not do due justice to the splendid humane effort that India has exerted in the past (and present) Bengal crisis. It is all too easy, sitting in an economically and socially cosy island some 5,000 miles from Bengal, to decry the efforts of the Indian authorities in their troubles. K. C. Tripathi should try to visualize 6 million Pakistani refugees suffering from disease and starvation swarming across the Oxfordshire border and having only the resources of the Oxfordshire County Council to organize the relief aid, to appreciate just how stupendous would be the task of helping the refugees.

It is a fairly simple matter for the "developed" countries to send a few spare planes loaded with surplus stores and medicines and then to expect someone else to do the dirty work of organizing the distribution of the relief aid dumped on their doorstep.

The people of India have made a huge sacrifice in relation to their gross national product and their generosity and humanity should be laudified and not decried.

Yours faithfully,

P. J. S. BROOKS

Cottage by the Ford,
Mill Lane,
Middle Barton,
Oxfordshire

Obituaries

Professor I. Vincke

IGNACE VINCKE, the eminent Belgian malariologist, died in Antwerp on March 13 at the age of sixty-five. He was born in Bruges where the family had long been established; his grandfather was Burgomaster and his father carried on the traditional occupation of growing bay trees for princely estates in Germany and the Low Countries. Ignace himself inherited "green fingers" and as a medical student he was awarded a Royal Medal for his discovery of a method for the sterile cultivation of orchid seeds. He graduated in medicine at the University of Ghent and, after taking the course in tropical médicine at the Institut Prince Léopold in Antwerp, embarked in 1931 for the Belgian Congo.

The starting point of Vincke's career was the study of the African anopheline mosquitoes; amongst which was *Anopheles dureni*, as the assistant of Albert Duren. He discovered sporozoites of a malaria parasite in this mosquito and for 5 years sought in vain the vertebrate host, which was clearly not man as the infected specimens were caught in uninhabited forest. In 1948 he traced the parasite to an arboreal rodent, *Grammomys surdaster*; he inoculated the blood into mice and rats and isolated a strain (*Plasmodium berghei*) which quickly became adapted as a model for the study of malaria in small laboratory animals. Within a year, the parasite was distributed throughout the world, and proved so useful that more than a thousand scientific papers have been written about it.

Strangely enough, Vincke's own interests veered from this discovery to the more pedestrian though more humanitarian pursuits of malaria control and eradication. But he continued his observations on the blood parasites of sylvatic rodents, and isolated another malaria parasite which was named *P. vinckei* in his honour, and a piroplasm which he named *Babesia rodhaini* in honour of his famous teacher, and both these organisms have been widely used in parasitological research. He worked on these problems, first in the Congo, then on various World Health Organization assignments and finally in the Department of Malariology in the Tropical Institute in Antwerp. His entomological interests remained and, as a result of his deep knowledge of the bionomics of mosquitoes, he was able both to apply DDT with intelligence in the rural zones of tropical Africa to destroy mosquitoes and to establish highly successful colonies in his insectaries in the Institut pour les Recherches Scientifiques en Afrique Centrale, and in Antwerp.

Vincke had a curious, unconventional personality. He loathed officialdom and once said, after a particularly frustrating period in the Congo, that "the only good DMS is a dead DMS"! He was quiet though mischievous (the late Professor Jacques Schwetz, also of the Congo Service, described him as a "méchant enfant"), charming and yet withdrawn, but with above all a great sense of modesty. The grand maître of Belgian tropical medicine, Jerôme Rodhain, appreciated the unusual talents of his pupil and foresaw a great future for him. His eminence was recognized with various awards, including the Prix Broden from Belgium and the Prix Carlos Chagas of Brazil, and by his elevation as Commander in the Order of Léopold.

Academician V. V. Parin

ACADEMICIAN Vasilii Vasilevich Parin, one of the leading physiologists and experts on space medicine in the Soviet Union, died on June 15, 1971.

Parin was born in 1903, in Kazan'. He graduated in 1925 from the Medical Faculty of Perm University and from 1930 to 1947 he held the post of head of the Department of Physiology at institutes in Perm, Sverdlovsk and Moscow. From 1942 to 1945 he was Deputy People's Commissar of Health of the USSR, and in 1944 he became a founder member and first Academic Secretary of the Academy of Medical Sciences of the USSR.

His academic career was interrupted in 1947, when he was imprisoned in a labour camp. After his release in 1954 he became head of the Physiology Laboratory of the Institute of Therapy of the Academy of Medical Sciences. Later appointments included head of the Faculty of Clinical and Experimental Physiology of the Central Postgraduate Medical Institute, director of the Institute of Normal and Pathological Physiology of the Academy of Medical Sciences, director of the Institute of Medico-Biological Problems of the Ministry of Health of the USSR, director of the Laboratory of the Control of Functions in Human and Animal Organisms of the Academy of Sciences of the USSR, and deputy academic secretary of the Department of Physiology of the Academy of Sciences. He was closely associated with the Soviet space programme from the earliest launches of rockets bearing test animals, and is understood to have been one of the principal organizers of the medical and biological experiments in the space programme.

He was on the editorial board of several physiological and medical journals and he published over 200 scientific and popular works on the regulation of blood circulation and space physiology and cardiology. He was also a member of Soviet delegations to a number of international conferences, a member of the International Astronautics Academy and an honorary member of the Rumanian

Academy of Sciences and the Purkinje Czechoslovak Medical Society. The awards he received included the Order of Lenin and the Order of the Red Banner of Labour.

Announcements

University News

Dr J. B. Lloyd, University College, Cardiff, has been appointed research professor in biochemistry in the **University of Keele.**

Dr Peter Harris has been awarded a personal professorship in the Department of Earth Sciences, **University of Leeds.**

The title of professor of physics has been conferred on **Dr P. V. March** in respect of his post at Westfield College, **University of London,** and that of professor of pathology has been conferred on **Dr T. Symington** in respect of his post at the Institute of Cancer Research, Royal Cancer Hospital.

Miscellaneous

The first **Florey fellowship** has been awarded to **Dr G. G. MacPherson,** MRC junior research fellow at the Sir William Dunn School of Pathology, University of Oxford, to enable him to work for two years on the physiology of the lymphatic system and its role in transplantation immunity, at the John Curtin School of Medical Research, Australian National University.

Bruno Mendel travelling fellowships have been awarded by the Council of the Royal Society to **Mr C. M. Colley,** University of Cambridge, and to **Miss M. Small,** Weizmann Institute of Science. Mr Colley will study the interactions between lipids and proteins in cell membranes at the State University, Utrecht, and Miss Small will work at the Chester Beatty Research Institute on the interaction between a lymphocyte activator from thymus and its possible target cells.

The Fondación Viviana Luckhaus international prize will be awarded in 1972 for a paper describing original research work related to blood platelets and/or their relationship to thrombosis and blood vessels. The prize will consist of an award of 18,188 Lay (3,000 dollars), a medal and a ticket and expenses for a one-week trip to Buenos Aires, and is open to investigators of all nationalities. Further information can be obtained from Dr Jorge Buraschi, Fondación Viviana Luckhaus, Hospital Juan Fernandez, Cerviño 3356, Buenos Aires, Argentina.

International Meetings

September 11–26, **Swiss National Autumn Fair,** Lausanne (Association des Intérêts de Lausanne, Rue Chancrau 3, Lausanne, Switzerland).

September 28–October 2, **Europrotection,** Paris (Europrotection, 8 rue de la Michodière, 75 Paris 2e, France).

September 29–October 1, **Research and Development,** The Hague (Financial Times Conference Department, 338 Strand, London WC2R 0LT).

October 4–6, **Turbulence in Liquids,** Rolla (John Short, Continuing Education, University of Missouri-Rolla, Rolla, Missouri 65401, USA).

November 24, **Computer aided Microscopy,** London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

Reports and Publications

not included in the monthly Books Supplement

Other Countries

Zoology Publications from Victoria University of Wellington, No. 53: Some Improvements in Zoological Microtechnique for Electron Microscopy. By H. W. Johnston and M. N. Loper. Pp. 7 (2 plates). (Wellington: Victoria University of Wellington, 1970.) [224

Smithsonian Contributions to the Earth Sciences, No. 5: The Ellende, Mexico, Meteorite Shower. By Royal S. Clarke, Jr., *et al.* Pp. iv+53. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $1.25. [234

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 186: Devonian Stratigraphy of Northeastern British Columbia. By G. C. Taylor and W. S. MacKenzie. Pp. 62. $1.50. Paper 70–2, Part B: Geological Survey of Canada—Radiocarbon Dates X. By J. A. Lowdon, R. Wilmeth and W. Blake, Jr. Pp. 472–485. $0.75. Paper 70–13: Lower and Middle Devonian Stromatoporoids from Northwestern Canada. By C. W. Stearn and P. N. Mehrotra. Pp. 32+6 plates. Paper 70–62: List of Translations in the Library of the Geological Survey of Canada, No. 1. Compiled by L. Swirsky. Pp. 25. $0.75. Index of Publications, Geological Survey of Canada, 1959–1969. Pp. 123. $2. (Ottawa: Queen's Printer, 1970 and 1971.) [234

US Department of the Interior: Geological Survey. Bulletin 1312–0: Mineralogy and Geochemistry of Some Belt Rocks, Montana and Idaho. By J. E. Harrison and D. J. Grimes. Pp. iv+49. $0.35. Professional Paper 534: Mississippian Rugose Corals Peratrovich Formation, West Coast, Prince of Wales Island, Southeastern Alaska. By Augustus K. Armstrong. Pp. iv+44+13 plates. $1. (Washington, DC: Government Printing Office, 1970.) [234

National Museums of Canada—National Museum of Man. Publications in Archaeology, No. 1: A Review of Alberta Archaeology to 1964. By Richard G. Forbis. Pp. ix+49. $1.50. Publications in Ethnology. No. 1: t'a: t': qsapa: A Practical Orthography for Nootka. By A. Thomas and E. Y. Arima. Pp. vi+35. $1.50. No. 2: The Girl Who Married the Bear. By C. McClellan. Pp. x+58. $1.50. Publications in History. No. 1: Two Centuries of Ceramics in the Richelieu Valley. By Helen H. Lambart. Pp. vi+34. $1. No. 2: The Rivers of the Potters. By Helen H. Lambart. Pp. viii+30. $1. No. 3: Plaisance. By John Humphreys. Pp. viii+24. $0.75. No. 4: Bibliograph of the Material Culture of New France. By Barbara Alexandrin and Robert Bothwell. Pp. vii+32. $1. National Museums of Canada—National Museum of Natural Sciences. Publications in Palaeontology. No. 3: A New Teleost from the Oldman Formation (Cretaceous) of Alberta. By David Bardack. Pp. vii+8. $0.50. No. 4: Comments on Dinosaurian Preservation in the Cretaceous of Alberta and Wyoming. By Charles M. Sternberg. Pp. vi+9. $0.50. No. 5: Biostratigraphy and Palaeontology of a Sangamon Deposit at Fort Qu'Appelle, Saskatchewan. By E. Khan. Pp. viii+82. $2. No. 6: A Fossil Ray, Possibly *Myledaphus* (Elasmobranchii: Batoidea) from the Late Cretaceous Oldman Formation of Western Canada. By Wann Langston, Jr. Pp. vii+15. $0.50. Publications in Zoology, No. 3: Shore and Freshwater Fish Collections from Newfoundland. By W. H. Van Vliet. Pp. vi+30. $1. (Ottawa: National Museums of Canada, 1970.) [234

Novos Taxa Entomologicos. No. 84 (November 1970): On Some New Genera and Species of Armoured Scale Insects (*Homoptera: Diaspididae*) from Southern Africa. By J. Munting. Pp. 14. (Lourenco Marques: Institut de Investigacao Cientifica de Mocambique, 1970.) [264

Institut Royal Météorologique de Belgique. Publications, Série A, No. 71: Distribution du Rayonnement Solaire en Belgique. Par R. Dogniaux. (Bruxelles: Institut Royal Météorologique de Belgique, 1971.) [264

The Physics Problems of Reactor Shielding—Report of a Joint ENEA/IAEA Specialist Meeting, Paris, December 1970. Pp. 175. (Paris: Organization for Economic Co-operation and Development, 1971.) 23 francs; £1.75; $5. [264

UNISIST—Synopsis of the Feasibility Study on a World Science Information System. By the United Nations Educational, Scientific and Cultural Organization and the International Council of Scientific Unions. Pp. 92. (Paris: Unesco, 1971.) 16 francs; $4; £1.20. [274

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature+ Nature Physical Science	**£24**	**$83**	**$90**
Nature+ Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, July 16 1971

NATURE Volume 232 JULY 30 1971

Responsibility without Government

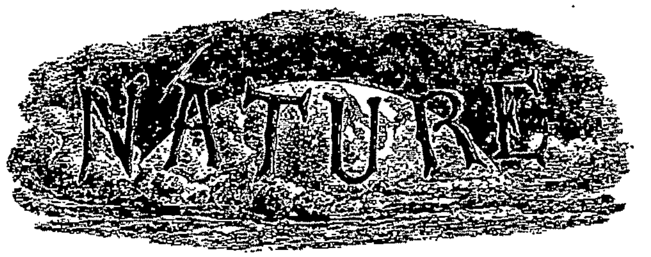
THE Select Committee on Science and Technology is entirely within its rights to complain, as it did on Wednesday, that the government is dragging its feet on planning for the future of research and development in Britain. In what appears to be a yearning for unattainable perfection, the British Government appears bent on resolving to its own satisfaction and in theory all possible questions before it actually sets out to tackle any one of them. The result is that the report of the committee under Sir Frederick Dainton on the reorganization of civil research, which has already been discussed by the Council for Scientific Policy —its legitimate parent—and passed on to Mrs Margaret Thatcher at the Department of Education and Science, has disappeared into never-never land. Would-be recorders of events have to be satisfied with such straws in the wind as they can find. Does, for example, the fact that the government has made an appointment for only one year as Secretary of the Agricultural Research Council imply that it expects a major reorganization within the year? Time may yet tell, but in the meantime uncertainty persists about the future of the research councils and their relationship with each other, with government departments concerned with specific functions and with the universities especially, because even a rapturous acceptance of the Dainton report would be unlikely radically to transform the functions which research councils at present undertake. There is no need for such a revolution but there is a sense in which procrastination creates problems out of nothing.

The select committee is also entirely right to protest at the way in which the government has done nothing to remove uncertainty, or even to make fuller use of the resources which exist, in the wider context of how civil research as a whole should be undertaken. The Atomic Energy Authority remains in a kind of limbo, with Aldermaston still within the civil field in spite of the near-certainty that it will eventually become a defence laboratory, with the laboratory at Harwell ploughing ahead so successfully at commercial developments of various kinds that it sits increasingly uncomfortably beneath the umbrella of atomic energy, and there is persistent uncertainty not merely about government policy about institutions such as the research associations but towards the whole principle of how and when governments should intervene in industrial research. It may be true, as the government appears to have decided off its own bat, that the time has come for a thoroughgoing review of policy on research. After all, it is now seven years since the report of the Trend committee advocated institutions for regulating the relationship between industry and government on research which have not for practical purposes been taken up (although there is an echo of them in Mr Anthony Wedgwood Benn's now defunct Green Paper on industrial research). It is also true that the issue is important enough to justify a philosophical and measured solution. The question is merely whether industrial research and development can wait that long—and also whether the problem will be the same a year from now.

By all accounts, responsibility for the new policy lies with Lord Rothschild. At the outset, the Council for Scientific Policy seems to have been fully informed of the status of the Dainton study and in particular of the intention that this should be merely one of several ingredients of a much fuller appraisal of present problems. One of the anxieties now is that a worthy piece of work should be not merely subsumed in a larger study but even made the basis for policy decisions which are put into effect without public discussion in advance. Yet all the experience of the past few decades has made it perfectly plain that juggling with the institutions of the scientific establishment, necessary though it may be, is possible only if the institutions concerned and those who work in them have had a chance to say their piece. On many occasions, the government of the day has been saved from error by precisely such discussion. It would therefore be a token not merely of good intentions but of good sense if the government were now to sanction the publication of the Dainton report. That would be something to be going on with. (At the same time, the Council for Scientific Policy could help its own case for public discussion by helping to ensure that the research councils under its own wing were more forthcoming with the publication of documents of public importance—the report on astronomy in the northern hemisphere which the Scientific Research Council has commissioned (see page 289) should, for example, be published without delay).

---

100 Years Ago



How, then, did life originate on the Earth? Tracing the physical history of the Earth backwards, on strict dynamical principles, we are brought to a red-hot melted globe on which no life could exist. Hence when the Earth was first fit for life, there was no living thing on it. There were rocks solid and disintegrated, water, air all round, warmed and illuminated by a brilliant Sun, ready to become a garden. Did grass and trees and flowers spring into existence, in all the fulness of ripe beauty, by a fiat of Creative power? or did vegetation, growing up from seed sown, spread and multiply over the whole Earth? Science is bound, by the everlasting law of honour, to face fearlessly every problem which can fairly be presented to it. If a probable solution, consistent with the ordinary course of nature, can be found, we must not invoke an abnormal act of Creative Power. When a lava stream flows down the sides of Vesuvius or Etna it quickly cools and becomes solid; and after a few weeks or years it teems with vegetable and animal life, which for it originated by the transport of seed and ova and by the migration of individual living creatures. When a volcanic island springs up from the sea, and after a few years is found clothed with vegetation, we do not hesitate to assume that seed has been wafted to it through the air, or floated to it on rafts. Is it not possible, and if possible, is it not probable, that the beginning of vegetable life on the earth is to be similarly explained?

From Nature 4, 269, *August* 3, 1871

OLD WORLD

SCIENTISTS' PAY

Protests Galore

THE series of meetings organized by the Institution of Professional Civil Servants (IPCS) to publicize the current salary dispute between the British Government and the scientists in its employ is now almost over but the climax is yet to come at the Central Hall, Westminster, on August 10. The support obtained so far has at least demonstrated that government scientists are wholeheartedly behind the IPCS. Whether their anger will be productive remains to be seen.

After the disagreement between the institution and the government, an arbitration tribunal is being set up under the chairmanship of Mr M. J. Mustill, QC. This will sit on August 12 and possibly August 13. A minor success for the institution has been to reverse the original decision to hold the tribunal *in camera* and the proceedings will now be relayed from Abbey Gardens, NW8, to the nearby Bishop Partridge Hall, which the institution hopes to fill with its members.

The final meeting at Central Hall is to be addressed by Dr Harold Turner, of the National Physical Laboratory, Teddington, who is chairman of the institution's Scientific Task Group, and by Mr William McCall, the General Secretary, with Mr J. C. McGloughlan of the IPCS in the chair. The institution is hoping for an overwhelming show of support but at the same time it is not giving any encouragement to the notion being circulated in government laboratories that everybody should take half a day's leave on August 10 in order to attend the meeting—thus effectively bringing all work to a halt on that afternoon. This would violate the promise already given by the IPCS that it would not resort to industrial action to achieve its claims.

A spokesman said earlier this week that the institution is quite optimistic that the outcome of the tribunal will bring a better offer than that already made. The government's view, however, is restated in a letter from the Prime Minister to Mr Airey Neave, the Member of Parliament for Abingdon, who has been taking a close interest in the scientists' claim. Mr Heath has refused to intervene in the dispute and has reiterated that the government had considered all the arguments advanced by the IPCS after it received the first pay offer, but in its view these arguments did not "justify any improvement in the offers that have been made".

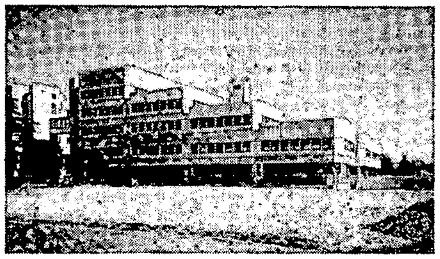
A matter of concern to the scientists is the apparent downgrading of their profession compared with other classes in the Civil Service, in particular the administrative grades who received a salary increase earlier this year. The institution's fear is that there is discrimination against the scientists but Mr Heath states that there is no question of this and "continued progress in science and technology is essential to the future of this country, and the government attaches great importance to the contribution made by the scientific staff within the Civil Service". It is doubtful whether this will mollify the scientists at this stage as the hard facts of the government's offer do not bear out Mr Heath's statement.

Whatever the outcome of the tribunal, the IPCS must consider whether the mechanism at present used to determine salaries within the Scientific Civil Service is the most appropriate. There is no doubt that in view of the latest experience most scientists would prefer not to have their salaries determined as a result of a pay research exercise but some method must be used that will ensure parity with salaries both inside and outside the Civil Service.

BIOLOGY

Old and New at Oxford

OXFORD'S biologists seem to have been celebrating with gay abandon recently. Two weeks ago, the zoologists and psychologists heralded the opening of their new palatial building and on July 24 the Botanic Garden was toasted with champagne on the occasion of its 350th anniversary.



Part of the laboratory built in Oxford for the departments of zoology and psychology. (Photo: Surveyor to the University.)

The garden was founded by Henry Danvers, Earl of Danby, who gave it walls strong enough to withstand the siege of the Civil War and remain unharmed through the succeeding centuries. Many distinguished botanists have tended the plants and worked at their scholarly pursuits within the walls. Although the Professor of Botany no longer personally superintends the activities of the garden, he is still its official keeper, and last week Professor C. D. Darlington, fulfilling one of his last functions before retiring next September, welcomed the Chancellor of Oxford University, Mr Harold Macmillan, and the guests who had braved the rain to join the celebration. The chancellor marked the occasion by planting a specimen of the scholar tree, *Sophora japonica*.

Among the non-botanical guests on this occasion were Professor J. W. S. Pringle of zoology and Professor L. Weiskrantz of psychology, who recently moved into their new building which was opened by Professor A. L. Hodgkin on July 12. The building, which cost £2 million, was designed by Sir Leslie Martin to bring together two departments which were previously scattered around Oxford. Now they occupy two sides of a complex which is linked by a spine housing common facilities such as libraries and lecture rooms. The furniture and fittings are sumptuous—the workbenches are made of teak; there are two electron microscopes on independently suspended floors; the zoology department is wired for closed circuit television. There is even a tropical climate room.

Brackish Water

THE committee that was set up last year to form an Estuarine and Brackish-Water Biological Association in the United Kingdom has announced plans for the association's inaugural symposium to be held at the Zoological Society of London on October 13, 1971. The need for such an association arises from the varied disciplines of the scientists who are interested in the properties of these waters and so the association will enable people working in such diverse fields as biology and sedimentology to have a common platform. Future plans for the association include regular meetings and the publication of a bulletin.

The committee feels that the essential unity of aquatic science can best be served by emphasizing the function of the previously neglected estuarine sector so that a bridge can be built between the freshwater and marine fields. The formation of the association will then be an important step towards the eventual integration of outlook for these disciplines.

The inauguration of the association will take place during the meeting in October. Membership will be open to anyone but the feeling of the committee is that it will probably be most attractive to research workers in appropriate fields.

ASTRONOMER ROYAL

New Astronomy

A NEW twist occurred last week in the search for a new Astronomer Royal with the announcement that the post is to be dissociated from the directorship of the Royal Greenwich Observatory. This move will please those who have been arguing for just such a break between the two jobs, which will take effect at the end of the year, with the retirement of the present Astronomer Royal and director of the Royal Greenwich Observatory, Sir Richard Woolley.

Now that observational astronomy is no longer the prerogative of optical observatories, the argument goes, it is anachronistic to insist that the Astronomer Royal should also be equipped to manage the optical telescopes associated with the Royal Greenwich Observatory. Indeed, as one astronomer has remarked of the quest for a suitable replacement that has been going on since Sir Richard's retirement was announced a year ago, logically there ought to be astronomers royal for X-ray and radio as well as optical astronomy, a post which, it has been suggested, might have the title of Master of the Queen's Astrophysik.

Such tongue in cheek proposals during the past year have, however, gone hand in hand with real concern that the best decision be made about the role of the Astronomer Royal and of the Royal Greenwich Observatory. One view has been that the post of Astronomer Royal should die when Sir Richard Woolley retires, but most astronomers would probably prefer to keep the old name, either linked to the Royal Greenwich Observatory or as an honorary title that can be held by any British astronomer, perhaps for a set period only.

Whether last week's announcement will make any easier the task of finding a successor to fill Sir Richard Woolley's second role as director of the Royal Greenwich Observatory is a debatable point. The overt explanation for separating the two posts is that it throws open the directorship to foreign astronomers—the Astronomer Royal cannot be non-British, although oddly enough the post of Astronomer Royal for Scotland has only once been held by a Scot. But Sir Brian Flowers may now find that some potential candidates will be discouraged by what amounts to a downgrading of the post. It is believed that a Dutch astronomer has already been unsuccessfully approached.

More important than the personality of the next Astronomer Royal, however, is that this watershed is being taken as an opportunity for a reappraisal of British astronomy. The time is particularly appropriate, for during the next few years many of the grand old names of British astronomy will reach retiring age, so that several crucial chairs will become vacant. To the extent that the Science Research Council can influence appointments within the universities, decisions taken during the next few years will affect British astronomy for several decades. Traditionally the Royal Greenwich Observatory has been at the heart of British astronomy, but since the war the new branches of astronomy that have developed outside the Royal Observatory system have called into question the wisdom of giving such a prominent role to an organization that was established primarily to provide astronomical information for mariners. This question came to a head a year ago when the Science Research Council was handed a review of the future of astronomy in the northern hemisphere that it had commissioned from a group of scientists that included astronomers, a space scientist, a nuclear physicist, and the two astronomers royal. As usual the report is being treated in a typically close-handed way by the Science Research Council, and is now unlikely to be published.

It is, however, understood that the most contentious proposal is that a British observatory to be established in the Mediterranean area be managed not by the Royal Greenwich Observatory, but by a new institution that would be linked with a group of universities. An association similar to the federation of ten universities that manages the Kitt Peak observatory in the United States is what the review committee had in mind, and is thought to have been accepted by all members of the committee with the exception of the two astronomers royal.

Although on paper at least the Royal Greenwich Observatory does not have control of the only large telescope that is available to British astronomers—the 98-inch Isaac Newton telescope—or the 150-inch telescope that is being built as a joint venture with the Australians, there seems to be a widespread feeling that the observatory exerts an influence on the running of the telescopes that is inimical to the progress of British astronomy.

In any case, the staff at the Royal Greenwich Observatory saw how the wind was blowing as long ago as November 1968—before the northern hemisphere review began in earnest—when all the scientific staff of the observatory signed a petition to the Queen to seek assurances that the directorship of the observatory would continue to be linked with the post of Astronomer Royal. At about the same time, Sir Brian Flowers wrote to the staff at the observatory saying that the staff would be consulted if there were any proposal to dissociate the directorship from the post of Astronomer Royal, a promise which the Institution of Professional Civil Servants says has not been kept.

In other words, the staff of the observatory are rightly concerned that the announcement last week marks the beginning of a downgrading of the observatory. The staff expect the first consequence to be that greater difficulty will be experienced in finding a strong candidate for the directorship. But at the backs of their minds is the fear that the Science Research Council will encourage the Royal Greenwich Observatory to become a service organization, at the behest of the universities.

Views on the appropriateness of such a future for the observatory depend on what attitude is taken to the usefulness of scientific institutions that are divorced from the universities and from industry (although the staff of the observatory will point to their strong links with the vigorous astronomy department at the University of Sussex). But for the time being at least, the plan to set up a new institution linked with a number of universities appears to have been shelved, apparently because of the cost although this is thought to have been modest. On the face of it, then, the present arrangements for managing the large optical telescopes will continue. Nevertheless, the next director of the Royal Greenwich Observatory will have to be prepared for criticism of the establishment from many astronomers who see the observatory as a white elephant. In particular, the £1.2 million which the Science Research Council spends on the Royal Greenwich Observatory, the Royal Observatory at Edinburgh, and on the observatories in South Africa is widely held to give poor returns compared with the running costs of the Hale Observatories of $1.7 million per year. In addition, the siting of the 98-inch Isaac Newton telescope adjacent to the Royal Greenwich Observatory a mile or so from the Sussex coast is still felt to be the most short-sighted decision in the management of British astronomy. The small amount of astronomy that it has been possible to carry out with the telescope compares unfavourably with the productivity of the Hale Observatories, for example, and many astronomers would be happy to see the telescope moved to the Mediterranean area at the first opportunity. It is true that there is little hope now that a site can be found in the Mediterranean that will produce the rapturous praise that astronomers reserve for the skies of Chile, but almost anywhere, the saying goes, will be better than Sussex.

NEW WORLD

AEC Bruised but not Cut

by our Washington Correspondent

AFTER the Food and Drug Administration, the Atomic Energy Commission probably ranks second in the list of the most heavily criticized federal agencies. During the past year it has had to face a barrage of criticism from environmentalists who oppose extension of the nuclear power programme, its safety procedures have been thrown into disrepute by a series of failures under test of the emergency core cooling device, and it has lately been placed in a compromising position by its advocacy of the nuclear test planned to take place in Alaska in the autumn.

This outspoken public disenchantment with the AEC's plans has not, however, gained a footing in Congress, for only last week both houses agreed to authorize more funds for the AEC than were requested in its budget, and only a few minor programmes have been trimmed by the Joint Committee on Atomic Energy which recommended the authorizations to Congress. Although the authorizations represent only the maximum funds that can be appropriated for the commission—its budget must now be considered by the Appropriations Committee—it seems likely that the AEC will be able to proceed with all the major parts of its planned programme for 1972.

The authorizations passed by Congress amounted to just over $2,321 million, $37 million more than the commission requested, and essentially the same amount as authorized for 1971. Chief beneficiaries of this Congressional largesse are the programmes for nuclear space propulsion (the Nerva rocket programme) and gaseous diffusion technology, which were increased by $37 million and $31 million respectively. The Joint Committee on Atomic Energy has, however, docked $21.5 million from the AEC's request for funds to establish a radioactive waste repository at Lyons in Kansas, and cut by half the commission's request for $1 million to develop a nuclear powered cardiac pacemaker, although the full amount was later restored by an amendment which was proposed in the Senate by Hugh Scott, the ranking Republican from Pennsylvania.

The joint committee left untouched the $263.6 million requested for physical research, although it has a cautionary word to say to the high energy physics laboratories operated by the AEC. When the 200 GeV accelerator at the National Accelerator Laboratory in Illinois becomes fully operational, it will require an annual funding of between $60 and $70 million which is more than half the $116 million requested for the whole high energy physics programme for 1972. The joint committee therefore asks the AEC to "examine the minimum level of support necessary to keep each of its high energy accelerator laboratories, including the NAL, viable and productive, and that it develops a priority listing of which accelerators should be kept operating should future funding be less than the minimum necessary to effectively support each of the six laboratories". The AEC has been asked to furnish the joint committee with its priority listing by the end of 1971.

Following the lead of the Senate Committee on Aeronautical and Space Sciences and the House Committee on Science and Astronautics, the Joint Committee on Atomic Energy has restored in full the funds requested for development of the Nerva nuclear powered rocket. In its budget request to the Administration, the AEC asked for $52 million for research and development on Nerva, but this was slashed by the Office of Management and Budget to $15 million—barely sufficient to keep the project ticking over, and probably a sufficient reduction to kill the project altogether.

What impressed the joint committee most was a series of joint hearings conducted with the Committee on Aeronautical and Space Sciences in February, during which a series of witnesses testified that the Nerva programme has so far been successful and is up to schedule. The joint committee therefore proposed, and Congress agreed without criticism, that the AEC should get the full $52 million requested, and that this should be split into $35.2 million for rocket propulsion development, $13.8 million for advanced research and development, and $3 million for operation of the Nuclear Rocket Development Station in Nevada (the Administration had deleted all funding for this latter facility).

The joint committee also looked favourably on Project Plowshare—the proposal to excavate natural gas by nuclear explosives. Congress last week authorized $8.1 million for this project—an increase of $3.1 million on the Administration's request. The idea is to use nuclear explosives to release natural gas from underground deposits more cheaply than by conventional means, but this proposal has already fallen foul of many sectors of opinion. Dr John Gofman, an ardent critic of the Atomic Energy Commission, for example, said last week that he regards the decision to proceed with Plowshare as "extremely unfortunate". Pointing out that "all these programmes eventually end up spewing radioactivity into the atmosphere", Gofman said that the concept that natural gas can be excavated by nuclear explosives and pumped into people's homes along with some radioactive debris is "so grotesque that we should have abandoned this project long ago".

The AEC estimates that Project Plowshare can bring natural gas to the consumer pipelines within five years, at an estimated government cost of $50 million, and to this end it proposes to test a device in 1972. If successful, it has been estimated that the project will double the nation's natural gas reserves, and apart from the economic advantages of such an achievement, its supporters justify Plowshare on the grounds that it will provide the market with a largely pollution-free fuel. But that justification would be something of a mockery if the project ended up contaminating the atmosphere with radioactivity, and if it also provided natural gas contaminated with radioactivity.

The joint committee has, however, sounded a more cautionary note in its deliberations on the AEC's proposals for the long term storage of radioactive wastes. The kingpin of the AEC's programme is to store such wastes in a disused salt mine in Lyons.

Kansas, and the commission requested $25 million for acquisition of land and for construction of facilities. But the joint committee has slashed this request by $21.5 million. In spite of a report by the National Academy of Sciences, which concluded that the use of bedded salt deposits represents the safest disposal method now available, the joint committee has taken the sensible step of requiring that the AEC makes further studies of the safety of the mines before they are acquired for use. In particular, the joint committee has asked for more information on heat transfer between the salt/shale interface, areal hydrology and geology, possible environmental damage, the transportation of waste to the salt mine and waste retrieval after burial. The AEC has, however, stated that such questions cannot be answered until small amounts of waste have been deposited in the mine, but that argument cut no ice with the committee, which stated in its report to Congress that "all reasonable questions raised must be answered from the standpoint of satisfying Kansas and Federal officials *before the Lyons facility is put into operation as a respository for industrial wastes*". (Committee's italics.)

The most controversial item in the AEC authorizations is the $19.7 million requested for the testing of a nuclear weapon in an underground site on Amchitka Island in the Aleutians off the coast of Alaska. The reason for the test, which was explained by the AEC in full only a day or so before the Senate debate on the authorizations, is to monitor the X-ray spectrum produced by the warhead of the Spartan anti-ballistic missile (the longer range missile that forms part of the Safeguard system). A statement sent by the AEC to Senator John O. Pastore, chairman of the Joint Committee on Atomic Energy, pointed out that the kill mechanism of the Spartan missile is X-rays released outside the atmosphere, and that this requires a low fission output so as to minimize radar blackout, and a high X-ray output.

The AEC therefore uses a mixture of economic, scientific and pragmatic justifications for the test. It says, for example, that failure to conduct the test would drastically reduce confidence in the missile, and that the test is necessary "to minimize the possibility of stockpiling a defective design", to measure the fission output, and to monitor the X-ray flux and yield. If the test does not take place, the AEC points out, "one would be forced to rely on theoretical calculations to determine X-ray flux and spectrum without the benefit of any meaningful experimental data".

Code-named Cannikin, the test is due to take place in October. With a yield of five megatons, it will be the largest underground explosion ever conducted by the United States, and it has already sparked off a wealth of political and scientific criticism. But amendments to the Authorizations Bill, introduced in the House by Mrs Patsy Mink, and in the Senate by Senator Mike Gravel, failed to prevent funds being authorized for the explosion, and the chief hope now is that President Nixon will veto the programme. Opposition centres around four chief points: that the test will release radioactive debris into the atmosphere directly through venting, and later by seepage of ground water; that the explosion may trigger an earthquake which could cause a tidal wave, resulting in substantial damage along the Pacific coast; that it could sabotage the Strategic Arms Limitation Talks (SALT) now taking place between the United States and the Soviet Union in Helsinki, and that in any case development of the Safeguard ABM system is likely to be a destabilizing influence on the arms race.

As far as the environmental consequences of the test are concerned, the AEC has consistently underplayed the likely effects of Cannikin. To be sure, even critics of the test acknowledge the fact that dire environmental consequences are a remote possibility, but they also point out that as long as the possibility exists the test should be scrapped. The most publicized risk involved in the test is that it may trigger off a massive earthquake in the region which could in turn cause a tidal wave, with devastating consequences for the Pacific islands, and even for California and other parts of the US Pacific coastline. The Aleutian islands are a particularly active earthquake area, and it has been estimated that the Cannikin blast will be equivalent to an earthquake of magnitude 7 on the Richter scale. (By comparison, the quake which shook California earlier this year was rated at 5.9.)

In an environmental policy statement which the AEC was required to publish under the terms of the National Environmental Policy Act of 1969, however, the commission stated "experience gained by observation of past nuclear detonations and earthquakes combines to provide extremely positive assurance that the Cannikin explosion cannot of itself cause a severe earthquake". But an *ad hoc* panel of scientists headed by Dr Kenneth Pitzer, which met under the auspices of the Office of Science and Technology in 1968, took a less rosy view of the situation. Its report stated: "There does not now appear to be a basis for eliminating the possibility that a large test explosion might induce, either immediately or after a period of time, a severe earthquake of sufficiently large magnitude to cause serious damage well beyond the limits of the test site. This possibility is more serious for tests greater than a megaton. . .". It has also been suggested that there is a one in a hundred chance that an earthquake bigger than the Cannikin blast will occur in the Aleutians in any two week period. What would happen if such an earthquake occurred simultaneously with the Cannikin test is a moot point.

Two underground tests conducted at the Nevada site have also been found to release more than ten times the energy released by the blasts themselves. But the Pitzer report pointed out: "Since the Amchitka area in Alaska is still more active seismically, the hazard of inducing an earthquake must be considered to be greater at that location than at either Nevada site." One point that the AEC emphasizes, however, is that the Milrow test—a one megaton "proving" test for the Cannikin blast, conducted at Amchitka in 1969—did not produce any sizable aftershock.

As far as leakage of radioactive debris is concerned, one fear which was suggested last week by Dr Robert Fleisher, director of the Committee for Nuclear Responsibility, is that if massive venting does occur (and some of the Nevada tests have resulted in quite large venting), radioactive fallout could be carried outside United States territory, in violation of the partial test ban treaty. There is also a fear that if there is an unsuspected fault close to the test site, leakage of radioactive material through groundwater could occur relatively quickly, resulting in danger not only to the wildlife in the Northern Pacific, but also to the Alaska coast. And this fear is intensified by suggestions that the Cannikin blast could also release radioactive debris remaining from the Milrow test. The AEC's environmental policy statement suggested, however, that "it is not conceivable that Cannikin would cause release of underground radioactivity from the cavities which remain from the earlier Milrow and Long Shot tests". Unfortunately, however, that statement was not accompanied by any discussion of why such a possibility is inconceivable.

In view of these serious doubts about the safety of the test, the Committee for Nuclear Responsibility, whose board of directors contains the names of James Watson, John Gofman, Paul Erlich, Linus Pauling and George Wald, has filed a suit against the Atomic Energy Commission, in an attempt to stop the test. Dr Robert Fleisher, executive director of the committee, said last week that the National Environmental Policy Act requires that if a project involves the possibility of serious ecological damage, it must be proved to fulfil an enormous need. He charges that the AEC's environmental policy statement does not provide such evidence, and that will be the basis of the committee's court action.

The most likely possibility for stopping the test rests, however, in its political repercussions. For one thing, it could seriously damage the chances of success in the SALT talks, which according to recent reports are progressing along hopeful lines. It has been suggested, for example, that the United States has proposed verbally, and will later propose in writing, an agreement that would halt the construction of both land-based and sea-based missiles, and also that as many as 300 missiles would be allowed in the US and the USSR to protect offensive missile sites. For President Nixon to cancel the Cannikin test would therefore help to establish his credibility in his desire to curb the arms race.

But a more immediate factor is the Presidential elections in 1972. Now that the possible consequences of the Cannikin blast have been aired in the press and in Congress, Nixon could well put off the test until after the 1972 campaign. If he in fact decided to go ahead on schedule, and an unlikely catastrophe did take place, his chances of re-election could be ruined. Some observers are very optimistic about the chances of a Presidential veto, and one critic of the programme suggested last week that the chances are "fifty-fifty or better". Another but less likely possibility is that the appropriations for the test could be deleted when they come up for debate in Congress. Senator Gravel is likely to oppose them in the Senate, and he will probably get more support then than he did last week when he proposed an amendment to the Authorizations Bill seeking to delay the test until after May 31, 1972. That amendment was defeated by a vote of 37 to 57, but with more exposure in the press, and if the SALT talks continue to progress towards a moratorium on the construction of nuclear weapons, Gravel may be able to muster enough votes, if not to defeat the appropriations outright, at least to provide Nixon with added justification for vetoing the measure.

FDA

New Name, Old Face

by our Washington Correspondent

If the Administration gets its way with Congress, the name of the Food and Drug Administration may soon be deleted from the list of Federal agencies, and in its place will be substituted the much more grandiose title of the Consumer Safety Administration. But this exercise in semantics will do little to appease the many critics of the way the FDA conducts its business, for instead of radical changes in the functioning of the FDA, what is being proposed is that it should have extra responsibilities grafted on to its present unsatisfactory structure. The proposal, in the form of a bill introduced into the Senate by the Administration, came under scrutiny by the Senate Commerce Committee last week, and it has also been given President Nixon's personal blessing.

The idea is that the FDA will cease to be solely a watchdog over food and drugs and that instead its responsibilities should extend over the whole field of product safety. This proposal was spelt out in some detail by Elliot L. Richardson, Secretary of Health, Education and Welfare, before the Commerce Committee last week. He said that when the bill is passed, "I propose to build upon the FDA framework by establishing a new Consumer Safety Administration to be composed of three major offices: the Office of Product Safety Regulation, the Office of Drug Regulation and the Office of Food Regulation." And, in a letter to Senator Frank E. Moss of Utah, chairman of the subcommittee considering the bill, Nixon left no doubt about the fact that the FDA will continue to operate in much its present form. After announcing his approval for the bill, the letter went on to say that "this unit (the proposed Consumer Safety Administration) will build upon the activities, personnel and facilities of the FDA, which has a long and distinguished history in the field of consumer safety".

The Administration's bill is a counter proposal to a bill introduced by Senator Moss and Senator Warren G. Magnuson which also seeks to extend the Federal Government's responsibility for the control of potentially dangerous consumer products (such as toys that are likely to cause actual bodily harm), but the essential difference between the two proposals is that the Moss–Magnuson bill seeks to set up a division of product safety outside the Department of Health, Education and Welfare. The chief idea behind such a measure is that the new agency could respond more readily to consumer interests if it were taken out of the bureaucracy of HEW, and it stems from a report published by the National Commission on Product Safety. Testifying before the Senate Committee on Commerce last week, David Elkind, Chairman of the National Commission on Product Safety, left no doubts about his dislike for the Administration proposal, calling the proposed Consumer Safety Administration a "paper tiger", Elkind said "the first step in creating a paralytic consumer protection function is to place it deep in the recesses of the already overburdened and unsuccessful Department of Health, Education and Welfare".

Why has the Administration chosen to oppose the Moss–Magnuson bill? Apart from the fact that to set up a unit outside the FDA may be regarded as the first step in dismantling the agency, the overt reason is one of administrative neatness. Richardson, for example, said that the FDA already has extensive laboratory and testing facilities, and that to set up a separate organization outside HEW may duplicate some of those facilities. He dismissed the idea, moreover, that an independent agency would be more receptive to consumer pressures than an expanded FDA, and came close to agreeing with critics of the FDA when he suggested, "I do not think that independent regulatory agencies have historically shown a greater resistance than other agencies to the pressures that often cause agencies to identify their interests with those of the industries they regulate".

If the Commerce Committee eventually decides not to go along with the Administration, but to support the Moss–Magnuson bill, the FDA would find itself in an embarrassing position, for Richardson confessed to the committee in reply to a question from Moss that he has already set up a division of product safety within the FDA, and Dr Charles Edwards, Commissioner of the FDA, later explained that in three to five years' time the Office of Product Safety would be the largest division of the FDA. He suggested that $19 million would be spent on setting up the new office in 1972, and that some of this money would be taken from the already undernourished and overburdened food and drugs offices.

According to Edwards, the new style FDA will parallel the Environmental Protection Agency in terms of function of operation, but Elkind was quick to point out that the essential difference between the two agencies is that the FDA will continue to operate from within the bureaucracy of the Department of Health, Education and Welfare, while the EPA was set up as an independent agency for the very reasons that the National Commission on Product Safety asked for an independent Consumer Safety Agency. The Administration's bill, he retorted, would "be as though the government were to fashion, like Dr Frankenstein, a man with beautiful sinews, long arms and piercing eyes, with just one weakness—an inability to hear from the consumer and respond to his needs".

NEWS AND VIEWS

X-Chromosome Inactivation in Mules and Hinnies

ACCORDING to the inactive-*X* hypothesis of dosage compensation (Lyon, *Nature*, **190**, 372; 1962), one or other of the two *X*-chromosomes of the mammalian female becomes genetically inactivated at an early stage of embryonic development and the choice of which *X* becomes inactivated in each cell is a random one, with the result that clones of cells arise within individual females, having either the maternally-derived or paternally-derived *X* in the inactive condition. The late DNA replication of one *X*, observed in the somatic cells of female mammals, and genetic inactivity of one *X* are generally considered to be related phenomena but as yet there is no final proof of this.

Horse–donkey hybrids have two qualities which make them unique material for testing the inactive *X* hypothesis: first, the horse and donkey *X*-chromosomes are morphologically distinguishable and, second, the *X*-linked genes which code for glucose-6-phosphate dehydrogenase (G6PD) in each species show different electrophoretic mobilities. A mixed expression of horse and donkey G6PD is generally found in cell cultures from female mules and hinnies but evidence that this derives from the presence of two cell populations, one with the donkey *X* active and one with horse *X* active, has only now been obtained. Hamerton, Gianelli and co-workers in an article on page of this issue of *Nature* have shown that cloning cells from original skin fibroblasts which show mixed G6PD expression yield clones which produce either horse or donkey G6PD. A small proportion of clones showing mixed expression were also found but, on recloning, these could again be broken down into the two component cell types. These data are thus consistent with the inactive-*X* hypothesis but, although they do not disprove Grüneberg's hypothesis (*J. Embryol. Exp. Morph.*, **22**, 145; 1969) that the activity of one or other *X* may range from 0 per cent to 100 per cent of the total *X*-chromosome activity of any one cell, they make it seem most improbable that such a mechanism operates in the mule, at least *in vitro*.

An impressive inverse correlation between late replication of the horse and donkey *X*-chromosomes and horse and donkey G6PD expression has recently been demonstrated by Cohen and Rattuzzi in the female mule (*Proc. US Nat. Acad. Sci.*, **68**, 544; 1971) and the two articles by Hamerton and Giannelli on pages 312 and 315 of this issue of *Nature* amplify these findings. With both the mule and the hinny, agreement was found between the frequency of cells in original fibroblast cultures with late replicating donkey or horse *X*-chromosome and the estimated frequency of cells in these cultures expressing donkey or horse G6PD, as determined by the yield of the two cell types in cloning experiments. Final proof of a definite relationship between late replication and genetic inactivity will be obtainable when late replication of the *X* is studied in horse and donkey G6PD clones.

Perhaps the most intriguing information provided by the present studies on horse–donkey hybrids is the discovery of extensive cell selection operating both *in vitro* and *in vivo*. In the *in vitro* studies this was best seen from the results of recloning mixed clones. If it is assumed that these mixed clones originated from two cells, one with the horse *X* active and one with the donkey *X* active, the proportion of sub-clones expressing the donkey G6PD is found to be significantly lower than that expected. Whereas this observation suggests powerful selective forces acting against cells with the donkey *X* active, it would be useful to subject this interpretation to test. This could be done by cloning cultures derived from artificial mixtures of the two cell types.

Hook and Brustman's article on page 349 provides striking evidence of organ differences in G6PD expression among a very large sample of female mules. Two organ types could be identified, one in which the horse G6PD predominates and one in which horse and donkey G6PD expression is not significantly different from that expected on a random basis. Because a predominance of donkey G6PD was never consistently found in any tissue studied, these data agree with Hamerton's in suggesting that selective forces operate against cells with the donkey *X* active, at least in some tissues and organs.

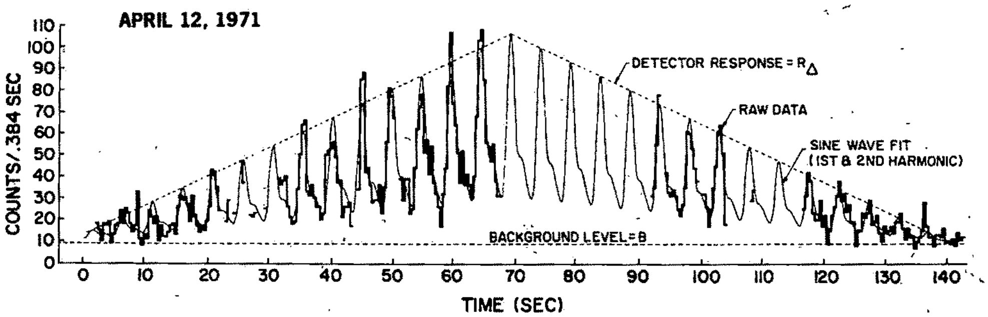
Earlier studies on the female mule had shown little agreement as to the frequency with which the horse and donkey *X*-chromosomes show late replication. In the light of the new evidence it seems most probable that this only reflected inter-animal variation. This could result either from the randomness with which one or other *X* is inactivated and/or from differences in the selection forces operating upon the two cell populations in different animals. In this context it will be interesting to see if selection operates against cells with a donkey *X* active even in animals which show a predominantly donkey G6PD expression. The new data do not contribute any evidence on the randomness of the *X* inactivation process in horse–donkey hybrids though they rule out the once real possibility that the paternally-derived *X* might be preferentially inactivated in the hybrid of each reciprocal cross.

New Class of Varying Stars

FOLLOWING the discovery of X-ray pulsations from Cygnus X-1, the data collected by the Explorer 42 X-ray satellite (Uhuru) have been intensively searched for evidence of any similar pulsations in other sources. To the surprise of most astronomers—and probably to their own surprise as well—R. Giacconi, H. Gursky, E. Kellog, E. Schreier and H. Tananbaum have now found that the source Centaurus

X-3 varies dramatically with a period of nearly 5 s, as shown in the figure. These variations are markedly different from the variations seen in pulsars, quite apart from the fact that in both Cyg X-1 and Cen X-2 there is no evidence of associated optical and radio pulsations (*Astrophys. J. Lett.*, **167**, L67; 1971).

The Cyg X-1 discovery itself caused quite a stir among astronomers and although this source has now been identified at radio frequencies (Braes and Miley, *Nature*, **232**, 246; 1971) it remains difficult to fit it into the accepted pulsar model. But the Cen X-3 discovery is even more remarkable, and although many people would be prepared to argue that the evidence for periodic variations in the X-ray flux from Cyg X-1 is so marginal that it could be discounted, it would be a brave man who ventured to suggest that the period seen in the flux from Cen X-3 is not a genuine effect (compare the figure here with that in *Nature Physical Science*, **231**, 91; 1971). The problem of the origin of these periodicities remains unsolved, but it is becoming increasingly clear that they cannot be interpreted in terms of the rotating neutron star model of pulsars.



X-ray variations of Centaurus X-3. Roughly 70 per cent of the power radiated at 2–6 keV is pulsed. From *Astrophys. J. Lett*, (**167**, L67; 1971).

In their latest report, Giacconi *et al.* discuss variations in the flux from Cen X-3 with time scales of days, minutes and seconds. Although the intensity of the source changed by a factor of 10 over January 11 and 12 of this year, the mean flux before and after this increase was remarkably constant. The almost sinusoidal rapid variation which is seen after this flaring has a period of 4.8713 s for the first 9,000 s after the flare, which changes over the next hour to a 4.8703 s period, this in turn lasting for roughly 28,000 s before changing, again over an hour, to a 4.8726 s period. This decrease by 1 ms followed by an increase by 2.3 ms poses serious problems in constructing models of the source. Furthermore, by April, the period of the variation had decreased by roughly 30 ms, that is, by 0.6 per cent.

In carrying out a search for evidence of any more rapid variation, the team have found only the "fundamental" 4.8 s period and a strong "first harmonic" at 2.4 s, using Fourier analysis of the January data. Any other rapid variations must occur with a time scale that is short compared with the 0.096 s integration time of the detector used. The presence of two harmonics in the variation, together with the sinusoidal shape of the curve which best fits the observed changes, also points to a pulsational origin, rather than one associated with rotation. Perhaps the pulsating white dwarf model of pulsars, discarded by theoreticians in favour of the rotating neutron star model, might be revived to explain this newly discovered phenomenon.

So Cen X-3, and in all probability Cyg X-1, cannot be fitted to the model of pulsars built up over the past three years. The sudden changes in period, the presence of overtones, and the simple fact that no optical or radio pulses are seen all point to the same conclusion. Do these sources form a new class of previously undetected objects? Or are they simply a previously unexpected manifestation of the pulsar phenomenon? In either case, there is a great deal of hard thinking ahead of the theoreticians. It may even be that Cen X-3 and Cyg X-1, as well as being different from pulsars, are entirely different sorts of object from each other. Indeed, the apparent differences between the spectra, Cyg X-1 following a power law and Cen X-3 seeming more like a blackbody, make it very likely that the sources have different origins.

Just as the development of optical astronomy earlier this century opened up unforeseen areas of research and led to the development of theories explaining the early and middle stages of stellar evolution, so high energy astronomy is now revealing equally unprecedented data, which may, when gathered into a coherent pattern, reveal the way in which stars move towards the end point of their evolution, producing short lived energetic phenomena such as pulsars and at least one or two types of regularly variable X-ray stars before they sink into obscurity as cold white dwarfs or neutron stars.

Confusion over Fossil Man

WHEN Leakey, Tobias and Napier redefined the genus *Homo* in 1964 they also described the type of the earliest known species of this genus, *Homo "habilis"*. The redefined genus as well as the new species have been the object of considerable discussion and dispute and the attribution of Olduvai Hominid 24 to *Homo habilis* by Leakey, Clarke and Leakey on page 308 of this issue of *Nature* will in no way lessen the controversy. The revised definition of the genus *Homo* included several cranial features that differed only slightly from, or overlapped with, those found in *Australopithecus*; indeed, many of the cranial criteria were so ambiguously flexible that perhaps only the odd gorilla would have been denied membership. It must be pointed out, however, that distinctions between genera are not subtle and overlapping features, but they reflect and represent major adaptations to a particular way of life; that is the "adaptive plateau" discussed by Sewall Wright, Ernst Mayr and others. It is difficult, therefore, for some palaeoanthropologists to accept as generically distinctive the differences in certain features in *Australopithecus africanus* and *Homo "habilis"* which are probably due only to normal variation within a single species. Moreover, the slow evolution of a single, widespread population of *Australopithecus* into *Homo* would seem more consistent with modern evolutionary theory than with the continued existence of several generically and specifically distinct groups of hominids.

Criticisms of theory aside, it is interesting and surprising to find that the new *Homo "habilis"* from Olduvai does not fulfil all the generic criteria outlined in the 1964 revision. The revision calls for a minimum cranial capacity of "about 600 cm^3". Although some gorillas have more than 600 cm^3, Olduvai Hominid 24 has only 560 cm^3. This is admittedly a preliminary estimate because there is "still some evidence of distortion in the reconstruction" and two inevitable questions arise: if cranial capacity cannot be accurately determined does this not undermine the validity of many of the cranial measurements? And does it not also undermine the validity of the conclusions that are drawn, at least in part, from those measurements?

One taxonomic criterion included in the 1964 revision was not phrased in equivocal terms: a member of the genus *Homo* will not have the concave or "dished" face characteristic of all known australopithecines. The dished face is a composite feature of considerable evolutionary significance and its absence in later hominines represents a diminution of the teeth and jaws, with reduced prognathism in the lower part of the face and emergence of the nose. The profile line drawing (Fig. 3 on page 310) clearly demonstrates the dished face of Olduvai Hominid 24 and serves to emphasize its overall similarity with the undoubted australopithecine from South Africa.

Olduvai Hominid 24 has been nicknamed "Twiggy" because its flattened shape before restoration resembled that notably unendowed figure. A badly crushed and distorted skull nearly two million years old presents some problems to those restoring it; it presents even more problems to those who try to decide, finally, what it is. On the basis of the evidence presented by Dr Leakey it is very difficult to agree that this specimen is "clearly *Homo*". Indeed it would seem to be excluded from that group on the basis of his own criteria are outlined in his 1964 article.

TRANSFORMATION

Responses to Cyclic AMP

from our Cell Biology Correspondent

EARLIER this year I drew attention in this column to the work of Puck and his associates and Pastan's group who respectively showed that dibutyryl adenosine cyclic 3′,5′ monophosphate causes Chinese hamster ovary cells growing in culture to assume reversibly a fibroblastic morphology, and causes the phenotypic reversion of transformed mouse fibroblasts. More recently *Nature's* Cell Physiology Correspondent (*Nature New Biology*, **232**, 35; 1971) commented upon the experiments of Sheppard, who has shown that doses of this substituted cyclic AMP not only restore the growth control and susceptibility to contact inhibition of populations of 3T6 cells and 3T3 cells transformed by polyoma virus but also reduce the susceptibility of these cells to agglutination by wheat germ agglutinin.

Myoglobin Sequence Completed

THE torrent of articles concerned, on the one hand, with the structure and function of haemoglobins and on the other with the characterization of variant haemoglobins, shows no sign of abating. A welcome addition, however, is the appearance, in next Wednesday's *Nature New Biology*, of three articles on the sequence of human myoglobin, the choice of an allosteric model for the haemoglobin-oxygen system, and the relation between structure and properties of the Japanese human haemoglobin variant Hb-Hiroshima.

Herra and Lehmann have repeated some parts of Hill's sequence studies on human myoglobin and disagree with him on only three points. They have also completed the sequence which largely agrees with that proposed from considerations of amino-acid composition and homology with the known sequence of sperm whale myoglobin. Their sequence allows the differences between seven myoglobins to be delineated and will no doubt stimulate discussion on the homology and evolution of mammalian myoglobins. The unique feature of human myoglobin is a cysteine residue at position 110 (G11), which it has in common with the α-chain of human haemoglobin. Apart from this feature, human myoglobin resembles the human α-chain to no greater extent than the β-chain, having thirty-seven identical residues with each. Of these several are discovered among the haem contacts of the α and β-chains.

The complete human myoglobin sequence will be of great value in determining the structural differences of the four known human skeletal myoglobin electrophoretic variants; the possible existence of neutral mutations can also be investigated. Herra and Lehmann report no differences in the fingerprints of human skeletal and heart myoglobin and the skeletal myoglobin of a case of Duchenne's muscular dystrophy. Further work on human myoglobin variants, which has in the past been an area of some confusion, will be awaited with interest.

Connoisseurs of theories of allosteric systems will no doubt appreciate a re-examination by Minton in next Wednesday's *Nature New Biology* of a recent treatment by Edelstein (*Nature*, **230**, 224; 1971) of the allosteric model for haemoglobin. Edelstein had concluded that the sequential model of Koshland, Nemethy and Filmer describes the oxygen binding curves of several species of human haemoglobin less well than does the two-state model of Monod, Wyman and Changeux. Minton points out that a tacit assumption in Edelstein's analysis is likely to be invalid, in which case it cannot be shown that any one of the two models is more appropriate to describe the haemoglobin-oxygen system.

In another article, Perutz and Shibata and their co-workers have re-investigated the Japanese haemoglobin variant Hb-Hiroshima, in which the magnitude of the Bohr effect is halved, the oxygen affinity at physiological *p*H is increased three times and the haem-haem interaction is somewhat reduced. This variant was thought to have the substitution β143 (histidine→aspartic acid), but the normal effect of 2,3-DPG on the oxygen affinity of Hb-Hiroshima was inconsistent with the proposed role of this residue in the binding of 2,3-DPG by normal adult haemoglobin.

Perutz and his colleagues have now been able to show, both by X-ray crystallography of deoxy Hb-Hiroshima and by chemical methods, that the replacement is in position β146. The physiological properties of Hb-Hiroshima can now be accounted for without difficulty. The result also confirms the earlier suggestion that half the alkaline Bohr effect is due to the salt bridge between the imidazole side chain of β146 (His) and the γ-carboxyl group of β94 (Asp). The reduced haem-haem interaction in Hb-Hiroshima is also related to the absence of a histidine residue at β146, which affects both the tertiary structure of the β-chains and the quaternary structure of the entire molecule.

In the latest issue of the *Proceedings of the US National Academy of Sciences* (**68**, 1648 ; 1971), Hsie, Jones and Puck describe their latest observations of Chinese hamster ovary cells exposed to dibutyryl cyclic AMP alone or in combination with a variety of steroid and other hormones. The change in morphology and pattern of growth of these cells on exposure to this substituted cyclic AMP is accompanied by a strict susceptibility to contact inhibition of growth and a decreased susceptibility to agglutination by wheat germ agglutinin. Moreover, in the presence of dibutyryl cyclic AMP Chinese hamster ovary cells fail to round up when exposed to concanavalin A or antibody lacking complement. The synthesis of collagen as judged by the intracellular concentration of hydroxyproline, which is of course a characteristic property of fibroblasts the expression of which may be modulated by virus transformation, is, however, increased six to ten times when these ovary cells are exposed to the drug.

Puck and his colleagues have also tested a wide range of male and female steroid sex hormones to discover whether the synergistic effect of testosterone on the morphological conversion induced by dibutyryl cyclic AMP is highly specific or common to many if not all steroids. They found that the male steroid hormones and some prostaglandins all have this property but the steroid oestrogens do not. Apparently the group have isolated mutant cells which have a fibroblastic morphology even in the absence of cyclic AMP; they believe that these cells carry single gene mutations and their analysis—an exciting prospect—should turn attention to the genetic control of morphological and biochemical transformation induced by this unique nucleotide.

It would be wrong, however, to run away with the idea that the multiplication of all cells is inhibited, or at least brought under strict contact inhibitory control, by cyclic AMP. For MacManus, Whitfield and Youdale, in an interesting article published earlier this year (*J. Cell Physiol.*, **77**, 103 ; 1971), reported that when rat thymic lymphocytes are exposed to epinephrine (adrenaline) the intracellular concentration of cyclic AMP and the activity of adenyl cyclase are both increased. And in due season these changes are followed by the induction of DNA synthesis and mitosis.

In other words, although cyclic AMP may suppress the unregulated multiplication of transformed fibroblasts and impose contact inhibition of Chinese hamster ovary cells, it, and its inducers, are mitogenic for suspensions of rat thymocytes, causing these cells to proliferate.

PHOTOCHEMISTRY

Dark Photobiology?

from our Biological Chemistry Correspondent

THE susceptibility of the nucleic acids to photochemical change on ultraviolet irradiation has been thoroughly explored. The mutagenic and lethal effects which follow such chemical modification of the bases are, for the most part, relieved by enzyme repair systems. Even in those unfortunate individuals where such repair is defective, it has been generally assumed that the resulting photochemical damage would be solely restricted to those cells on the surface of the organism which are exposed to ultraviolet irradiation.

This restriction is now questioned by the elegant demonstration that pyrimidine dimers can be formed as a result of the generation of electronically excited molecules without light. Lamola selected trimethyl-1,2-dioxetane for this purpose because it is known to thermally decompose into excited state acetone and acetaldehyde and such carbonyl compounds have triplet-state energies closely matched to those of thymine and cytosine.

As expected, when the dioxetane was decomposed at 70° in the dark in the presence of *Escherichia coli* DNA, Lamola was able to isolate pyrimidine "photodimers" from both thymine and cytosine residues (*Biochem, Biophys. Res. Commun.*, **43**, 893; 1971). Although he estimates the efficiency of this process to be not much more than 1 per cent of the photosensitized reaction, the hunt must be on for other electronically excited promoters both to raise the level of dimerization and to provide a biological model.

There is no speculation in the photochemical modification of those tRNAs which contain 4-thiouracil because this base is excited by medium-wavelength ultraviolet light at 334 nm. Favre, Yaniv and their colleagues (*J. Mol. Biol.*, **58**, 367 and 381 ; 1971) now provide some of the facts about the results of such irradiation. A 4-thiouracil at position-8 becomes covalently linked to a cytosine at position-13 in $\mathrm{tRNA}_{\mathrm{I}}^{\mathrm{Val}}$ and in five other tRNAs which all have those residues in common. Thus it seems likely that these six tRNAs have similar structures.

The nature of the linkage is not clear, though it is distinct from that in known photodimers and survives borohydride reduction of the tRNA in a modified form which provides a new fluorescent chromophore in the molecule. This fluorescence can be used to probe changes in the tertiary structure of the modified tRNAs and reveals that they have experienced very little change in segments of the tRNA other than that containing residues 8 and 13.

Contractile Microfilaments

A SURPRISING result has emerged from recent structural and chemical studies of motile processes in cells. The proteins actin and probably myosin as well, which were formerly thought to be present only in muscle cells, are in fact widely distributed in a variety of cell types. Microfilaments, structures 40–60 Å in diameter, which have been shown in some cases to be composed of an actin-like protein, appear to be involved in protoplasmic streaming, cytokinesis, formation of a ruffled membrane, nerve outgrowth and various morphological movements. In next Wednesday's *Nature New Biology*, Allison, Davies and de Petris describe the participation of microfilaments in phagocytosis and pinocytosis by mouse peritoneal macrophages.

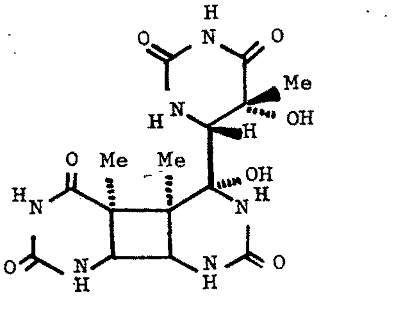
Two techniques have been responsible for recent advances—an apparently specific test for the identification of actin-like microfilaments and a specific inhibitor of microfilament function. Heavy meromyosin forms a complex with actin in which the asymmetric myosin molecule is at 45° with the actin filament leading to a characteristic "chevron" appearance. Glycerination, which allows entry of the myosin, enables microfilaments to be identified within the cell. It was first shown by S. B. Carter (*Nature*, **213**, 261 ; 1967) that the drug cytochalasin B breaks down microfilaments, and various functions of microfilament have been blocked by this compound (N. K. Wassells *et al.*, *Science*, **171**, 135 ; 1971).

Allison and his collaborators show that macrophages contain bundles of filaments in the peripheral cytoplasm which bind heavy meromyosin. Cytochalasin reversibly blocks cell movement, ruffled membrane formation and pinocytosis in these cells. Phagocytosis of bacteria is also blocked even though the bacteria still attach to the plasma membrane. Treatment with colchicine, a specific inhibitor of microtubule dependent processes, does not effect pinocytosis.

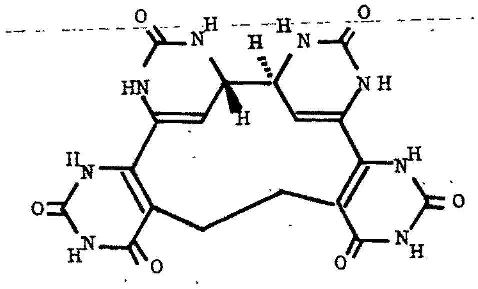
The ability to distinguish between the functions of microtubules and microfilaments by specific drugs and the isolation of both proteins have opened the way for an understanding of the functioning of fibrous proteins in intracellular and morphogenetic movements.

The thiouracil-cytosine linkage does, however, have small effects on the biological activity of the tRNA which is shown in a reduced affinity for the synthetase and a slower rate of incorporation of valine from the charged tRNA into polypeptides during protein synthesis.

The photoproducts so far isolated from DNA never contain more than two pyrimidines linked together. Wang, however, has isolated and characterized trimers and tetramers from irradiation of pyrimidines in ice matrices and in water (*J. Amer. Chem. Soc.*, **93**, 2554 and 2768; 1971) which could prove to have biological counterparts. The trimer in particular has the interesting property of reverting either to thymine and thymine dimer or to thymine and a thymine-adduct.



I



II

The structure (I) of the trimer and that of the tetramer (II), produced on irradiation of a thymine-uracil photoproduct, have been elucidated by Flippen and Karle (*J. Amer. Chem. Soc.*, **93**, 2556 and 2762; 1971) by X-ray diffraction.

ANTIBODIES

Garnering γGlobulin

from our Molecular Biology Correspondent

A 6 Å electron density map of an immunoglobulin has now been achieved by Davies and his colleagues (Sarma *et al.*, *J. Biol. Chem.*, **246**, 3753; 1971) and is in gratifying accord with what has been deduced about the shape of the molecule by electron microscopy and other methods. In the crystal the molecules have a dyad symmetry axis, and the electron density distribution is interpretable in terms of three globular elements, joined together so as to form a T-shape, the stem consisting of the so-called Fc portion which is attached by means of a rather narrow hinge to the two Fab components. The hinge gives access to proteolytic enzymes, which cleave at this point, and is surmised from a variety of lines of evidence to be flexible when the molecule is in solution. The size and shape of the Fc unit are in good agreement with data derived from a study of the crystals of this fragment alone from Poljak's laboratory.

In a companion article, Lebaw and Davies (*ibid.*, 3760) show electron micrographs of the immunoglobulin crystal. They utilize the magic of optical integration—whereby multiple prints of the micrographs are made, advancing the photographic paper each time under the enlarger by a distance corresponding to the periodicity. The picture of a well-dug field then gradually resolves itself into a sharply-defined pattern, in which the repeating unit stands out as a T or Y shape, somewhat smaller than the hydrated molecules observed by X-ray diffraction in the unfixed crystal, but exactly like the isolated molecules observed in the electron microscope by Valentine and Green some years ago.

These γG-immunoglobulin molecules consist of two heavy and two light chains, joined together by disulphide bonds, but other forms exist with a higher level of complexity. Thus electron microscopy has shown macroglobulin (immunoglobulin M) to consist of a cyclic pentamer of γG-like (7S) subunits. The units are joined by disulphide bonds between their Fc regions. The determination of the sequence of events in the assembly, first of the four-chain structure, then of the pentameric form, has been one of the recent triumphs of the field, and some details of the mechanism are described in the latest article by Askonas and Parkhouse (*Biochem. J.*, **123**, 629; 1971). They showed previously that inside the tumour cells, in which the macroglobulin is made, only the 7S subunits are present. By the time the newly synthesized protein is secreted assembly to the complete pentamers has occurred. When the subunits from lysed cells are allowed to stand in air, no pentamers

---

The Truth about Juan de Fuca

THE appealing simplicity of seafloor-spreading is probably an illusion on two counts. First, it would be surprising if a conceptual model so simple were to be rigorously applicable to a real Earth which is manifestly so complex, although the seafloor spreading model in its most basic form has done much to unify the Earth sciences. Current ideas on spreading will in the course of time no doubt come to be seen as a first approximation rather than as a detailed hypothesis for the behaviour of the Earth. But second, and perhaps more obviously, even if the seafloor is being, and has been, formed in a simple way, the final picture is likely to be quite complex. For notwithstanding the comparative youth of the oceans, changes may have taken place in the structure of the ocean floor since spreading began. Indeed, considering the state of activity in the oceanic crust it would be surprising if they had not.

As Peter and DeWald show in next Monday's *Nature Physical Science*, a good example of the latter type of complexity is the ocean floor off the north-west coast of the United States. Here the Juan de Fuca, Gorda and Explorer Ridges, each of which is offset from the others and each of which trends in a north-easterly direction, were clearly formed as a single rift but have since been subject to numerous fractures. The first job is thus to try to construct an authentic map of the anomalies and faults in the region; and this is what Peter and DeWald have done, partly with the help of data previously published and partly with thirteen new east–west magnetic traverses carried out off the coasts of Washington and Oregon.

Examination of the pattern of magnetic offsets clearly shows the existence of a previously undiscovered fracture zone separating the mountainous ridge topography from the Tufts Abyssal Plain. This Peter and DeWald have termed, appropriately, the Juan de Fuca Fracture Zone. But, more importantly, the discovery of the new fracture taken in conjunction with the complete anomaly map has enabled Peter and DeWald to end the speculation about why the original ridge broke up and why its component parts now trend north-east. The fact is that some of the anomalies west of the Juan de Fuca Ridge are missing and obviously disappear along the newly discovered fracture zone which coincides with a 550-metre change in seafloor relief. This must indicate deformation involving rotation, strike-slip faulting and the over thrusting of small crustal plates; and according to Peter and DeWald the most likely cause is compression in the roughly north-east–south-west direction. What is now quite clear is that the ridge trends in this region cannot be attributable to anything as gradual as a slow realignment produced by a change in the direction of seafloor spreading.

are formed. The finished pentamers are easily reduced, and electrophoretic analysis shows that 7S monomers are first formed—as well, apparently, as some oligomeric intermediates. At higher concentrations of reducing agent the intra-subunit disulphide bonds begin to break, and HL pairs (one heavy with one light chain) appear, showing that the internal H–H disulphide bonds are next in order of lability. Finally, the remaining (H–L) links break and single heavy and light chains are produced. The 7S monomers, allowed to oxidize in air, regenerated the inter-subunit disulphide bonds and the pentamers were once more formed in high yield. When the free thiols were blocked with iodoacetamide no pentamers appeared.

Evidently then, the freshly synthesized, intracellular, subunits, which resist all attempts to make them polymerize, differ in the reactivity of their thiols, and Askonas and Parkhouse conjectured that these might be chemically protected. An obvious mechanism for easily reversible modification would be the formation of a mixed disulphide with a thiol present in the cell. Their surmise proved correct: treatment of the molecule with a reducing agent in low concentration converted it to a form that readily generated pentamers on standing. Exposure to the alkylating agent iodoacetamide before this reduction step had no detrimental effect, whereas alkylation after the reduction caused total inhibition of polymerization. The nature of the blocking group is unknown; cysteine or glutathione are obvious candidates, though an especially attractive alternative would be an intra-chain disulphide bond. Such a situation would be reminiscent of the formation of streptococcal protease from its zymogen by reduction of a disulphide bond. One must suppose that the liberation of thiols and polymerization occur during the passage of the immunoglobulin monomers through the membrane, where, it will be recalled, the disulphide-exchange enzyme is known to reside.

The earlier stage of synthesis—the association of light and heavy chains to give 7S subunits—has been studied by Parkhouse (*ibid.*, 635). The most stable disulphide bonds—those between light and heavy chains—are formed first, and the two HL fragments then associate. Pulse-labelling studies show that the light chains are present in a sizable pool.

Other disulphide-linked oligomers (IgA) are secreted by tumour cells of a different kind, and Parkhouse (*FEBS Lett.*, **16**, 71 ; 1971) has shown that here too only the 7S form is present in the cell. Oligomers up to the tetramer are formed; the disposition of the linking groups in this case evidently does not favour pentameric cyclization. In this species, moreover, the association between heavy and light chains is non-covalent only.

HIGH ENERGY PHYSICS

The Elusive Quark

from a Correspondent

RECENT experimental studies at Livermore and Berkeley of more than 100,000 cosmic ray tracks from extensive air showers have failed to reveal any tracks made by fractionally charged particles and so another search for quarks has proved fruitless (A. F. Clark *et al.*, *Phys. Rev. Lett.*, **27**, 51 ; 1971).

The hunt for quarks has been going on since their existence was independently proposed by Murray Gell-Mann and George Zweig in 1964. They have been looked for since then with particle accelerators, in cosmic radiation, and in investigations of macroscopic material, for example seawater: most of these searches rely on the fact that the quarks should possess nonintegral electric charges of one-third, or two-thirds of that of the electron, and should therefore yield tracks in particle detectors with substantially lower ionizations than conventionally charged particles.

Although no substantiated evidence for the quark has been revealed by these investigations, there have been a few false hopes. In 1969, an Australian group, led by C. B. A. McCusker of the Cornell-Sydney University Astronomy Centre, claimed that certain anomalies, observed in cloud chamber studies of cosmic ray ionizations, were most likely to have been caused by quarks of charge $\frac{2}{3}e$ (*Phys. Rev. Lett.*, **23**, 658 ; 1969).

Five candidates for such quark events were reported from a total of some 6,000 cloud chamber photographs. This result was greeted with some scepticism by the physics community and was soon under severe attack: an article by R. K. Adair of Yale University and H. Kasha of Brookhaven (*Phys. Rev. Lett.*, **23**, 1355 ; 1970) indicated that the anomalously low ionizations reported by McCusker *et al.* were probably due to low energy electrons and muons associated with the air showers. A more recent report of a quark sighting by a group from Ohio State University, and based on a cosmic ray bubble chamber photograph, has also received very little support (W. T. Chu *et al.*, *Phys. Rev. Lett.*, **24**, 917 ; 1970).

Escaping Atmospheres

A CONTINUAL battle rages between the high-speed molecules trying to fly off from the Earth's atmosphere and the gravitational attraction trying to restrain them. For some planets, like the Earth and Venus, gravity has triumphed over levity up to now, but for the Moon, and probably Mercury, most of the escaping molecules have eluded their gravitational jailers; on Mars the issue is still in doubt, and a thin atmosphere persists, rather precariously.

The escape of molecules from the outer atmospheres of planets is determined chiefly by the temperature prevailing in the region, usually called the exosphere, where the mean free path of molecules is greater than the scale height—the height in which pressure and density decline by a factor of 2.8. In an article in next Monday's *Nature Physical Science*, S. J. Bauer describes a method for estimating this temperature in planets like Venus and Mars and hence the atmospheric escape rate.

If the molecules in the exosphere have thermal velocities higher than the escape velocity from the planet, the molecules travelling upwards are not very likely to suffer further collision, and will usually escape. The escape velocity for the Earth is between 10 and 11 km s^{-1} and this is the thermal velocity corresponding to a temperature of about 4,000 K for hydrogen atoms (or 16,000 K for helium atoms). The actual temperatures at the base of the Earth's exosphere, at a height of about 500 km, are determined largely by the heating at lower heights (100 to 200 km), where solar extreme ultraviolet radiation is absorbed. On Earth it so happens that the solar radiation does not usually produce exospheric temperatures greater than 2,000 K, and even these temperatures are only maintained during a high maximum of solar activity, like that in 1957 and 1958. So, although there is no guarantee that the Sun will continue to be so cooperative, the present loss of hydrogen from the Earth's temperature is not too serious. There is always some loss, however, because the high-velocity tail of the Maxwellian distribution of thermal velocities can make its escape unchecked.

For the Earth, Bauer has calculated that the exospheric temperature at a strong solar maximum (2,000 K) is about 2.5 times that at solar minimum (800 K). For Venus and Mars, he suggests that the corresponding factor is about 1.7, and concludes that Earth and Venus have exospheres which, although subject to some loss, are stable even at solar maximum, whereas the atmosphere of Mars is stable only near solar minimum and suffers large losses at solar maximum.

The experimental technique used by Clark *et al.* was similar to that used by McCusker *et al.* and was based on cloud chambers triggered by coincidence counters. Artificially produced low-droplet count tracks were used to determine the detection efficiency for quark-like tracks, and from this the US group claim that their scanning team would have picked up four of the original five quark candidates found by the Sydney team. They concluded that no quarks had been observed, so that, for the present at least, quarks must remain essentially a convenient mathematical abstraction.

The story, however, is by no means over. On the theoretical side, the now fashionable parton picture of particle structure has stimulated renewed interest in quarks. Parton is the name given by theorist Richard Feynman of the California Institute of Technology to the unknown constituents of the nucleons and attempts have been made to equate the parton and the quark. On the experimental side, quark searches have been proposed for the giant new accelerator at Batavia, Illinois, and without a doubt the physicists working with CERN's new intersecting proton storage rings will maintain a sharp vigil for anomalously ionizing radiation. Meanwhile, most high energy physicists are still keeping an open mind on quarks, and awaiting further developments.

NARCOTICS

Antagonist Activities

from a Correspondent

FUNDAMENTAL to the symposium of the British Pharmacological Society on agonist and antagonist activities of narcotic analgesic drugs, held at the University of Aberdeen on July 12 and 13, was the concept that nearly all drugs of the group discussed possess both agonist and antagonist properties. This concept originated from the findings of L. Lasagna and H. K. Beecher in 1954 that the morphine antagonist nalorphine itself has analgesic properties, and of J. E. Villarreal about ten years later that some powerful analgesics of this group have enough antagonist activity to precipitate abstinence effects in morphine-dependent monkeys. The concept was quantified by H. W. Kosterlitz and his co-workers, who measured in the isolated ileum of the guinea-pig the opposed activities of drugs of this group, believed to correspond with their agonist and antagonist actions in the central nervous system. Using this simple model, these workers at the University of Aberdeen were able to place these drugs in descending order of the ratio of the agonist to antagonist activities that each possessed, making a series beginning with the powerful analgesics heroin and morphine and ending with the seemingly "pure" antagonist naloxone. At the symposium, the validity of this procedure was supported by measurements of the antinociceptive and morphine-antagonist potencies of six drugs in this series in the rat and mouse by Drs H. O. J. Collier and C. Schneider (Miles Laboratories Limited, Stoke Poges). These measurements placed the drugs in an essentially similar order to that obtained with the guinea-pig ileum.

These experiments, both *in vitro* and *in vivo*, showed that the analgesic pentazocine possesses appreciable antagonist activity, placing it at a point some distance down the series from morphine in the direction of naloxone. That pentazocine is freer of addictive liability than is morphine or heroin supports the idea that this liability diminishes as the antagonist activity of an analgesic increases. A paper by Dr G. F. Blane (Reckitt and Colman, Hull) and other contributions indicated that the advent of pentazocine has changed the search for new drugs of this group by lessening the acceptability of compounds with a high agonist to antagonist ratio and hence high addictive liability.

With the increase in drug addiction, much of the work reported at the symposium was slanted towards problems of dependence on opiates. The directions explored owed something to suggestions about the mechanism of drug dependence advanced during the 1960s by A. and D. B. Goldstein, W. D. M. Paton, H. O. J. Collier and others. Thus, Drs E. L. Way (University of California, San Francisco) and B. M. Cox (Chelsea College of Science and Technology, London) have found that drugs blocking protein synthesis inhibit the development of dependence as well as tolerance towards morphine. Again, Drs T. C. Muir and D. Pollock (University of Glasgow)

Agglutinins and the Cell Surface

DURING the past eighteen months plant agglutinins, or lectins as they are sometimes called, have become a major topic of conversation in many cancer research laboratories; for, as Burger and his colleagues at Princeton and Sach's group at Rehovot have shown, these substances preferentially agglutinate certain spontaneously and chemically transformed cells and some cells transformed by DNA tumour viruses. Moreover, a considerable collection of circumstantial evidence has been accumulated which suggests that the changes in the cell surface which can be monitored by measuring susceptibility to agglutination are intimately and perhaps causally connected with the regulation of cell division (see, for example, *Nature*, **228**, 502; and **228**, 512; 1970).

It has generally been assumed that, because transformed cells are more readily agglutinated by lectins such as concanavalin A and wheat germ agglutinin, transformation must result in the exposure of receptor sites which bind these molecules. In other words, the simple idea was that the transformed cell has more of these binding sites exposed on its surface than does the untransformed cell and that this surface change was somehow involved in the unregulated growth of transformed cells. In next Wednesday's *Nature New Biology*, however, the research groups of Ozanne and Sambrook and Cline and Livingston put a cat in this particular dove-cote.

Using either concanavalin A or wheat germ agglutinin labelled with ^{125}I or concanavalin A labelled with ^{3}H, they have shown that although BHK hamster cells and mouse 3T3 cells transformed respectively by polyoma virus and SV40 are more readily agglutinated than the corresponding untransformed cells, the untransformed and transformed cells bind, on a per milligram of protein basis, about the same amounts of both concanavalin A and wheat germ agglutinin.

In other words, there is no correlation between the amount of lectin a cell can bind and its susceptibility to agglutination by that lectin. The increased susceptibility to agglutination of transformed cells does not result from the exposure of more lectin-binding sites at the cell surface. The most convincing and elegant proof of these statements is provided by Ozanne and Sambrook who have measured the binding capacity and agglutinability of hamster BHK cells transformed by the temperature sensitive mutant of polyoma virus *ts*3. Growing at 32° C these cells are agglutinated by low concentrations of concanavalin A or wheat germ agglutinin but when they are shifted to 38.5° C much larger amounts of the lectins are needed for agglutination. The amount of lectin bound, however, is the same at both temperatures.

Clearly, increased susceptibility to agglutination reflects surface changes more subtle than an increase in the number of lectin-binding sites and as Ozanne and Sambrook say "it seems difficult to postulate a general mechanism of growth control based solely on the number of exposed lectin binding sites".

reported that, in the rat, the response of smooth muscles to adrenergic or cholinergic stimulation greatly increased after the withdrawal of chronic treatment with morphine—reaching a peak three days later.

In another direction, the mechanisms of dependence suggested some years ago require an interaction of morphine with a humoral mediator involved in the activity of neurones in the brain. In this direction, some setbacks and some encouraging findings were recorded. On the one hand, the evidence adduced by Drs A. Goldstein (Stanford University) and I. Marshall (St Mary's Hospital Medical School, London) was against the proposition, stoutly defended by Dr Way, that a change in the level or turnover of brain 5-hydroxytryptamine is important in morphine dependence in the mouse. On the other hand, C. B. Smith and J. E. Villarreal (University of Michigan, Ann Arbor) reported that continued treatment with morphine and other addictive opiates increases the incorporation of ^{14}C-tyrosine into the noradrenaline and dopamine of the brain of the mouse. The increased turnover of brain catecholamines can not be induced by strong antagonists of morphine and is blocked by naloxone; in the author's opinion it is correlated with addictive liability in this group of drugs.

BACTERIOLOGY

More Pollution

from a Correspondent

THE summer conference of the Society for Applied Bacteriology was held at the University of Liverpool from July 13 to 15 and took the form of a symposium on the microbial aspects of pollution. The contributions and subsequent discussions interpreted the title in the broadest possible terms so that well discussed topics, such as the pollution of the aquatic environment, and lesser known forms of pollution, such as the disposal of infected laboratory materials, were included. Few new data were presented, but they illustrated the importance of microbiological processes in the armoury for combating today's pollution problems.

Dr A. L. Downing (Water Pollution Research Laboratory, Stevenage) warned microbiologists not to get too excited at the prospect of an increased number of jobs for their profession in this expanding field because the introduction of new and more stringent existing standards for effluent quality could well result in non-biological methods becoming increasingly more important.

Problems pertaining to the eutrophication of surface waters were covered in detail by four speakers. Professor P. L. McCarty and Dr R. T. Haug (Stanford University, California) discussed the various methods available for the removal of nitrogen from sewage effluents by the microbiological processes of nitrification and denitrification and presented evidence for supporting the use of aerobic and anaerobic upward-flow submerged filters. Dr N. P. Burman (Metropolitan Water Board, London) pointed out, however, that the removal of nitrogen and phosphorus from sewage effluents was not always the answer to eutrophication problems, and gave the example of the Thames valley reservoirs where eutrophic conditions were inevitable because sufficient quantities of these elements were available from sources other than sewage.

Aerobic and anaerobic biological treatment of sewage and sewage sludges was discussed from both the microbiological and applied points of view and it was particularly encouraging to hear that the lead given by the detergents industry which changed from the production of biologically "hard" to "soft" materials is seriously being considered by industries producing other widely used materials. First, Dr H. O. W. Eggins (University of Aston) spoke on the possibilities of the plastics industry producing materials with parts in the chemical chain susceptible to microbial attack which would cause depolymerization and allow the subsequent attack of the smaller molecules by other microbes. Second, Dr S. L. J. Wright (Bath University of Technology) stated that a particularly attractive idea concerning the degradation of herbicides was the possibility of spraying the treated soil with an appropriate active microbial culture after an optimum time; preliminary experiments had already met with success. Third, Dr R. E. Cripps (Shell Research Ltd, Sittingbourne) spoke of his work on the relation between the chemical structure of pesticides and the possibilities of their degradation by microbial populations.

Chromosome Replication in Two Directions

FOR the double stranded and covalently closed circular DNA molecule which constitutes the chromosome of *Escherichia coli* to replicate in a semiconservative fashion the two parental strands of DNA must separate. There is, of course, evidence indicating that this separation is achieved by breaking phosphodiester bonds of the backbone of one of the two strands such that the nicked strand can be unwound from the circular strand. (Whether a single nick suffices or the chromosome is repeatedly nicked during replication is in the long run immaterial, for the net result is the same.) Moreover, genetic experiments have shown that replication begins and proceeds sequentially from a fixed point on the chromosome, the origin of replication. In theory at least replication might proceed in one or in both directions from this origin and until now it has generally been assumed that replication is unidirectional, proceeding 360° from the origin back to it. In next Wednesday's *Nature New Biology*, however, Masters and Broda challenge this notion; their experiments lead to the conclusion that replication occurs in both directions from the origin.

Masters and Broda have, in essence, measured the frequency of occurence of particular genetic markers situated at either side of the origin of replication in cultures of cells growing at different rates. They used the generalized transducing phage P1 which picks up *E. coli* genes at particular frequencies dependent on the number of copies of the genes present in the cell and the ease with which they can be integrated into the phage genome.

Ratios of the frequency of particular genetic markers measured in this way fall on a two directional gradient when plotted against the markers' genetic map positions. Moreover, a series of elegant transduction experiments with various male and female strains of *E. coli* indicate that the relative frequency of transduction of a particular marker depends on the position of that marker in the chromosome.

In dividing cells it seems that there are more copies of markers immediately adjacent to both sides of the origin of replication than there are copies of genes distal from the origin and genes close to the origin are more often transduced than genes distal from it. Both these findings indicate that replication proceeds in both directions from a fixed origin. Furthermore, assuming that the current genetic map accurately represents the length of DNA between markers, it seems that the rate of replication in the two directions is not the same. The terminus of replication is not 180° from the origin and replication in a clockwise direction about the chromosome seems to be faster than replication in the counterclockwise direction. In other words, Masters and Broda claim that replication of the *E. coli* chromosome is bidirectional but asymmetric and, as they note, Caro and his colleagues have reached the same conclusion from quite different experiments.

Why Hydrology?

JOHN C. RODDA
Institute of Hydrology, Wallingford, Berkshire

The International Hydrological Decade is now more than six years old and decisions about future international cooperation must soon be taken if the impetus given to hydrology during this period is not to be lost.

WHY hydrology? What is the point of studying the circulation and distribution of the world's freshwaters? Why bother with this science concerned with the rain and what happens to it when, in Britain at least, there always seems to be so much of it? Perhaps these questions and others like them were relevant twenty or thirty years ago, but not today; not when the consumption of water in the United Kingdom is rising at such a rate that demand will probably double by the end of the century and when the dry weather flow in a large number of rivers consists almost entirely of treated effluent from sewage works. In fact the Central Advisory Water Committee has recently made it clear[1] that re-use of this treated water will become increasingly important for supply purposes in the future.

Then at the other extreme, there are the floods that affect different parts of the country each year and can cause damage costing several million pounds annually. Fortunately loss of life occurs less frequently, but there can always be other disasters on the scale of that at Lynmouth in 1952. In fact there is some indication that the incidence of heavy rain is increasing; for example, on July 10, 1968, parts of Somerset and Gloucestershire recorded more than 130 mm of rain in 18 h, and in September of the same year parts of Kent, Surrey and Sussex experienced rainfalls of similar intensities. Even these rates of rainfall are, however, low on a world scale that reaches 1,500 mm a day for some Indian Ocean islands. One consequence of these relatively low intensities is that soil erosion is not the problem in Britain that it is, for example, in New Zealand. Nevertheless rivers draining the moorlands and mountains transport considerable amounts of sediment during flood, particularly when hill pastures have been ploughed recently. This also applies to almost any part of the country where building and construction are in progress on a large scale.

Facing the Hazards

Flood, erosion, drought and pollution are high on the list of hazards confronting man and they are also the chief problems facing the hydrologist. Can floods be forecast and drought severity predicted? Can the controls of the quality of the water flowing in a river be understood satisfactorily? These are questions that involve hydrologists the world over, but the fact that vast sums of money are spent in water supply schemes, flood alleviation works and sewage treatment plants, in order to mitigate the effects of these hydrological hazards, is usually taken for granted. In the same way, minimal attention is paid to the continuing research programmes into water problems which provide a basis for the design of these schemes and works. The aims and objects of the International Hydrological Decade (IHD), for example, are relatively little known. Other worldwide scientific programmes such as the International Geophysical Year (IGY) and International Biological Programme (IBP) have received far wider publicity, but perhaps none of these is as basic to the needs of modern civilization. The objectives of the IHD are to foster international collaboration and cooperation in hydrology and to take stock of the world's freshwater resources, to increase the effectiveness of research into such problems as floods and the effect of man on the hydrological cycle, and to improve the knowledge needed for the future management of water resources.

The IHD started in 1965 and was the outcome of an idea conceived in the early 1960s. At a preliminary meeting, representatives of fifty-seven nations agreed that their governments should support the United Nations Educational Scientific and Cultural Organization (UNESCO) in launching an international cooperative programme in hydrology; now the IHD involves more than 100 countries as well as other international organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the World Meteorological Organization (WMO). More than fifty technical items appeared in the original programme, and these included improving methods of measuring the basic factors of precipitation, evaporation and river discharge and making more effective use of maps for hydrological purposes. Proposals were made for standardizing methods of observation, for training and educating hydrologists in developing countries and for giving support to symposia on important topics. Progress up to 1969 was reviewed at the Mid-decade Conference, which was held in Paris in the December of that year, and both the programme for the remaining five years and plans for hydrology after the conclusion of the decade were also discussed. This question is now being considered by a special working group set up to make proposals for a long term UNESCO based programme in scientific hydrology. One problem is how to devise a means of fostering hydrology internationally, which is not just a revamping of IHD ideas spread over a longer time; another is that developing countries have special needs that may not be catered for in an intensely scientific programme. There are also the roles of the other UN agencies to be considered and the position of the International Association of Scientific Hydrology (ASH), one of the members of the International Union of Geodesy and Geophysics (IUGG). The FAO, for example, has its special interest in applying hydrology to the betterment of agriculture and the WMO is embarking on its own programme for operational hydrology. What is more, the meeting of the World Meteorological Congress in April 1971 gave consideration to the Organization's future role in hydrology and discussed to what extent it should cater for the needs of hydrological services in addition to those of the meteorological services. The question really is whether the WMO should become the World Hydrometeorological Organization at some future date. There are hydrometeorological services in a number of countries such as Sweden and the USSR, and these could be the basis of an obvious extension for WMO. Another possibility would be to found a new UN agency with interests spanning the entire field of hydrology and water resources. This would avoid the apparent "takeover" of hydrology by an allied discipline or the alternative—that it becomes an insignificant part of the programme of a large UN agency. On the other hand, there is the important question of finance and the fact that UNESCO, WMO and FAO and possibly WHO, the International Atomic Energy Agency (IAEA) and the UN Water Resources Development Centre would find it exceedingly difficult to divorce themselves from their present responsibilities in these fields.

Divided Responsibilities

It may seem rather odd that these international complexities have a parallel in Britain, but here too the responsibilities for water are divided. Depending on whether the fluid is considered a commodity, a danger, as a means of transport or as a subject demanding attention from the research worker, different bodies are involved. Water undertakings, sewage disposal authorities, government departments, the Central Electricity Generating Board and British Waterways have their own, sometimes competing, interests, to say nothing of industry, agriculture and those who think of water for sport, recreation and as an amenity. As a rule the unit of operation is small. In England and Wales there are more than 200 water supply undertakings, 1,300 sewage disposal authorities and 26 river authorities. As far as research is concerned there are 20 or more government bodies involved, as well as the universities. In fact the recent Natural Environment Research Council (NERC) Survey of Hydrological Research in the United Kingdom[2] revealed a tremendous wealth of research, much of it long term, being undertaken in a wide variety of establishments. One suggestion is that the most substantial parts of this research should, in future, come under the aegis of a new Central Water Authority[3]; but in view of NERC's existing statutory responsibilities and present commitments for research in hydrology, perhaps a strengthening of these responsibilities and better coordination centred on NERC would be a more appropriate solution.

Of course, it could be argued that the organization of hydrology, whether nationally or on a global basis, has little relevance to the drought stricken Indian village or the wide areas which the Danube has flooded. Yet the sharing of a river basin with other nations is in itself a compelling reason for international cooperation. Hence there are commissions or some form of control body for a number of the world's principal rivers, the countries concerned often being assisted by one or more UN agencies. Then there is the need to standardize instruments, methods of observation, methods of data processing and analysis so that hydrological information knows no frontiers. For example, there are considerable differences in the instruments used in various countries for the measurement of rainfall and evaporation—two basic elements in the water balance. To produce a rainfall map of Europe is an extremely difficult task, the most important reason being that each country uses its own type of gauge, which often differs radically in size, shape, material and method of installation from those used by its neighbours.

Evaporation is even more of a problem, for few countries have widespread networks of evaporation pans or tanks and some have none of these instruments at all. Not many have the detailed records of climate that are needed for determining evaporation indirectly, by the Penman method for example. There is a comparison of evaporation pans and tanks sponsored by WMO in progress at the moment. A number of meteorological stations around the world, for example Kew Observatory, have been equipped with US National Weather Service Class A pans, the GGI 3000 and 20 m^2 pans of the USSR Hydrometeorological Service and several other types of instruments; a number of years of data has now been amassed. A similar but unsatisfactory series of comparisons of rainfall gauges has been based on the Interim Reference Precipitation Gauge; unfortunately this type of instrument, a 1 m high gauge fitted with a wind shield, was as susceptible to aerodynamic effects as any other type of elevated gauge—these effects cause acceleration of air moving over the top of the gauge and consequently a diversion of drop trajectories away from the orifice. This problem can be almost completely overcome by installing a gauge in a shallow pit so that its rim is level with the ground surface and surrounding the gauge with an anti-splash grid. Proposals for a project based on this type of gauge were made at a WMO meeting at the Institute of Hydrology in July 1970 and the comparison of national rain gauges with these instruments commenced in June this year. Tests of these ground level gauges have been carried out in some countries already. In the USSR, comparisons of national standard gauges and ground level gauges made at a large number of sites have resulted in the re-drawing of precipitation maps and an upward scaling of values. Results from a number of sites in the United Kingdom indicate that the existing 1 foot high standard gauge network measures less rain than actually reaches the ground. Annual rainfall totals are between 3 and 7% greater than those recorded over most of south-east England, while the deficit can be up to 20% for unfavourable sites in the north and west.

Basin Programme

One of the most successful aspects of the IHD has been the representative and experimental basin programme which has aided the improvement of basic countrywide hydrological networks in less well developed countries and has been a stimulus to research in the more developed ones: these studies of complete catchments and their water budgets highlight the need for specialized instruments and improved methods of data processing and analysis. Several countries have planned their networks of representative and experimental basins; the one designed to sample the principal hydrological regions and the other to sample the chief changes in the use of land. In others, Britain for example, the growth of these catchment studies has been rather haphazard. A survey of basin studies undertaken by NERC in 1968 showed that there were almost fifty such projects in progress within the United Kingdom. Certain parts of the country and certain objectives were adequately covered, for example studies of the effects of afforestation in upland Britain. On the other hand, there were only two studies being undertaken in lowland England on the consequences of urbanization—one of the most radical of all land use changes. The hydrological significance of snow, particularly in the Scottish Highlands, was also not being investigated—quite a serious gap which still remains.

The situation in Australia is rather different. About eighty physical regions have been delimited using indices of geomorphology, geology and rainfall, and a representative basin is being established in each[4]. Various government organizations and universities are participating in these activities which are being promoted by the Department of National Development. Records from each basin are being assembled in computer compatible form and will be made available to the CSIRO Division of Land Research, Canberra, for analysis. For the analysis, a rainfall-runoff model has been devised[5] and tested with data from a basin near Alice Springs. Predictions of storm runoff are produced from observations of rainfall, potential evaporation and a number of other factors.

The New Zealand network of representative basins has also been planned rationally[6]. The two islands have been divided into about ninety hydrological regions, a representative basin being established in nearly all of them by the Water and Soil Division of the Ministry of Works. These catchments sample the principal precipitation zones, the chief soil types and the important geomorphological areas; they range in size from 1 to 250 km^2. There are also a number of experimental basins in operation such as those at Moutere near Nelson, where the significance of various changes in land use is being studied in twelve small catchments[7]. One experiment at this site is concerned with cultivating a gorse catchment and studying the resulting change in the water budget and the differences in the distribution of stream discharge. Peak flows were found to increase, annual runoff totals were nearly doubled, but there were fewer days when runoff occurred. This and similar studies are particularly important for New Zealand, where immense

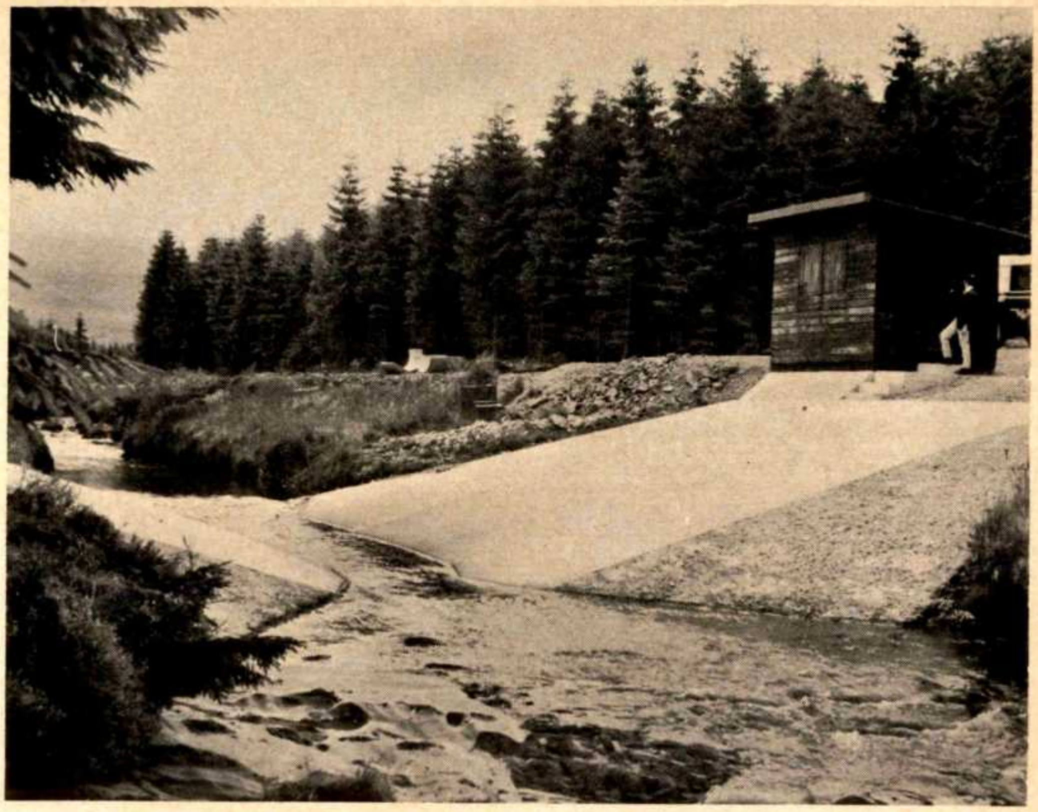


Fig. 1 Trapezoidal flume used by the Institute of Hydrology for gauging flows from the forested headwater catchment of the River Severn in the Plynlimon experimental catchment where the hydrology of forest and upland sheep pasture is being compared.

problems have resulted from imported land use practices and their effects on soils and water.

Measuring Advancement

The nature of these problems and the strenuous efforts being made to find solutions to them may have been one of the reasons for holding in December 1970 the symposium on the results of research on representative and experimental basins at Wellington, New Zealand. This symposium, organized by the Royal Society of New Zealand and arranged by the International Association of Scientific Hydrology with the support of UNESCO, was attended by over 150 hydrologists from more than thirty countries. Earlier in the year, the World Water Balance symposium had been held at Reading University and had attracted more than 250 participants from about fifty countries. After the Symposium about sixty of the participants went on a tour of Central Wales and the Midlands. One of the sites visited was the Institute of Hydrology's Plynlimon Catchments, where a long term study of the contrasting headwater basins of the Wye and Severn was commenced in 1967. The basin of the Wye is almost entirely sheep pasture, while the adjacent Severn is nearly all planted softwood forest—an ideal situation for investigating the hydrological consequences of these land use differences. For large areas of upland Britain are being afforested, but the effects of this change in land area river flows are not clear. Are floods reduced in severity? Do flows diminish more rapidly during dry periods? By measuring rainfall, evaporation, and changes in soil water storage, in addition to river flows, it is anticipated that the magnitude of any alterations to the hydrological regimes of these areas can be established, as well as the causes. Data are now being collected from specially constructed river gauging stations in the two basins (Fig. 1) and from fifty rain gauges and fifty soil moisture sites distributed over the catchments. Five automatic weather stations will be installed shortly for assessment of evaporation to supplement the two conventional stations that have been operating for some time. Initial results show that there are substantial differences between the two areas that must result from the contrasting land usage. Both the Reading and Wellington meetings took place within the framework of the IHD and they provided valuable forums for the exchange of ideas and the promotion of contacts between individuals. Indeed it has been said that the IHD has been better for hydrologists than for hydrology, but it is difficult to see how the benefits can be separated.

Measurement of the advancement of a science is not, of course, an easy task; progress is never continuous but rather a series of breakthroughs and periods of consolidation. Sherman's inceptive unit hydrograph paper[8] was the breakthrough in the 1930s, followed by Gumbel's work[9] on extreme value theory and Penman's studies of evaporation[10] a decade later. But similar progress after 1950 is difficult to pinpoint in the same way. Perhaps the computer revolution and particularly the spate of mathematical models of catchment behaviour that has resulted is one of the most noteworthy features. Advances in methods of analysis have certainly been rapid recently, so much so that faults in the basic measurements are becoming one of the chief hindrances to further progress. Britain seems to be well placed, for there are few countries with equal experience in instrumentation, particularly in rainfall measurement, stream gauging and soil moisture assessment. It could even be argued that the problems resulting from Britain's relatively recent start in scientific hydrology are rapidly being overcome. Hydrology is now an established science, a science that is extremely valuable to the community at large, though not one that captures the interest it deserves.

[1] *The Future Management of Water in England and Wales* (Central Advisory Water Committee, HMSO, 1971).

[2] *Hydrological Research in the United Kingdom (1965–1970)* (Natural Environment Research Council, 1970).

[3] *Seventh Annual Report* (Water Resources Board, HMSO, 1971).

[4] *The Representative Basin Concept in Australia*, Hydrological Series No. 2 (Department of National Development, Canberra, 1969).

[5] Chapman, T. G., *Proc. IASH Symposium on the Results of Research on Representative and Experimental Basins, Wellington*, 126 (1970).

[6] Toebes, C., *Proc. IASH Symp. Budapest*, 147 (1965).

[7] Scarf, F., *J. Hydrol. (New Zealand), Proc. IASH Symp. of Wellington*, **9**, No. 2, 142 (1970).

[8] Sherman, L. K., *Engineering-News-Record*, **108**, 501 (1932).

[9] Gumbel, E. J., *Amer. Geophys. Union Trans.*, Part III, 836 (1941).

[10] Penman, H. L., *Proc. Roy Soc.*, A, **193**, 120 (1948).

Prospects for Carbon Fibres

R. JEFFRIES

The Cotton Silk and Man-made Fibres Research Association, Shirley Institute, Manchester

The relatively high price of composites reinforced with carbon fibres still means that they will only be used when their strength, stiffness and lightness are absolutely essential. But in the future, industries as diverse as the chemical industry and shipbuilding could reap the benefits of a cheaper product.

CARBON fibres made from cellulose fibres have been known for a considerable time. They were not sufficiently strong or stiff to be of any real value for the reinforcement of composite materials and were manufactured for their thermal insulation and ablative properties, and for use in filter beds and so on. A team at the Royal Aircraft Establishment (RAE) first made carbon fibres with tensile properties good enough to warrant the term "high performance" in relation to reinforcement[1,2] and at about the same time, at the Aero Engine Division of the former Rolls-Royce Ltd, similar techniques were also being developed for producing these high performance carbon fibres; rapid progress was made in translating this laboratory work into engineering practice. The RAE and Rolls-Royce carbon fibres are produced by the controlled thermal degradation of a special grade of acrylic fibre made by Courtaulds Ltd (for example refs. 2–5). In the three-stage heating process (for example refs. 2–7) the fibre is first oxidized by heating in air at 200° to 300° C; it is then carbonized by heating in an inert atmosphere to about 1,000° C and finally the carbonized fibre is graphitized to a greater or lesser extent by heating (also in an inert atmosphere) to a temperature in the region 1,500° to 3,000° C. Carbonization removes almost all nitrogen and hydrogen from the acrylic fibre and a proportion of the carbon, leaving a fibre made substantially from carbon—hydrogen cyanide and ammonia are the chief gases evolved[8–13]. Graphitization "crystallizes" the carbon fibre to some extent, that is it causes the carbon structure to organize itself into a more regular arrangement similar to (though not the same as) natural graphite. The mechanical properties of the final fibre depend on the temperature of the graphitization stage of the preparation process and it is therefore possible by this means to produce different types of carbon fibre.

An important feature of the process is the maintenance of the high orientation in the acrylic precursor throughout the carbonization processes[2,3,5]; to this end, tension is applied to the fibre during the early stages of the process to prevent any shrinkage and structural disorientation. It is partly the use of tension which distinguishes the process of Watt and his co-workers from that of Shindo[8] who also studied the preparation of carbon fibre from an acrylic precursor.

Each stage of the heating programme has presented considerable development problems, particularly in turning the process into a large scale operation; for example, the oxidation stage is exothermic in character and liable to get out of control[6].

These problems were solved, however, and production of the fibre in commercial quantities has been under way for some time. Two forms of process have been developed: in the first the material is prepared by a batch process, in which carbon fibres are produced with lengths of about a metre. A continuous process has more recently been developed which yields material with lengths up to about 1,500 m.

Properties and Performance

High performance carbon fibres are black, lustrous, rather slippery to handle and are at present produced with diameters of 8 to 9 μm (ref. 3). Their mechanical properties are outstandingly good (Table 1, from refs. 1, 14–17) and values quoted by other manufacturers of carbon fibre (Rolls-Royce, Courtaulds, and Morganite) are all similar in magnitude. Each value of strength and modulus in Table 1 is divided by specific gravity to give the specific strength and modulus; these specific values should always be considered when comparing materials on a weight for weight basis. The carbon fibres (both the high strength and high modulus types) are far superior to the steel. The glass fibres (particularly the S type) are similar in strength to the carbon fibres but have a much lower modulus. It is this very large stiffness that makes carbon fibre such an important reinforcing material for engineering applications; high strength needs to be associated with high modulus (that is, high stresses have to be borne at low extensions) in most engineering materials. The carbon fibres show completely elastic behaviour up to the breaking point and so there is no irreversible extension or creep. The fibres are comparatively weak and of low modulus in the direction perpendicular to the fibre axis; for instance, the Young's modulus of graphite at right angles to the graphite planes is only about 5×10^6 pound inch^{-2} (ref. 18).

As the temperature of the third stage of the heat treatment[16] is increased in the range of 1,000 to 2,500° C the modulus steadily increases and the tensile strength at first increases, reaches a maximum at about 1,500° C, and then decreases. It is thus possible to prepare a high modulus fibre, at the expense of some strength, or a high strength fibre at the expense of some modulus. The difference between the "high modulus" and "high strength" fibres in Table 1 arises from the differences in the temperature of the final heat treatment. Courtaulds and Morganite manufacture both high modulus and high strength fibres; Courtaulds type HT and Morganite type II are high strength, Courtaulds type HM and Morganite type I are high modulus. Courtaulds also make a Type A fibre which is of somewhat lower strength and modulus than the type HT, but has certain other advantages that will become clear later[3].

The standard deviation of the strength values of carbon fibres is about 25% (refs. 1 and 14); this is because of structural flaws which cause premature breakage. By comparison, the Young's modulus of the fibre, being an intrinsic property of the fibre and therefore not influenced by flaws, is much less variable and the standard deviation is only about 5% of the mean. Flaws of a discrete, "macroscopic" nature may, however, be the strength-controlling factor only for carbon fibres prepared at temperatures up to 1,300° C (refs. 19 and 20); at higher treatment temperatures some other strength-controlling mechanism seems to take over. It has been suggested[21] that the strength of the fibres may be governed in some circumstances by microcracks formed during cooling from the processing temperature.

Carbon fibres are very resistant to heat in inert conditions and the volatilization temperature is more than 3,000° C; they begin to oxidize in air at 410 to 450° C, however. The thermal and electrical conductivities along the length of the fibre are lower than the values for a heat treated pyrolytic graphite in the direction parallel to the planes in the graphite structure[22], showing the effect of the small crystallite size in the fibres.

Fibre Structure

The structure of carbon fibres is similar to that of graphite—the atoms are in layers or planes and the planes are arranged almost parallel to each other. In true graphite, however, the atoms in each plane are arranged in a regular manner with respect to those in the adjacent planes, but in carbon fibres there is no such regular arrangement and the structure is described as turbostratic[22].

The planes of atoms are well oriented in the direction of the fibre axis[1]—in the case of a high modulus fibre to within 10° of the axis. This high degree of orientation is the reason for the high modulus of the fibres[1], and any treatment that increases the orientation invariably increases the modulus, regardless of whether the increased orientation is imparted by stretching the precursor acrylic fibre[23,24], by the application of tension during the oxidation stage[25,26], or by stretching the final carbon fibre during the graphitization process[27-29].

Table 1 Comparison of Properties of High Performance Carbon Fibres with Other Materials

Property	RAE carbon fibre High modulus	RAE carbon fibre High strength	Steel wire	S-glass fibre	E-glass fibre	Bulk steel	Bulk aluminium	Bulk titanium	Cotton	Textile viscose	Polyester fibre
Tensile strength (10^3 pound inch^{-2})	300	430	400–600	650	500	145	67	135	70	40	90
Extension at break (%)	0.5	1.3	~2	~5	~5				9	20	22
Young's modulus (10^6 pound inch^{-2})	60	33	30	12.5	9	30	10.5	16	0.6	1–2	1.8
Specific gravity	2.0	1.74	7.8	2.5	2.5	7.8	2.8	4.5	1.55	1.51	1.38
Specific tensile strength	150	250	51–77	255	200	18.5	24	30	45	26	65
Specific Young's modulus	30	19	3.8	4.9	3.5	3.8	3.75	3.5	0.4	0.65–1.3	1.3

The carbon fibres are composed of aggregates of crystalline regions separated by grain boundaries and pores. These crystallites in graphitized fibre are thought to be about 40 to 100 Å wide[21,22,30,31] and about 60 to 120 Å long in the fibre direction[21,22,30]; the dimensions are somewhat less in ungraphitized carbon fibre[22,31,32]. This polycrystalline nature has already been mentioned in connexion with the electrical and thermal conductivities of the fibres.

The specific gravity of RAE carbon fibres (Table 1) is much lower than that of true graphite, which is about 2.26. This may be partly the result of imperfect graphitization but is more likely to reflect the presence of a pore structure in the fibre. Johnson and Tyson[30] consider that the voids separate the short crystals in the graphitized material, and have a mean width less than 10 Å.

There is evidence[21,22,33] that RAE carbon fibres have some fibrillar character and the suggestion has been made that the strength of the fibre is a function of the interfibrillar bonding. Evidence has recently been adduced that sheet-like structures are also present in carbon fibres[33].

Composites

If the exceptional properties of carbon fibres are to be utilized for engineering purposes they must be fabricated as a "reinforced plastic"; the fibres are aligned in the required direction or directions and embedded in a suitable resin. There are three types of technique for preparing "directional" composites[34-36]. In the "leaking mould" technique, the fibre is combined in the mould with a solventless liquid polyester or epoxy resin and then pressed; the excess resin is forced out of the leaky mould. In the "prepreg" method, layers of fibre are impregnated with heat-setting resin in dilute solution and dried. These preimpregnated layers are then moulded together in the normal way under heat and pressure, and cured. Filament winding techniques involve the building up of layers of fibre (intermixed with resin) by winding the fibre onto a rotating former; more or less continuous lengths of fibrous material are obviously needed for this technique. Random composites are made by mixing chopped carbon fibre with resin and subjecting the mixture to the usual moulding techniques.

The mechanical properties of the carbon fibre-resin composite depend on the mechanical properties of the fibre, the volume fraction of the fibre in the composite, the orientation of the fibre in the composite, and the strength of the fibre-resin bond. Phillips[35] has described the various properties of unidirectional composites (those containing fibres oriented in only one direction) made with RAE high modulus fibre and a polyester resin at a volume fraction of fibre of about 40% (50% by weight). The properties in the direction of fibre orientation, as listed by Phillips[35], are shown in Table 2. In spite of the diluting effect of the resin, the specific strength and modulus values of the composite in the direction of fibre orientation are still much superior to that of any of the three bulk metals in Table 1. The modulus and flexural strength of the composite are almost unaffected by immersion in cold or boiling water[37]—the carbon fibre-resin interface is weakened by less than 5%, whereas the interface between glass fibre and resin is weakened by as much as 15%. The fatigue life of the composite is very good, and the damping properties are also good. The properties of carbon fibre reinforced plastics, made with Courtaulds and Morganite carbon fibres, and also with carbon fibres produced in the United States have been summarized by Dauksys and Ray[38].

The properties of carbon fibre reinforced plastics depend to some extent on the strength of the bond between the fibre and the resin. Too weak a bond leads to a low interlaminar shear strength (ILSS) whereas too strong a bond causes the

Table 2 Properties of Carbon Fibre–Resin Composites

Property	Unidirectional carbon fibre–resin composite[35]	Bulk steel	Bulk aluminium	Bulk titanium
Tensile strength (10^3 pound inch^{-2})	105	145	67	135
Young's modulus (10^6 pound inch^{-2})	22	30	10.5	16
Specific gravity	1.54	7.8	2.8	4.5
Specific tensile strength	68	18.5	24	30
Specific modulus	14	3.8	3.75	3.5
Fatigue resistance in fluctuating tension (50,000 ± 20,000 pound inch^{-2} stress level)	$> 20 \times 10^6$ cycles to failure			

material to be brittle; a compromise has therefore to be reached[38,39]. The ILSS of carbon fibre reinforced plastic made with untreated carbon fibre (particularly high modulus fibre) is lower than is desirable for many purposes but both Courtaulds and Morganite now market both high modulus and high strength fibres that have been specially treated to improve the fibre-resin bond and thus increase the ILSS. Courtaulds also market a carbon fibre which, although somewhat less strong and stiff than the other two types, produces composites of satisfactory ILSS even without treatment; it is cheaper than the other types, and has a breaking strain of more than 1% which is advantageous for some purposes. An alternative means of increasing the ILSS is to whisker the carbon fibres, that is, to cover the fibre with large numbers of randomly oriented, short whiskers[40,41]. Giltrow and Lancaster[42-44] have shown that the presence of carbon fibre in a resin reduces the coefficient of friction and rate of wear.

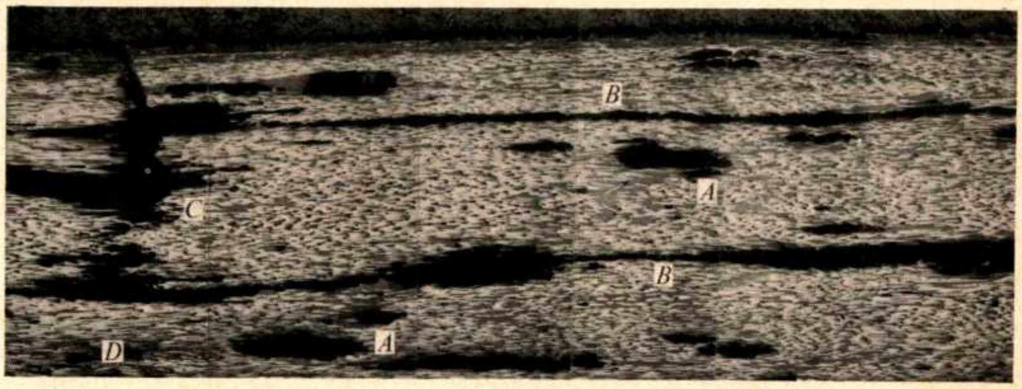


Fig. 1 Photomicrograph of a longitudinal section of a carbon fibre resin unidirectional composite tested to failure.

Fig. 1 shows a photomicrograph (at a magnification of about 40) of a longitudinal section of a carbon fibre resin unidirectional composite tested to failure in a three-point short-beam bending test; the composite contains 60% of fibre in an epoxy resin. The photomicrograph illustrates the various faults that can be present in this type of composite as manufactured (this is, in fact, a poor specimen of composite chosen to reveal these various faults), and also the additional features which appear as a result of stressing the composite to failure in this type of test. In the photograph, the fibres show up white, the resin grey, and voids and cracks appear black. The three types of fault present in untested specimens are voids (marked A in the photograph), places where the concentration of fibre is very low, and misaligned fibres. Each of these faults are potential weak points in the composite, particularly the voids; it is likely that, in the case of this specimen, the failure mechanism originated at voids. When a composite is tested in the three-point test, the specimen first fails in shear, and the long cracks (B) are the result of this type of failure. Further stressing of the specimen leads to compressive failure, characterized by the crack (C) perpendicular to the fibre direction. In some instances of tests of this type, tensile failure near point D takes place.

The incorporation of carbon fibres into magnesia, glasses and aluminium is being investigated[45] and there is thus a possibility in the future of toughened ceramics and metals. One difficulty in the reinforcement of metals with carbon fibre is the tendency for a chemical reaction to take place. This difficulty can be overcome by coating the carbon fibres with a barrier layer of an unreactive third material (for example, tungsten[46]).

Development Potential

Whiskers of graphite, prepared for example by a high pressure carbon arc technique[47], have extremely high tensile properties; tensile strengths of 3×10^6 pound inch^{-2} and a Young's modulus of 100 to 130×10^6 pound inch^{-2} have been achieved[47], but it has been estimated that the theoretical strength and modulus of the graphite crystal are 14.5 to 20×10^6 and 145×10^6 pound inch^{-2}, respectively[48]. There is therefore ample room for development in the tensile properties of the acrylic based carbon fibre, particularly in the strength. Whiskers are, however, almost continuous crystals of graphite, whereas carbon fibres are polycrystalline and will therefore suffer from inherent limitations in strength and modulus as a result of the structural imperfections associated with the crystallite boundaries.

Carbon fibres made from acrylic precursor fibre are by no means the only form of carbon fibre available. Carbon fibres made from cellulose have been available for many years and, though of relatively modest tensile properties, they have attributes that make them suitable for a range of applications (for example, thermal insulation). Union Carbide have improved the orientation, and thus the mechanical properties, of cellulose carbon fibres by stretching them during graphitization at more than 2,000° C; these stress-graphitized fibres (the Thornel fibres) have mechanical properties not too far below those of acrylic based carbon fibres.

Why have only acrylic and cellulose fibres been used on any large scale as precursors for carbon fibres? Although a complete answer to this question is not possible, the basis of the answer is probably reasonably well understood. The organic fibres that can be carbonized fit into two groups: those that give a carbon fibre that is graphitizable and those that give a carbon fibre that is not in reality these are probably two extremes, with all carbon fibres lying somewhere in between. The graphitizable carbon fibres probably have superior tensile properties (after heating to graphitizing temperatures) than the equivalently heated non-graphitizing carbon fibres[49]. Carbon fibres from acrylic fibres are graphitizable and this is probably one reason for their superior tensile properties. But why are they the best of the currently available graphitizable carbon fibres? The answer probably lies, partly at least, in the high orientation of the acrylic based carbon fibres, which in turn originates in the particular nature of the chemical reactions taking place in the acrylic degradation process (principally in the oxidation stage under tension). These reactions stabilize and preserve the orientation during the later stages of the heating process.

Carbon fibres are being produced from precursors other than organic fibres, however. The Kureha Company in Japan, for example, makes carbon fibre from pitch. The Kureha fibres available are, however, of low orientation and have similar mechanical properties to glass fibres (that is, they are of comparatively low modulus). A research group at the University of British Columbia are also producing carbon fibres from low cost pitch materials: excellent mechanical properties (similar to those of acrylic based carbon fibres) have been reported for pitch based fibres that have been stretched during graphitization at 2,000° C to 2,800° C (ref. 50). The British Coal Utilization Research Association's Industrial Laboratories are making strong carbon fibres from coal products and fibres stronger than E-type glass fibre have successfully been prepared[51].

Composite Applications

The most likely immediate applications for composites reinforced with carbon fibres are those in which their combination of strength, stiffness, and lightness is at a premium and in which the cost of the material is less important[34–36]. The aircraft and space industries meet both these conditions and will, no doubt, provide numerous applications in the future for carbon fibre reinforced plastic, despite the much discussed difficulties with the fan blades of the Rolls-Royce RB211 engine. It seems likely that several other aircraft components (helicopter blades, aircraft structure parts, aircraft containers and so on) will also eventually be made from carbon fibre reinforced plastic. The advantages of carbon fibre reinforced plastics over metals in space vehicles (for example, rocket motors and satellites) are particularly obvious because of the large amount of fuel needed to put a small weight into space.

The cost of carbon fibres, and difficulties in the use of composite materials for some purposes, have restricted the number of "earthbound" uses of high performance carbon fibre. At the Atomic Energy Research Establishment, Hanwell, carbon fibre composites have been used in chopper disks in neutron experiments[3] and Dowty Rotol Ltd make fan blades from the material[52]; Bonas Brothers use carbon fibre reinforced plastic to make a special component for a high speed loom[53]. The high strength and stiffness of RAE-type carbon fibre has also been utilized with benefit in the panels of the Ford GT40 sports car.

This present rather limited use of carbon fibre reinforced material is, however, a phase that will soon pass, and there is no doubt that in the not too distant future the use of the material will expand rapidly. Some possible applications include the manufacture of boats and submarines for which the fact that carbon fibre composites are little affected by water is a particular advantage and pressure vessels of all types. The relative chemical inertness of carbon makes the use of carbon fibre composites of considerable potential value in chemical plant environments that are severe, both chemically and physically[54]. Any engineering device involving rotating members will obviously be improved if manufactured from carbon fibre reinforced plastic, because the centrifugal forces in the rotating part depend on the weight of the part. There are also improvements to be gained in the manufacture of wires and cables for high stress-bearing situations and engine components for high performance internal combustion engines. Composite material containing chopped fibre has also been shown to have excellent friction and wear characteristics[42–44]. The high strength and modulus of carbon fibre reinforced plastic allows a good balance to be achieved on sports equipment—adequate strength can be allied to a proper distribution of weight.

Research at Shirley Institute

Certain aspects of the development work on carbon fibres and composites have clearly required knowledge and expertise of a textile, rather than engineering, character. For this reason both the former Ministry of Technology and the former Rolls-Royce Ltd sponsored research work on carbon fibres at the Shirley Institute. This many sided research is confidential in nature and it is only possible to mention briefly two pieces of work. The first, performed under contract to the Ministry of Technology, is the development of a novel method for spinning yarns from the comparatively short lengths of carbon fibre produced by the batch process. Ordinary textile machinery and processing could not be used because the carbon fibres are too smooth and slippery. Work was also carried out under contract to the former Rolls-Royce Ltd on the development of a new weaving method for assembling filaments into a wide, flat, continuous sheet.

The Institute now possesses widely based expertise and facilities for research on carbon fibres and composite materials, and is continuing a high level of effort in this field, both in its general research programme and in the rapidly expanding sponsored sector of its activities.

I am grateful to the Composite Section of Shirley Institute for the photomicrograph of the tested specimen of composite.

[1] Watt, W., Phillips, L. N., and Johnson, W., *The Engineer* (May 27, 1966).
[2] Johnson, W., Phillips, L. N., and Watt, W., *BP*, **1**, 110, 791 (1968).
[3] *Carbon Fibres* (edit. by Smith, E. A.) (Morgan-Grampian, London, 1970).
[4] *Carbon Fibres* (Report from the House of Commons Select Committee on Science and Technology, HMSO, 1969).
[5] Gunston, W. T., *Sci. J.*, 39 (February 1969).
[6] *New Sci.*, 564 (June 13, 1968).
[7] *Chemical and Engineering News*, 35 (August 26, 1968).
[8] Shindo, A., *Rep. Govt. Ind. Res. Inst. Osaka*, No. 317 (1961).
[9] Burlant, W. J., and Parsons, J., *J. Polymer Sci.*, **22**, 249 (1956).
[10] Miyamichi, K., *et al.*, *J. Soc. Fibre Sci. Tech., Japan*, **22**, 538 (1966).
[11] Turner, W. N., and Johnson, F. C., *J. Appl. Polymer Sci.*, **13**, 2073 (1969).
[12] Watt, W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[13] Watt, W., *Nature*, **222**, 265 (1969).
[14] Standage, A. E., and Prescott, R., *Nature*, **211**, 169 (1966).
[15] *Carbon Fibres for Reinforced Plastics* (Royal Aircraft Establishment, 1967).
[16] Moreton, R., Watt, W., and Johnson, W., *Nature*, **213**, 690 (1967).
[17] *Fibre Data Summaries* (edit. by Ford, J. E.) (Shirley Institute Pamphlet No. 91, 1966).
[18] Watt, W., *Interlab. Symp. Carbon Fibres*, 2 (1969).
[19] Johnson, J. W., *Amer. Chem. Soc. Polymer Prep.*, **9**, 1316 (1968).
[20] Thorne, D. J., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[21] Brydges, W. T., Badami, D. V., Joiner, J. C., and Jones, G. A., *Amer. Chem. Soc. Polymer Prep.*, **9**, 1310 (1968).
[22] Johnson, W., and Watt, W., *Nature*, **215**, 384 (1967).
[23] Moreton, R., *Royal Aircraft Establishment Tech. Rep. 69114* (1969).
[24] Moreton, R., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[25] Watt, W., and Johnson, W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[26] Watt, W., and Johnson, W., *Amer. Chem. Soc. Polymer Prep.*, **9**, 1245 (1968).
[27] Johnson, J. W., Marjoram, J. R., and Rose, P. G., *Nature*, **221**, 357 (1969).
[28] Logsdail, D. H., *Amer. Chem. Soc. Polymer Prep.*, **9**, 1324 (1968).
[29] Johnson, W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[30] Johnson, D. J., and Tyson, C. N., *Brit. J. Appl. Phys. Phys. Ser. 2*, **2**, 787 (1969).
[31] Saunderson, D. H., and Windsor, C. G., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[32] Spencer, D. H. T., Hooker, M. A., Thomas, A. C., and Napier, B. A., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[33] Johnson, D. J., and Tyson, C. N., *Proc. Roy. Microsc. Soc.*, **4**, 19 (1969).
[34] Phillips, L. N., *Royal Aircraft Establishment Tech. Rep. 67088* (1967).
[35] Phillips, L. N., *Fibre Sci. Tech.*, **1**, 3 (1968).
[36] Phillips, L. N., *Composites*, **1**, 101 (1969).
[37] Pearce, D., *Interlab. Symp. Carbon Fibres*, 8 (1969).
[38] Dauksys, R. J., and Ray, J. D., *J. Comp. Materials*, **3**, 684 (1969).
[39] Morley, J. G., *Phys. Bull.*, **20**, 479 (1969).
[40] Milewski, J. V., Shaver, R. G., and Withers, J. C., *Materials Engineering* (May 1968).
[41] Prosen, S. P., and Simon, R., *Reinforced Plastics and Composites World* (September/October 1967).
[42] Lancaster, J. K., *Proc. Inst. Mech. Eng.*, **182**, Pt. 1, 33 (1967/68).
[43] Giltrow, J. P., and Lancaster, J. K., *Nature*, **214**, 1106 (1967).
[44] Giltrow, J. P., and Lancaster, J. K., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
[45] *The Engineer*, 499 (March 29, 1968).
[46] *Fulmer Research Institute Newsletter* (June 1969).
[47] Bacon, R., *J. Appl. Phys.*, **31**, 283 (1960).
[48] McCreight, L. R., Rauch, sen., H. W., and Sutton, W. H., *Ceramic and Graphite Fibres and Whiskers*, 56 (Academic Press, 1965).
[49] Badami, D. V., *New Sci.*, **45**, 251 (1970).
[50] Hawthorne, H. M., Baker, C., Bentall, R. H., and Linger, K. R., *Nature*, **227**, 946 (1970).
[51] *The Engineer*, 30 (July 2, 1970).
[52] Peters, D. M., *Design Engineering*, 104 (February 1969).
[53] *The Engineer*, 38 (November 13, 1969).
[54] Judd, N. C. W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).

New Hominid Skull from Bed I, Olduvai Gorge, Tanzania

M. D. LEAKEY, R. J. CLARKE & L. S. B. LEAKEY

Centre for Prehistory and Palaeontology, PO Box 30239, Nairobi

Restoration of a hominid skull recovered from Bed I at Olduvai Gorge has revealed it to be a member of the genus *Homo*. Although the new cranium resembles *H. habilis* from Bed II, it differs in some respects from the type of *H. habilis* from Bed I. This may be a case of sexual dimorphism.

THE cranium described by L. S. B. L. later in this article (Olduvai Hominid 24) was found by P. Nzube in October 1968 (ref. 1) at a site in Olduvai Gorge that lies in the eastern part of the gullies known as DK, approximately 300 m east of the living site in lower Bed I that was excavated during 1962 and 1963.

The Site

The cranium was embedded in a mass of calcareous matrix measuring approximately 20 × 12 × 8 cm. All the bones were covered by matrix with the exception of a small portion of the right supra-orbital region and the posterior part of the palate. Indeed, so few identifiable parts were visible that it is remarkable it was recognized as hominid. The deposit on which it was found consists of a tuffaceous clay that can be traced over a wide area in Bed I, extending from the eastern part of the Gorge as far west as the FLK sites. This horizon overlies the basalt and is in turn overlain by the marker tuff IB for which an apparently reliable average date of 1.8 m.y. has been obtained by K/Ar dating[2]. At the hominid site (DK East), the tuffaceous clay is 3 m thick but the thickness varies considerably at different localities, depending on the surface configuration of the underlying lava. The clay at several other sites has yielded artefacts and fossil bones, including the molar tooth of Olduvai H. 4, found at MK during 1959, that has been attributed to *Homo habilis*[3] as well as the only remains of a chalicothere known from Olduvai.

After the discovery of the cranium the surface deposit was sieved extensively and an area of approximately 400 m^2 was eventually worked over. Three complete and some broken teeth were recovered as well as substantial portions of the maxillae, part of the occipital, several fragments of the parietals and the left supra-orbital region of the frontal bone. Some fragments were found low down the erosion slope and at a depth of just under 1 m from the present ground level: others lay on the surface, close to the block of matrix containing the cranium. It is evident that the specimen had been exposed for many years and that, as fragments broke off, some rolled down the slope and were buried under hill wash, while others became detached more recently and remained on the surface near the parent block. No trace of the canine and incisor teeth was found and it is likely that they broke off first and were transported downhill by erosion, possibly as far as the present river course.

The matrix in which the cranium was embedded is indistinguishable from the limestone concretions that frequently occur within the tuffaceous clay that overlies the basalt at DK and elsewhere (the mandibular fragment containing the molar of Olduvai H. 4 was similarly embedded). These concretions often form round fossil bones that can be seen as central cores when the blocks are broken open.

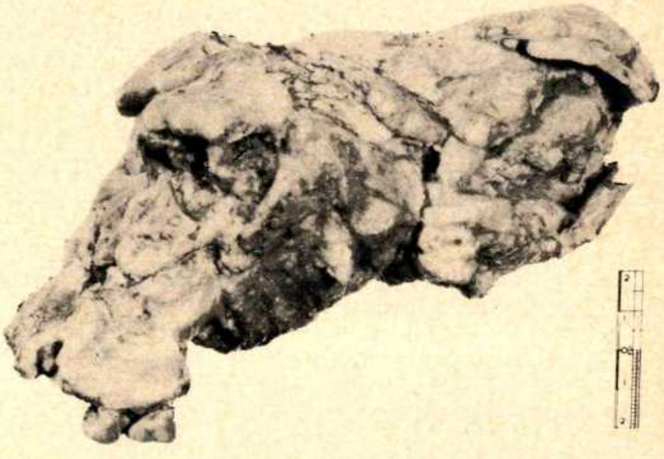


Fig. 1 Cast of Olduvai H. 24 after partial cleaning and before reconstruction.

Crocodile remains predominate among the faunal material from this site and more than 2,000 teeth were found. Tortoise plates, shells of Urocyclid slugs, fish vertebrae and scales, bird bones and pieces of ostrich eggshell were also relatively common. Mammalian bones and teeth included those of primates, rodents, carnivores, equids, suids, giraffids and bovids. There were also more than 37,000 unidentifiable bone fragments. The state of preservation of these remains is very similar to that of the cranium and many were also found embedded in limestone concretions.

Associated Stone Industry

Apart from a few quartzite flakes and chips, no artefacts were found, but the living floor previously excavated at DK—which included the circle of lava blocks considered to represent an artificial structure—has yielded a large series of artefacts that represent the contemporary stone industry[4]. This site consists of a living floor within the same tuffaceous clay that occurs at DK East and is similarly overlain by Tuff IB. In some places the accumulation of occupation debris rests on the surface of the lava, so that the living floor is at a slightly lower level than the layer of limestone concretions from which the cranium is believed to have eroded. Artefacts recovered from the living floor and from the clay above amount to 1,198 specimens, consisting of 154 tools, 187 utilized pieces and 857 debitage. The industry is typical of the Oldowan as it occurs throughout Bed I. It includes choppers of various forms, generally made on water-worn lava cobbles, polyhedrons, discoids, sub-spheroids, heavy and light duty scrapers and burins. Although the tools are of the usual Oldowan types, a proportion of the choppers, polyhedrons and discoids are smaller than those from higher levels of Bed I.

The associated faunal material includes all the species noted at DK East with the addition of *Elephas* and *Deinotherium*. Crocodile remains are similarly the most numerous.

Restoration of Olduvai H. 24

The tools used by R. J. C. in the removal of matrix from Olduvai H. 24 were a chisel-ended dental pick and a small hammer, a diamond drill and an S. S. White Industrial Air–Abrasive machine. Acetic acid could not be used because although the exposed pieces of bone had become hardened by weathering, those not exposed were softer than the matrix. Parts of the matrix contained impressions of bone fragments that had fallen out during weathering. Some of these were found during excavation and it was possible to fit them back into position and thus build up the cranium into the form it had been before they became detached. It was apparent that the specimen was reasonably complete but had been badly crushed and distorted, the chief pressure having been downward and backward from the brow region, with the result that the nasal bones, although complete and perfectly preserved, were lodged down behind the infra-orbital region. The glabella, still attached to the nasals, was crushed, and the brow ridges were squeezed close together on top of the glabella.

The vault fragments were first separated and then completely cleaned of matrix under a binocular microscope. The facial fragments were more difficult to separate because they were interlocked and rammed against each other, but once they were free of matrix there were perfect joins between the nasals and the rest of the face and between the right brow ridge and the right orbital margin, just above the frontozygomatic suture.

The base of the cranium had been depressed into the brain cavity and was rammed against the right petrous, and the basioccipital had been pushed under the vomer.

After thorough cleaning, consideration of sutures, bone thickness and curvature it was found that all the vault fragments but one joined together and that there was contact from the foramen magnum almost to the glabella. The sides of the vault, however, were not present. The posterior part was

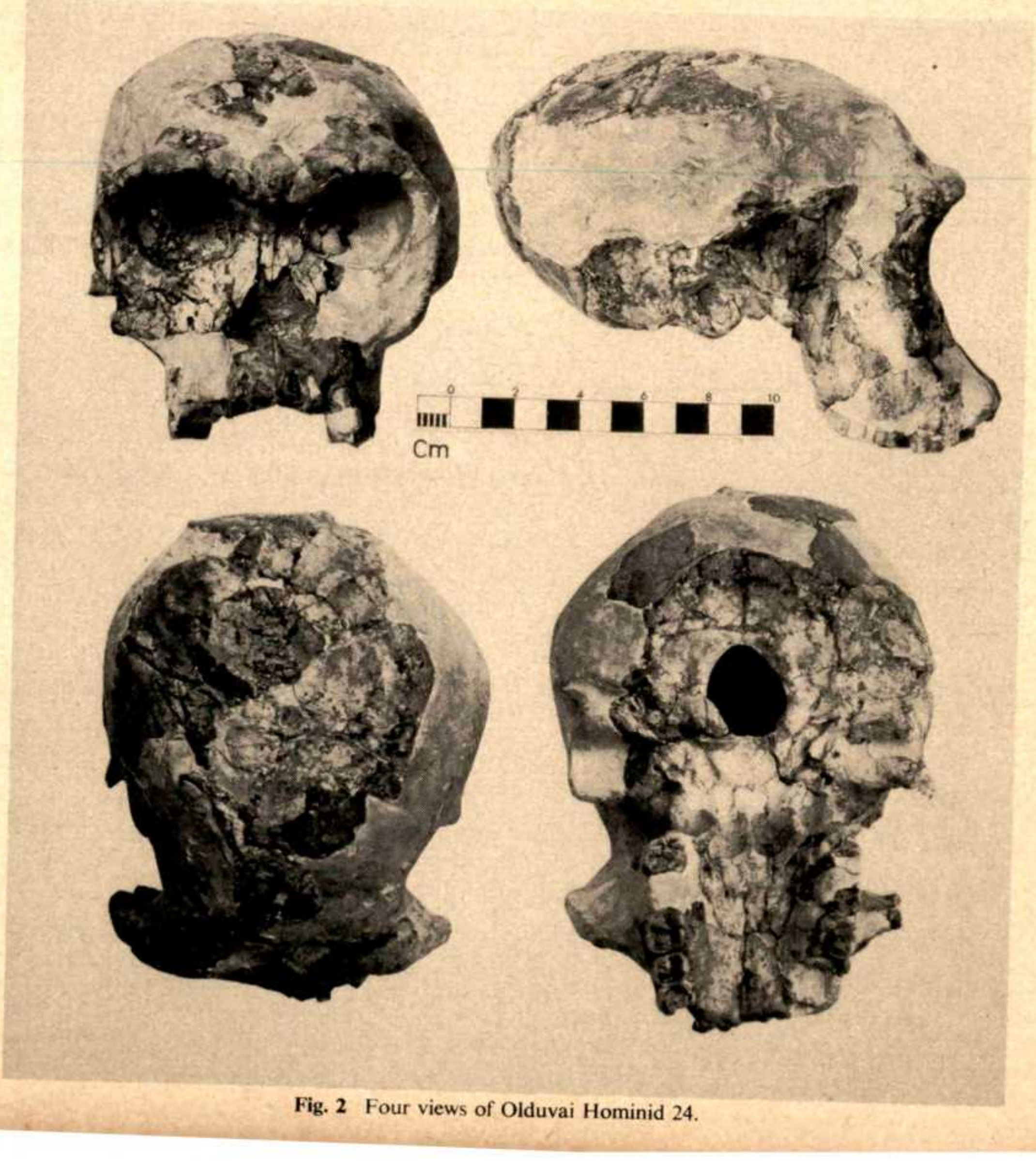


Fig. 2 Four views of Olduvai Hominid 24.

considerably crushed and in order for the joins in this region to lock properly it was necessary to straighten out the buckled lambdoid region by breaking through the bone along one crushed bend and by partially cutting and breaking the bone just above the suture.

The crowns of the left P^4, M^1 and right M^1 that were found during sieving fitted back directly on to the palate, but the left P^3 and the fragment of right M^2 had to be placed in position with plaster supports. A small but perfect contact between the mesiolingual fragment of the right M^3 and the major portion of this tooth could be seen under the microscope. The two pieces were fitted together and were placed in position on the palate by means of an impression that had been left in the matrix.

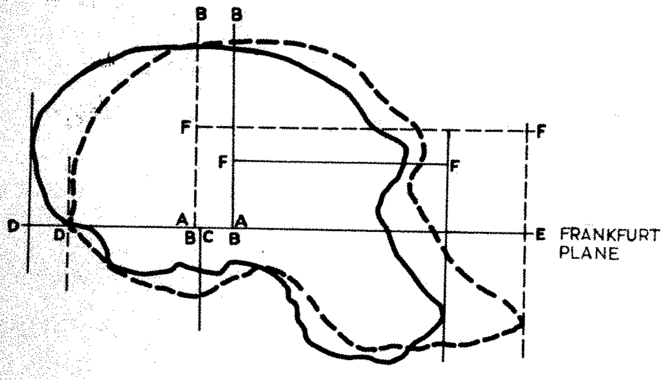


Fig. 3 Profile of Olduvai Hominid 24 (solid line) superimposed on that of *Australopithecus africanus* ST. 5 (broken line).

The cranium is still warped slightly and it is doubtful whether it can be completely straightened out, particularly as the sides of the vault are missing. The right petrous and tympanic plate are still in their crushed position and it would be a major operation to attempt to restore these bones to their proper relationship. The base of the cranium is also still depressed into the brain cavity and its thinness in places and the fact that it is covered with a network of fine cracks inhibit the possibility of restoration. The crushing of the whole cranium has also to be taken into account when considering the cranial capacity, which must inevitably have been greater than the absolute capacity as measured now.

Description of Olduvai H. 24

This specimen now consists of the following parts: almost the entire supra-orbital region of the frontal, the greater part of the vault, a considerable part of the occipital bone, nearly all the sphenoid, nearly all the right temporal bone and a considerable part of the face and palate, including the nasal bones. (Measurements are given in Tables 1 and 2.)

(1) The frontal bone. The supra-orbital region on the right side is almost intact as well as most of the glabella region. A large part of the left supra-orbital region is also preserved but it does not extend quite as far as the external orbital angle. There is also a small piece missing between the left supra-orbital fragment and the glabella region. On the right side the external orbital angle is intact and in normal articulation with the orbital process of the zygomatic. The supra-orbital region as a whole, while forward projecting, is not very massive. The glabella contact with the nasal bones at nasion is preserved and nasion lies well behind the overhanging glabella. Behind the supra-orbital region there is a depressed valley that is more pronounced above the glabella than on either side. In such parts as are preserved the frontal bone rises from this valley in a low curve towards bregma. There is sufficient of the frontal bone to be reasonably certain of the glabella–bregma length, which is approximately 90 mm.

On the right side of the cranium the region immediately behind the external orbital angle and the supra-orbital torus is well preserved. Part of this area is also present on the left side so that the minimum frontal width can be closely estimated. The figure is 75 mm.

(2) The parietals. Although neither of the two parietal bones is intact, the whole of the sagittal suture is preserved so that the bregma–lambda cord and arc can be measured with reasonable accuracy: these are 76.5 mm and 81.0 mm respectively. On either side of the sagittal suture the parietal bones extend laterally with only a slight downward curve for about 50 mm. Although the lateral parts of both parietals are missing, these bones must then have turned abruptly downwards if they were to meet the squamous parts of the temporal bones. This can clearly be seen on the more complete right side of the cranium. The sagittal suture is very largely fused but can still be traced. This condition is somewhat surprising in an individual whose third molars were barely erupted and not yet in occlusion. It suggests that the eruption of the third molars in early hominids based on analogy with modern man may not be a reliable indication of age.

(3) The occipital bone. A large part of this bone is well preserved, especially on the right side. There is enough of the nuchal area intact as well as continuous contact from lambda to opisthion to make a reasonable reconstruction of the upper half of the occipital and it is possible to estimate, with a fair degree of accuracy, the lambda–opisthion arc and cord: these measure 87.5 mm and 71.0 mm respectively. The arc/cord index (Martin's index 125)[5] is 81.14. By doubling the measurement from the right asterion to the midline of the occipital it is also possible to estimate the bi-asterionic width, which is approximately 99 mm. The occipital index (Martin's index 129) is approximately 72.0.

Viewed in profile, when the skull is orientated in the Frankfurt plane, the occipital region is seen to project far beyond a vertical line through the external auditory meatus, a feature seen in *Homo* but not in *Australopithecus*. The occipital condyles lie between the external auditory meati and the foramen magnum is elongate. The basi-occipital projection is short.

Table 1 Measurements of Olduvai H. 24 Cranium (in mm)

Glabella to inion cord	147.0
Glabella to bregma cord	88.5
Bregma to inion cord	78.5
Bimastoid crest breadth	122.0
External orbital angle width	107.0
Minimum frontal width	75.0
Foramen magnum length	28.5
Foramen magnum width	25.0
Length nasal bones	19.5
Nasion to prosthion	60.0
Nasion to orale	61.0
Nasion to left side of pyriform aperture	38.5
Nasion to naso-spinale	36.0
Naso-spinale to prosthion	25.0
Maximum breadth of pyriform aperture	25.0
Palatal width at alveolae of M^1	37.0

Table 2 Measurements of Teeth of Olduvai H. 24 (in mm)

Left side	Bucco-lingual breadth	Mesio-distal length
P^3	(12.0)	9.0
P^4	(12.5)	9.1
M^1	(13.0)	12.6
M^3	(14.5)	12.0

(4) The sphenoid bone. Nearly all the sphenoid is preserved including the pterygoid plates but not the pterygoid hamuli. In general appearance this bone is very similar to that seen in *Homo*, but this area requires detailed study in order to elucidate its significance taxonomically.

(5) The temporal bones. In the right temporal only the superior portion of the squamosal and part of the zygomatic process are missing. The mandibular fossa is perfectly preserved and is deep and narrow. The external auditory meatus is large and rather parallel-sided. The posterior part of the tympanic plate rises steeply to separate the meatus from the mastoid region. The tip of the mastoid process is broken off and the original size can only be estimated approximately from the preserved part. The zygomatic process is slenderly built and lacks the wide lateral shelf and outward flaring generally seen in *Australopithecus*.

The greater part of the left temporal bone is missing except for a small fragment in the medial region of the mandibular fossa.

(6) The face and palate. (*a*) The nasal bones. Both nasal bones are preserved with only the tips of the lateral processes on either side slightly damaged. The contact of the two bones in the midline is marked by a distinct keel which continues almost to the end of the nasal area. The margins of the pyriform aperture are not quite intact, but at its widest point it measures approximately 25 mm. The nasal length from nasion to the left lateral lip of the nasal aperture is 42.5 mm and nasion to nasospinale 36.0 mm. The nasal index (Martin's index 148) is 69.4, that is hyperchamaerrhine. There is a well developed anterior nasal spine at the centre of the pyriform aperture and from this point a very distinct keel runs downwards in the mid-line on the naso-alveolar clivus. (*b*) The zygomatic bones. The right zygomatic bone is reasonably well preserved; only the zygomatic process and a chip from the frontal process are missing. The root of the zygomatic arch lies approximately above a point between the first and second molars. The left zygomatic bone is missing. (*c*) The maxillae. The right maxilla is almost complete; parts of the alveolar process are missing, particularly in the region of M^2, and at the canine jugum the bone is also missing. The left maxillary bone is less complete, the zygomatic process, part of the nasal surface, and much of the posterior part of the alveolar process are missing. At the incisor sockets the alveolar margin seems to be preserved lingually but is broken labially. At present the margin runs straight across between the two canine alveoli and is not curved; but it is difficult to assess the original form on account of the damage. The whole of the median palatal suture is preserved. The right side of the bony palate has been displaced posteriorly by approximately 2 mm. The palate is seen to be reasonably deep (depth of alveolar processes at the first molars is approximately 15.0 mm). (*d*) The palatine bones. The palatine bones are intact and the areas of their contact with the maxillary tuberosities and with the sphenoid are well preserved.

(7) The teeth (see Table 2) present in the cranium as now reconstructed are as follows: (*a*) The crown of the left M^3. This is slightly broken on the distal aspect. The occlusal surface is undamaged and is very wrinkled with no sign of wear. (*b*) The left M^1 is intact and is firmly rooted in the maxilla. The enamel of the crown shows a degree of flat wear and the dentine is only exposed in a very small area on the protocone. (*c*) The left P^4 is complete. Its bucco-lingual width is less than that of M^1 and it is rather rectangular in form. (*d*) The left P^3 is partially preserved, the mesio-lingual area is broken away. (*e*) the right M^3 is partially preserved, the paracone and part of the protocone are broken away. The tooth compares closely with the intact left M^3. (*f*) The right M^2 is incomplete; it is broken diagonally so that the lingual and distal area is missing. (*g*) The right M^1 is nearly complete, only the protocone is missing. Its wear and structure are like those of the left first molar.

In addition to these teeth several fragments of molars and premolars were also recovered during sieving. It has not yet been possible to fit these fragments on to the cranium.

(8) Cranial capacity. It is not possible at this stage to give a close estimate of cranial capacity but preliminary determinations give a figure in the order of 560 cm^3.

(9) Endocranial region. Considerable parts of the endocranial morphology are preserved.

Comparisons with Other Hominids

There is still some evidence of distortion in the reconstruction. This has resulted in the vault of the skull being lower than it was originally and the backward projection of the occipital is now exaggerated.

The frontal bone does not exhibit the marked post-orbital constriction that is seen in the australopithecines from South and East Africa, nor does the supra-orbital element of the frontal bone curve backwards to the same extent. Seen in both lateral and vertical views the supra-orbital torus and glabellar region form a more or less straight line whereas in all known species of *Australopithecus* the glabella tends to project further forward with the lateral edges of the supra-orbital torus swinging backward. The parietal bones extend outwards from the sagittal suture with only a slight downward curve at first, and then bend steeply downwards to meet the squamosal parts of the temporal bones. The parietal region of the brain is thus more expanded than in *Australopithecus*, where the parietals usually begin to curve downwards close to the mid-line. Even allowing for the backward distortion of the occipital region this area of the skull extends further behind a vertical line through the external auditory meatus, when the cranium is in the Frankfurt plane, than in the australopithecines. The mandibular fossae are deep and very similar morphologically to those of *Homo sapiens* and wholly unlike those seen in *Australopithecus*. The nasal bones are long and slender with a marked keel in the mid-line.

The discovery of this skull, which is clearly a member of the genus *Homo* as defined in 1964[6], although it has certain primitive morphological features, raises an important issue. Although it is clearly not an australopithecine it nevertheless differs in certain important respects from the incomplete type of *Homo habilis* (Olduvai H. 7). Yet it resembles the paratype from Bed II (Olduvai H. 13) in both cranial and dental characters. At the same time, the dentition of the type of *Homo habilis* shows considerable resemblances to that of Olduvai H. 16, from lower Bed II, Maiko Gully, although differing in some respects. The problem therefore is whether or not there are two forms of *Homo* in Beds I and II or whether the differences between the two groups can be accounted for on the basis of individual variation and sexual dimorphism.

Although only part of the upper dentition is preserved in the cranium from DK, there are other specimens from Beds I and II which have a comparably small or even smaller tooth size and are morphologically similar. These clearly belong within the same group. They include a complete upper molar (Olduvai H. 6) from the site of *Australopithecus boisei* and an isolated molar (Olduvai H. 21), also probably from Bed I.

In Olduvai H. 24 from DK and in H. 13 from MNK certain important diagnostic characters of the skull are preserved, including the area of the temporal bones around the external auditory meatus and most of the occipital bones. These features, together with the morphology of the teeth, clearly indicate that both specimens belong within the genus *Homo*.

Although the occipital bone of the type specimen of *Homo habilis* (Olduvai H. 7) is not preserved it is evident from the structure of the parietal bones, and especially the form of the lambdoid suture, that the occipital bone must have been morphologically similar to that of *Homo erectus*, that is to say, the lambdoid suture is in the form of a low, wide arch and not V-shaped. This form is also seen in the skull of Olduvai H. 16 from lower Bed II. In contrast, the form of this suture in both Olduvai H. 24 from DK and Olduvai

H. 13 from MNK and, indeed, the whole form of the occipital bone, is very similar to that seen in *Homo sapiens* today: the line of the suture descends sharply from lambda in the form of an inverted V. There are thus two types of occipital morphology, one represented by Olduvai H. 24 and Olduvai H. 13 and the second by the type of *Homo habilis* and Olduvai H. 16.

The teeth of Olduvai H. 7 and H. 16 are megadont and within the size range of *Australopithecus africanus*; yet, in other respects, they display a number of morphological differences, especially in the premolars.

Distinct differences from the australopithecines are also evident in the cranial morphology, particularly in the parietals. Although the frontal bone of the type of *Homo habilis* is missing, a large part of this bone is preserved in Olduvai H. 16. Both the minimum frontal width and the width between the temporal crests is greater than in any known australopithecine.

Until the discovery of Olduvai H. 24 it was considered possible that the difference in morphology and in size between the cranial parts and dentition of the type of *Homo habilis* from Bed I and those of the paratype from Bed II (Olduvai H. 13) might have resulted from the elapse of a prolonged time interval. Because Olduvai H. 24 is chronologically near to the type of *Homo habilis* and yet more closely resembles the paratype from Bed II this explanation is no longer tenable. There remains the question of sexual dimorphism and it is possible that Olduvai H. 13 and H. 24 represent females of *Homo habilis* with the type and H. 16 representing the males. The difference in the morphology of the occipital region in the two groups, however, cannot be disregarded and suggests the possibility of taxonomic variation.

Beyond doubt, the new specimen represents the genus *Homo* as defined by Leakey, Tobias and Napier and differs fundamentally from the australopithecines. At the same time it is not entirely certain that it necessarily represents a female of *Homo habilis*.

We thank Dr A. Walker of the University of Nairobi for checking the text and measurements and for most valuable comments.

Received April 2, 1971.

[1] Leakey, M. D., *Nature*, **223**, 754 (1969).
[2] Curtis, G. H., and Hay, R. L., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J.) (Scottish Academic Press for Wenner Gren Foundation for Anthropological Research, in the press).
[3] Leakey, L. S. B., and Leakey, M. D., *Nature*, **202**, 3 (1964).
[4] Leakey, M. D., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.) (University of Chicago Press, Chicago and London, 1967).
[5] Martin, R., *Lehrbuch der Anthropologie* (Fischer, Stuttgart, 1959).
[6] Leakey, L. S. B., Tobias, P. V., and Napier, J. R., *Nature*, **202**, 5 (1964).

Non-random *X* Chromosome Expression in Female Mules and Hinnies

J. L. HAMERTON, B. J. RICHARDSON & PHYLLIS A. GEE

Department of Medical Genetics, Children's Hospital, Winnipeg, and Departments of Paediatrics and Anatomy, University of Manitoba

W. R. ALLEN & R. V. SHORT

ARC Unit of Reproductive Physiology and Biochemistry, Animal Research Station, Huntingdon Road, Cambridge, and
Department of Veterinary Clinical Studies, School of Veterinary Medicine, Madingley Road, Cambridge

The behaviour of *X*-linked glucose-6-phosphate dehydrogenase locus in a mule and two hinnies provides support for Lyon's single active *X* hypothesis.

THERE have been several studies of *X* chromosome DNA replication and the behaviour of *X*-linked genes in the female mule[1-6] but the results are not in agreement on the frequency with which the two parental *X* chromosomes (X^D and X^H) show late DNA replication. Here we present additional data on the expression of the *X*-linked glucose-6-phosphate dehydrogenase (*Gd*, E.C.1.1.1.49) locus in a female mule and two female hinnies. (The hinny is the reciprocal cross *Equus caballus* ♂ × *E. asinus* ♀.) The mule and one hinny were less than one year old and the third animal was a 35 day old hinny foetus obtained by hysterotomy. All were bred at the Veterinary School and ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, England.

Fibroblast cultures were established from the three animals by standard methods. The medium used was McCoy's 5a (modified) supplemented with 15% foetal calf serum (McFC15). Cloning of fibroblasts was carried out as follows[7]. Healthy exponentially growing fibroblast cultures were trypsinized, and the cell suspension was diluted as necessary to a final dilution of about thirty cells per ml. One drop of this suspension (0.03 ml.) was placed in each well of an appropriate number of Falcon Microtest II culture plates. (Each plate contains ninety-six wells of 0.5 ml. capacity. For each experiment five or six plates were seeded.) Then 0.2 ml. of McFC15 was added to each well and the plates were covered and incubated in a 100% humidity incubator in an atmosphere of 5% CO_2 in air. They were examined after 3 and 5 days and wells containing one colony were marked. The plating efficiency varied from 20–40%, which was similar to that obtained in conventional plating experiments using the same cell suspensions.

After 10–14 days, wells containing single colonies were trypsinized and the cell suspension transferred to 60 mm Falcon Petri dishes. When these dishes were confluent, the cells were either transferred to culture bottles to allow more extensive growth or, alternatively, the G6PD types were determined on the cells in the Petri dishes without further passage. Cells from each original culture and from a number of clones were stored at −70 or −196° C in a cryoprotective medium containing 15% glycerol. Lysates were prepared from both original cultures and clones by freezing and thawing in 0.015 M Tris-borate buffer (*p*H 7.8) containing 0.005 M EDTA, 0.02 g% 'Triton X-100', 2 mg% NADP and 0.05% (v/v) 2-mercaptoethanol. Electrophoresis ('Cellogel', Chemetron, Milan) was carried out for 1.25 h at 4° C at a potential gradient of 45 V/cm and the gels were stained for glucose-6-phosphate dehydrogenase[5].

Table 1 Expression of Glucose-6-phosphate Dehydrogenase in Horses and Donkeys and their Hybrids

Animal No.	Type	Material	Gd^D	Gd^H	Source
Female hybrids					
100	Mule	E	±	+	Cambridge *
		F	−	+	
138	Hinny	E	±	+	Portugal
139	Hinny	E	−	+	Portugal
143	Mule	E	−	+	Portugal
187	Hinny	E	±	+	Portugal
188	Hinny	E	±	+	Portugal
189	Hinny	E	±	+	Portugal
190	Hinny	E	±	+	Portugal
		F	−	+	
191	Hinny	E	−	+	Portugal
192	Hinny	E	+	±	Portugal
195	Mule (foetus)	F	−	+	Cambridge †
196	Mule (foetus)	F	−	+	Cambridge †
200	Hinny (foetus)	F	−	+	Cambridge †
202	Mule	E	−	+	Cambridge
		F	±	+	Cambridge
203	Hinny	F	±	+	Cambridge
		E	±	+	
217	Mule	E	±	+	Cambridge
Male hybrids					
	Mule (n=1 foetus)	F	−	+	Cambridge
	Mules (n=4)	E	−	+	Portugal and Cambridge
	Hinnies (n=7)	E	+	−	Portugal and Cambridge
Parental species					
Males					
	Donkeys (n=7)	E	+	−	Cambridge
	Horses (n=2)	E	−	+	Cambridge
Females					
	Donkeys (n=12)	E	+	−	Cambridge
	Horses (n=8)	E	−	+	Cambridge

* This animal (No. 100) reported by Hamerton *et al.*, 1969.

† 195 45D gestation
196 60D gestation
200 35D gestation
201 ♂ mule 35D gestation
All obtained by hysterotomy

+ Strong; ±, faint; −, absent; E, erythrocytes; F, fibroblasts.

Gd is seen as a single band in both parental species, Gd^H moving slower than the Gd^D (refs. 2 and 8). The faint slower bands seen in both parental species and the hybrids after starch gel electrophoresis represent breakdown products. The earlier samples from animals 100–196 were typed on starch as described earlier[2]. A number of other hybrid animals of both sexes as well as the parental species have been studied using both erythrocytes and fibroblasts without cloning. The *Gd* types are summarized in Table 1, and clearly confirm *X*-linkage of *Gd* in the Equidae. The parents of the three hybrids that were cloned are included in this table and show the expected *Gd* patterns. The interrelationship of the various hybrids which we have studied in detail is shown in Fig. 2. The hybrids, with one exception, develop a strong Gd^H band and either a faint or no Gd^D band. The exception (animal 192) has a stronger Gd^D band than Gd^H band. This inter-animal variation may explain the lack of agreement between different workers[3,4].

The *Gd* results obtained on 303 clones derived from three hybrids are given in Table 2. Two hundred and ninety-seven of these clones showed either the Gd^H or Gd^D band. Six clones were mixed (Gd^H/Gd^D), two of these were recloned, and 106 out of 107 of these sub-clones showed either Gd^H or Gd^D while one was again mixed (Table 3). In all animals, irrespective of the cross, the frequency of the two types is significantly different from 50% ($P > 0.0005$). This results from a deficiency of clones showing Gd^D.

Recloning the two mixed clones at a later passage showed that the proportion of Gd^D sub-clones (0.16) is significantly lower than expected, if it is assumed that these mixed clones originated from two cells, one Gd^D and one Gd^H. This suggests powerful selective forces acting *in vitro*.

A number of conclusions can be drawn from these results.

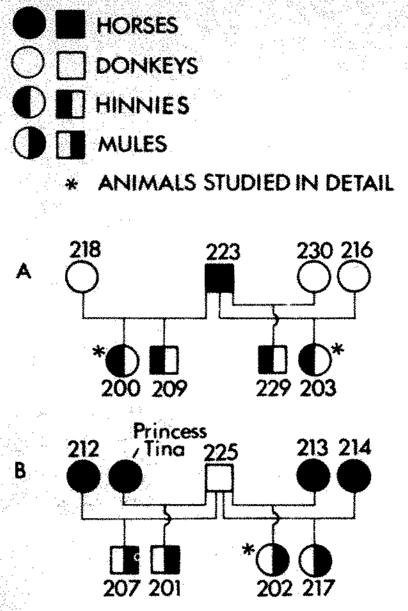


Fig. 1 Pedigree charts of the mules and hinnies studied. The numbers refer to numbers in Table 1. *A*, Hinnies; *b*, mules.

First, the mixed expression seen in the hybrids and some of the original cultures derived from them can be accounted for by the expression of one *X* chromosome only in any given cell and its descendants, as postulated by Lyon[9,10], and not from partial activity of both *X* chromosomes as postulated by Grüneberg[11] in his complemental *X* hypothesis. It could be argued, however, that the minor allele is expressed in less than 10% of the cells, in which case it would not be detected by the methods used here without sub-cloning. It seems unlikely that in a system where if the expression of an allele is equated to 100, all relative activities from 100 : 0 to 0 : 100 can reversibly occur for two alleles as suggested by Grüneberg[11], we would expect to find 81.7% of one type, 16.6% of the other and only 1.7% mixed, which are the results obtained here. Second, it can be concluded that active cell selection over a fairly short period of time occurs during *in vitro* fibroblast culture and that this selection would usually seem to be against those cells expressing Gd^D irrespective of whether the X^D is maternal or paternal in origin. This indicates that the maternal cytoplasm does not preferentially activate the maternal *X* chromosome or effect subsequent cell selection in these hybrids. Third, studies of *X*-linked loci using electrophoretic methods seem to demonstrate activity of a given locus only when it is expressed in more than 10% of the cell population. Original cultures from animals 202 and 203 both showed a minor fast band (Gd^D) at an early passage; this was subsequently lost during culture. In both these animals the frequency of Gd^D cells as demonstrated by the typing of clones was between 0.1 and 0.3 in different experiments. The original cultures from the foetus (200) never showed a fast Gd^D band; in spite of this, 9.0% of clones showed expression of Gd^D. These results are interesting in the light of the results reported by Sharman and his co-workers[12–14] on *X*-inactivation in marsupial hybrids in which they demonstrated preferential late replication of the paternal *X* chromosome and inactivation of the paternal *Gd* and phosphoglycerokinase (*PGK*) loci. Finally, the results obtained here agree with other data showing that the proportion of late replicating X^D ranged from 0.67–1.00 in six female mules and eight female hinnies[6]. If late replication of a given chromosome represents its genetic inactivation, then the autoradiographic results suggest that the proportion of cells expressing Gd^D should vary between 0.33 and zero which fits very closely to the results obtained here. Similar results have recently been reported for four mules by Cohen and Rattazzi[5].

Table 2 *Gd* Expression in Clones derived from Fibroblasts from Three Equine Hybrids

Animal No.	Expt.	No. Gd^D	No. Gd^DGd^H	Total	Proportion Gd^D
200	VIII	4	–	40	0.100
(Hinny ♀)					
35 D Foetus	X	6	1	68	0.088
Total		10	1	108	0.093
202	II	5	3	11	0.454
(Mule ♀)	IV	3	–	20	0.150
	VI	7	–	41	0.171
	XII	14	1	42	0.333
Total		29	4	114	0.254
203	VII	1	–	3	0.333
(Hinny ♀)	IX	3	–	26	0.115
	XIII	8	1	52	0.154
Total		12	1	81	0.148
Grand total		51	6	303	0.168

Table 3 Expression of *Gd* Locus in Sub-clones from Two Mixed (Gd^DGd^H) Clones from Animal 202

Clone	Expt.	No. Gd^D	No. Gd^DGd^H	Total sub-clones	Proportion Gd^D
Cl	XIV	9	1	58	0.155
C8	XV	8	–	49	0.163
Total		17	1	107	0.159

These findings support the single active *X* hypothesis originally put forward by Lyon[9,10]. They demonstrate clearly activity of only one *Gd* allele in any given cell, which has also been shown for man[15,16]. Unfortunately, the other *X*-linked enzyme systems *PGK* and α-galactosidase which we have tested have failed as yet to show different mobilities in horse and donkey and so cannot be tested. Furthermore, these data demonstrate *in vitro* cell selection in favour of those cells carrying an active X^H. Examination of uncloned fibroblasts and erythrocytes from these animals also shows predominant expression of Gd^H (Table 1) suggesting that the proportion of cells with an active X^H *in vivo* are reflected in our *in vitro* results and that these are not therefore due to differential plating efficiencies of the two cell types. The one animal showing equal expression of the two types is unavailable for further study. Lyon[9,10] has postulated *in vivo* cell selection to account for variation in expression of sex linked loci in the mouse and man and the demonstration of such strong selective forces *in vitro* due to the activity or inactivity of a single *X* chromosome lends support to the hypothesis that similar selective forces may be acting *in vivo*. Finally, the close agreement between the autoradiographic data[2,4–6] and the genetic data presented here adds further support to the hypothesis that late replication is the cytological manifestation of genetic inactivity. A final proof of this would be provided by autoradiographic studies on the two clonal types derived here. These studies are at present in progress.

We thank Mrs G. Anderson, Mrs E. Cameron and Miss V. Niewczas-Late for technical assistance. This work was supported by a grant to J. L. H. from the Medical Research Council of Canada. Financial support from the Children's Hospital Research Foundation of Winnipeg is also gratefully acknowledged. W. R. A. is supported by the Thoroughbred Breeders Association. Collection of material from some of the animals in Portugal was supported by a grant to J. L. H. and R. V. S. from the Wellcome Trust. We thank Dr M. J. Freire and his veterinary colleagues for Portuguese samples.

Received March 29; revised June 7, 1971.

[1] Mukherjee, B. B., and Sinha, A. K., *Proc. US Nat. Acad. Sci.*, **51**, 252 (1964).
[2] Hamerton, J. L., Giannelli, F., Collins, F., Hallett, J., Fryer, A., McGuire, V. M., and Short, R. V., *Nature*, **222**, 1277 (1969).
[3] Mukherjee, B. B., Mukherjee, A. B., and Mukherjee, A. B., *Nature*, **228**, 1321 (1970).
[4] Hamerton, J. L., and Giannelli, F., *Nature*, **228**, 1323 (1970).
[5] Cohen, M. M., and Rattazzi, M. C., *Proc. US Nat. Acad. Sci.*, **68**, 544 (1971).
[6] Giannelli, F., and Hamerton, J. L., *Nature*, **232**, 315 (1971).
[7] Cooper, J. E. K., *Texas Rep. Biol. Med.*, **28**, 29 (1970).
[8] Trujillo, J. M., Walden, B., O'Niel, P., and Anstall, H. B., *Science*, **148**, 1603 (1965).
[9] Lyon, M. F., *Ann. Rev. Genet.*, **2**, 31 (1968).
[10] Lyon, M. F., *Phil. Trans. Roy. Soc.*, B, **259**, 41 (1970).
[11] Grüneberg, H., *J. Embryol. Exp. Morphol.*, **22**, 145 (1969).
[12] Sharman, G. B., *Nature*, **230**, 231 (1971).
[13] Richardson, B. J., Czuppon, A. B., and Sharman, G. B., *Nature*, **230**, 154 (1971).
[14] Cooper, D. W., Vandeberg, J. L., Sharman, G. B., and Poole, W. E., *Nature*, **230**, 155 (1971).
[15] Davidson, R. G., Nitowsky, H. M., and Childs, B., *Proc. US Nat. Acad. Sci.*, **50**, 481 (1963).
[16] Linder, D., and Gartler, S. M., *Amer. J. Human Genet.*, **17**, 212 (1965).

Non-Random Late Replication of *X* Chromosomes in Mules and Hinnies

F. GIANNELLI

Paediatric Research Unit, Guy's Hospital Medical School, London SE1

J. L. HAMERTON

Department of Genetics, Children's Hospital, Winnipeg, and Departments of Paediatrics and Anatomy, University of Manitoba

Autoradiography of lymphocytes and fibroblasts has shed more light on the behaviour of *X* chromosomes in mules and hinnies.

THE process of DNA replication and genetic activity in *X* chromosomes of female mules and hinnies has been studied[1-6] without any agreement on the relative frequency with which the two parental *X* chromosomes (X^D and X^H) become late replicating and genetically inactive. Additional autoradiographic results on six female mules (*E. asinus* ♂ × *E. caballus* ♀) and eight female hinnies (*E. caballus* ♂ × *E. asinus* ♀) are presented here. One of the hinnies (No. 203) was bred in Cambridge[5], three of the mules (Nos. 27, 33, 100), subject of a preliminary communication[2], were obtained from various sources in Great Britain, while the remainder of the hybrids were from Portugal.

Lymphocyte and fibroblast cultures were established by standard methods[7,8]. Mixed lymphocyte cultures, using female donkey and horse blood were set up as controls, for the hybrid cultures. ^{3}H-Tdr (specific activity 5 Ci/mmol, Amersham) in a concentration 0.3–0.5 µCi/ml. was added to the leucocyte cultures 3–4 h before collection and colcemid was added 1.5 h before termination of the culture. Fibroblast cultures were labelled with 0.3 µCi/ml. 6 h before termination and colcemid was added 3 h later. Slides were prepared by air drying, stained lightly with lacto-acetic orcein and suitably spread metaphase plates were photographed. Autoradiographs were prepared by standard methods[2,9]. The cells which had previously been photographed were relocated and examined for a differentially labelled *X* chromosome. (This was defined as a medium sized chromosome which had at least twice as many autoradiographic grains as any other chromosome of similar size. This definition was adoped because the donkey has a submetacentric autosome much larger than the *X* and with a large heterochromatic paracentric region which is often densely labelled in late S.) The morphology of the differentially labelled *X* chromosome (*DLX*) was then determined by examination of the photographs taken before autoradiography. The two *X* chromosomes in the female hybrids are quite different, one (X^D) being submetacentric, while the other (X^H) is metacentric[1,2] (Fig. 1).

Twenty-one suitable metaphase plates from each of two hinnies and one mule (Nos. 100, 188, 203) were selected for quantitative autoradiographic studies, and karyotypes prepared. The total autoradiographic grains for each cell and for each of the five longest medium sized submetacentric and metacentric chromosomes were then counted. Grain counts were also made on samples of cells from the mixed leucocyte cultures, selected either at random or for the presence of a differentially labelled *X* chromosome.

The blood and skin cultures from the fourteen animals studied contained varying proportions of labelled cells and of cells with a differentially labelled *X* chromosome (Table 1). This did not influence the relative frequency of cells with a differentially labelled X^D or X^H. The relative frequencies differed significantly from 0.5 in every hybrid from which enough cells could be analysed and in each case there was an excess of DLX^D (Table 4). In the mixed leucocyte cultures, on the other hand, the number of horse cells which has a DLX was greater than the number of donkey cells with a DLX (Table 2).

The relative frequencies of DLX^D and DLX^H in cultures from individual mules or hinnies did not differ significantly from the overall frequencies for their own group (Tables 1 and 3). The hinnies behave in a similar fashion to the mules, but are possibly less homogeneous and seem to show a slightly higher frequency of DLX^H (Table 3).

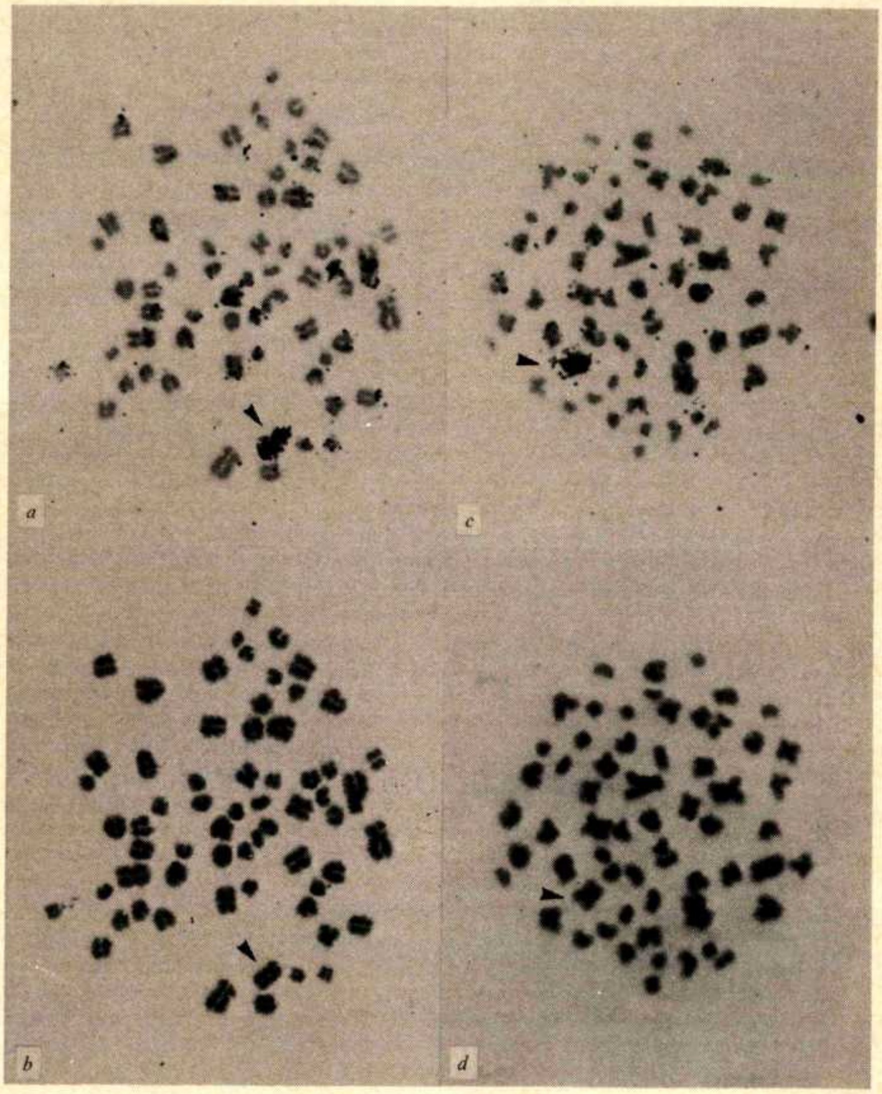


Fig. 1 Two metaphase plates from the same hybrid, the first showing (*a* and *b*) a submetacentric chromosome (arrowed) and the second (*c* and *d*) a metacentric chromosome (arrowed) differentially labelled. These chromosomes presumably represent, in order, the donkey and horse-derived X chromosomes.

In the sample of twenty cells selected at random from a mule and each of two hinnies (Table 4) the grain counts over the most densely labelled medium sized submetacentric and metacentric chromosomes showed the same regression line on the total cell grain counts, but the former chromosome had clearly higher grain counts than the latter. If these chromosomes are taken to represent respectively the X^D and X^H chromosome, these data seem to agree with the qualitative observation that the X^D chromosome is more frequently differentially labelled. Furthermore, the proportion of X chromosome grains counted over X^D seems to be very similar in the mule and the two hinnies.

Finally, in the mixed leucocyte cultures the regression of the grains counted over the differentially labelled X chromosomes, the most densely labelled X-like chromosomes, on the total cell grain counts was very similar in the horse and donkey cells. The covariance analysis (Table 5) showed that the grain counts over the X^H and X^D were very similar after allowance had been made for the labelling over the rest of the complement.

These results show that in both crosses irrespective of the direction of the cross, X^D is consistently more often differentially labelled than X^H. This differs from the findings of Mukherjee and his co-workers[1,3] but is in broad agreement with the observation of Cohen and Rattazzi[6].

Mukherjee and Sinha[1] used both longer labelling and colchicine times for blood cultures, while for skin cultures Mukherjee *et al.*[3] used a shorter labelling time than ours. Their results, however, differ in the same direction in both culture systems from those reported here; this suggests that the discrepancy cannot be explained by a variation in labelling procedure.

In the present study the DLX was defined as the chromosome of compatible morphology which had at least twice as many grains as any other chromosome of a similar size. This is in agreement with the illustration of Mukherjee and Sinha[1] who did not discuss their criteria in detail, but is less strict than that used in their later article[3] in which the DLX is defined as the chromosome with a grain count of at least 50% the total grain count over the whole complement.

In our opinion, this definition is too strict because of the difficulty of counting fifty grains or more over an *X*-like chromosome, which means that all cells with a total grain count of 100 or more over the remainder of the complement would have to be rejected. To see whether a more strict definition of *DLX* could affect the results presented here, however, all cells in which the grain counts over the *DLX* were not greatly in excess of twice that over any chromosome of similar size were rejected, leaving only those cells with a "very hot" *DLX* available for analysis. This procedure did not alter the ratio of DLX^H to DLX^D (Table 1). It seems reasonable to conclude from this that the way in which the *DLX* is defined is unlikely to account for the differences observed. Could any peculiarity or intrinsic difference in the labelling of X^D and X^H, not associated with the process of facultative heterochromatinization of the *X* chromosome, for example, a higher rate of DNA synthesis in X^D than in X^H at some stage of late S, be responsible for the difference which we have observed between the frequency of differentially labelled X^D and X^H in the hybrids' cultures ?

Data from the control cultures suggest that this is not so. The mixed lymphocyte cultures showed that the horse lymphocytes have a G_2 similar to that of the donkey and have a differentially labelled *X* chromosome at least as frequently. Admittedly a mixed lymphocyte culture is not a perfect control for cultures from hybrids in which each chromosome must be compared with the haploid sets from both parental species. But covariance analysis of grain counts over the *DLX* in the mixed lymphocyte cultures shows that, when account is taken of the labelling over the rest of the complement, DLX^D and DLX^H label in a similar fashion during the part of S studied This is confirmed by the similarity of the regression of grain counts over the presumptive X^D and X^H on total complement grain counts (TGC) in the three animals studied quantitatively (Table 4). Furthermore, an analysis of lymphocyte cultures from the hybrids shows that with an increase in labelling time the proportion of labelled cells increases and that of cells with a *DLX* decreases, while the relative frequency of DLX^D and DLX^H remains the same (Tables 1 and 6).

Finally, can these results be explained on the basis of an error in the identification of the X^D due to the presence o

Table 1 Autoradiographic Data from Six Mules and Eight Hinnies

Animal No.	Tissue cultured	No. of cells scored	Total No. of labelled cells	No. of cells with a *DLX*		No. of cells with a "very hot" *DLX*‡	
				Total	No. DLX^D	Total	No. DLX^D
Mules							
27	Skin	70	65	14	13	8	8
33	Blood *	1,000	35	27	24	19	18
	Skin	105	77	45	43	33	33
100	Blood *	755	453	68	60	55	50
	Blood †	15	9	3	3	2	2
	Skin	45	40	8	8	8	8
143	Blood *	12	2	1	1	1	1
	Blood †	18	16	7	7	5	5
144	Blood *	22	13	8	7	7	6
	Blood †	142	124	33	28	28	24
145	Blood *	12	7	1	1	0	0
	Blood †	17	14	2	2	2	2
Totals	Blood *	1,801	510	105	93	82	75
	Blood †	192	163	45	40	37	33
	Skin	220	182	67	64	49	49
Hinnies							
138	Blood *	9	5	2	2	1	1
	Blood †	3	2	0	0	0	0
139	Blood *	27	12	6	6	5	5
187	Blood *	172	155	28	22	21	18
	Blood †	17	16	0	0	0	0
188	Blood *	168	120	13	11	8	8
	Blood †	23	22	0	0	0	0
189	Blood *	326	133	18	12	14	10
190	Blood *	152	127	12	8	9	6
	Blood †	37	32	1	1	0	0
191	Blood *	159	135	5	5	3	3
	Blood †	32	30	4	3	3	2
203	Blood *	165	105	23	18	17	13
	Blood †	131	121	211	17	19	16
Totals	Blood *	1,178	792	107	84	78	64
	Blood †	243	223	26	21	22	18

* 3 h incubation with ^{3}H-Tdr. † 4 h incubation with ^{3}H-Tdr.

‡ *DLX* with at least 2.2 times the grains counted over any other chromosome of similar size.

morphologically similar late replicating autosome which mimics the behaviour of the DLX? Our definition of the DLX precludes this explanation unless the DLX^H had less than half the grains counted over this autosome much more often than the DLX^D. This could occur either if the DLX^H usually had lower grain counts than the DLX^D or if it was in the differentially labelled state less often. The data presented do not fit the first of these alternatives so that it seems reasonable to accept the second.

Table 2 Autoradiographic Data from Mixed Lymphocyte Cultures

Cell type	No. of cells scored	No. of labelled cells	Cells with a DLX No.	Proportion	Difference in proportions	s.e.	P
Horse	158	144	33	0.2291	0.1205	0.0515	$0.05 > P > 0.025$
Donkey	96	92	10	0.1086			

A further possibility which might account for our results is biased selection of cells or chromosome identification. To avoid this, the analysis of autoradiographs was conducted as independently as possible of the morphological chromosome analysis (a chromosome was classified as differentially labelled before analysis of the unlabelled photographs, and karyotypes were made by an individual who was unaware of the autoradiographic results). It is difficult, however, completely to exclude bias in an analysis of this type. The most serious cause of observer bias is the *a priori* expectations. In the study described here our intention was to investigate the relationship between differential labelling and genetic inactivity of one X chromosome, and it was expected that a 1:1 relationship would be found between the DLX^H and DLX^D in accordance with earlier findings[1]. Preliminary observations on differential labelling in cells with three mules[2] showed this was not so and it was considered a tentative hypothesis that the reciprocal cross might show the reverse, namely a greater frequency of DLX^H than DLX^D. On both counts therefore the results reported here are contrary to *a priori* expectations and lead to the conclusion that X^D is significantly more often differentially labelled than X^H in both mules and hinnies.

These results must now be considered in relation to the Lyon hypothesis (LH). In contrast to the suggestion of others[1,3] the mule may not provide a simple cytological test of random inactivation for reasons which have already been discussed[4]. These hybrids are, however, useful in studying the relationship between differential labelling of one X chromosome and genetic inactivation. Data on glucose-6-phosphate dehydrogenase (*Gd*)[5] show that the frequency with which Gd^D is expressed in fibroblast clones derived from three hybrids is in almost exact inverse proportion to the frequency with which X^D is observed to be differentially labelled in both lymphocytes and fibroblasts. Furthermore, qualitative data on expression of Gd^D show that, with the exception of one animal, it is expressed far more weakly than Gd^H in all the hybrids studied[10]. Cohen and Rattazzi[6] have reported a close correlation between the frequency of DLX^D and the expression of Gd^D in four further female mules. In each animal the X^D was more frequently late replicating and, correlated with this, Gd^D was the minor component. Hook and Brustman[10] have studied *Gd* expression in organs from 37 female mules. They found that in most tissues (blood, pancreas, brain, cervical cord, kidney and parotid gland) Gd^H is preferentially expressed. In thyroid and lung there appeared to be random expression while in the liver Gd^D seemed to be preferentially expressed.

The excellent correlation observed in our studies and those and Rattazzi[6] between differential labelling and the expression of an X-linked locus implies a definite relationship between these two phenomena; proof of such a relationship will be provided by autoradiographic studies on the clones discussed in the previous article[5]. These are in progress. A comparison of X chromosome behaviour and *Gd* expression in both the mule and the hinny suggests that neither the maternal cytoplasm nor the maternal environment is important in determining which X chromosome becomes genetically inactive and differentially labelled in the female hybrids or in determining which cell type proliferates preferentially

Table 3 Observed Proportion of Cells with an X^D Differentially Labelled and their 95% Confidence Limits

Hybrids Type	No.	Tissue	Proportion DLX^D	95% Confidence limits
Mules	27	S	0.928	0.661–0.998
	33	B	0.889	0.708–0.976
		S	0.955	0.848–0.995
	100	B	0.887	0.790–0.950
		S	1.00	0.631–1.00
	143	B	1.00	0.631–1.00
	144	B	0.854	0.708–0.944
	145	B	1.00	0.292–1.00
	Total	B	0.887	0.824–0.932
		S	0.955	0.875–0.991
Hinnies	138	B	1.00	0.158–1.00
	139	B	1.00	0.541–1.00
	187	B	0.786	0.590–0.917
	188	B	0.846	0.545–0.981
	189	B	0.667	0.410–0.867
	190	B	0.692	0.386–0.909
	191	B	0.889	0.517–0.997
	203	B	0.795	0.647–0.902
	Total	B	0.789	0.747–0.885
Total		B	0.841	0.801–0.853

S, Skin fibroblasts; B, lymphocytes.

The significant excess of cells with an inactive and differentially labelled X^D is most easily explained by a difference between the two X chromosomes either in respect to the mechanism of "induction" of facultative heterochromatin or to the increased fitness which an active X^H confers on the hybrid cells resulting in selection in their favour. There are at least two alternatives which might account for a difference between X^D and X^H in regard to the mechanism of heterochromatinization; first, that X^D is simply more prone to become heterochromatic, or, second, that heterochromatinization commences earlier in the donkey than in the horse and that this is retained in the hybrids and so leads to an earlier involvement of X^D compared with X^H. The studies reported in the previous article[5] on fibroblast clones suggest *in vitro* cell selection in

Table 4 Comparisons between the Grain Counts over the Most Densely Labelled X^D and X^H-like Chromosomes) $?X^D$, $?X^H$) in a Random Sample of Cells from Mule 100 and the Hinnies 188 and 203

Hybrid No.	No. of cells analysed	Comparison of mean grain counts over $?X^D$ and $?X^H$	Comparison of the regression coefficients of the logarithm of $?X^D$ and $?X^H$ grain counts on TGC*	Comparison of the contribution (D) of $?X^D$ to the grains counted over the presumptive X chromosomes in the mule and the two hinnies
100	21	$\bar{Y}_1=12.85$ $\bar{Y}_2=9.00$ $\bar{Y}_1-\bar{Y}_2=3.85$ $\text{s.e.}(\bar{Y}_1-\bar{Y}_2)=1.35$ $P<0.02$	$b_1=0.000956$ $b_2=0.001864$ $b_1-b_2=-0.000908$ $\text{s.e.}(b_1-b_2)=0.000869$ $P>0.3$	$D_m=0.5882$
188	20	$\bar{Y}_1=8.40$ $\bar{Y}_2=6.10$ $\bar{Y}_1-\bar{Y}_2=2.30$ $\text{s.e.}(\bar{Y}_1-\bar{Y}_2)=0.964$ $P<0.05$	$b_1=0.00209$ $b_2=0.00199$ $b_1-b_2=0.00010$ $\text{s.e.}(b_1-b_2)=0.00077$ $P>0.9$	$D_h=0.5892$ $D_m-D_h=-0.0010$
203	18	$\bar{Y}_1=15.61$ $\bar{Y}_2=10.61$ $\bar{Y}_1-\bar{Y}_2=5.00$ $\text{s.e.}(\bar{Y}_1-\bar{Y}_2)=2.25$ $P<0.05$	$b_1=0.00102$ $b_2=0.00205$ $b_1-b_2=-0.00103$ $\text{s.e.}(b_1-b_2)=0.000613$ $P>0.1$	$\text{s.e.}(D_m-D_h)=0.023$ $P>0.8$

* Scatter diagrams have shown that the regressions of the logarithms of the $?X$ chromosomes grain counts on the total complement grain counts (TGC) are linear.

1, $?X^D$; 2, X^H; m, mule; h, hinny.

favour of cells with an active X^H; studies by Hook and Brustman[10] suggest that *in vivo* cell selection occurs in different organs.

Our finding of one animal (No. 192)[5] with a preponderance of Gd^D, on which unfortunately autoradiographic studies were not possible, suggests that there may be inter-animal variation in the expression of *Gd*. Inter-animal variation could perhaps account also for the discrepancy between our autoradiographic results and those of Mukherjee and his colleagues[1,3].

Table 5 Comparison by Covariance Analysis of the Grain Counts over Differentially Labelled X Chromosomes (A) or the Most Densely Labelled X-like Chromosomes (B) in Horse–Donkey Mixed Blood Cultures

Analysis	A chromosome X^D	X^H	B chromosome $?X^D$	$?X^H$
No. cells analysed	10	33	20	20
Mean grain counts	$\bar{Y}_1=30.21$	$\bar{Y}_2=26.00$	$\bar{Y}_1=25.65$	$\bar{Y}_2=19.95$
Adjusted mean	$\bar{Y}'_1=29.55$	$\bar{Y}'_2=28.17$	$\bar{Y}'_1=22.90$	$\bar{Y}'_2=22.70$
Difference	$\bar{Y}'_1-\bar{Y}'_2=1.38$		$\bar{Y}'_1-\bar{Y}'_2=0.20$	
s.e. of difference	1.88		2.55	
Probability	$0.5>P>0.4$		$0.95>P>0.90$	

Scatter diagrams were made before these analyses. These have shown a linear relationship between the presumptive X chromosome grain counts and the counts over the rest of the complement. The variance of Y was fairly constant over the range of total grain counts used and this was similar in the two samples compared. The regression coefficients of the individual samples used in this comparison were significantly different from zero, but did not differ significantly from each other.

Our data and those of Cohen and Rattazzi[6], however, clearly show that X^D tends to be more often heterochromatic than X^H in the tissues of female horse–donkey hybrids which have been available for study.

We thank Drs R. V. Short, W. R. Allen and M. J. Freire, with his colleagues in Portugal, for samples of material. Collection of Portuguese samples was supported by grants to J. L. H. from the Wellcome Trust. During this work J. L. H. and F. G. were supported by the Spastics Society. J. L. H. also acknowledges support from ARC and the Canadian Medical Research Council. We also thank Mrs F. F. Collins, Mrs J. Hallett, Miss A. Fryer, Miss V. M. McGuire and Mr L. Grixti for technical assistance.

Table 6 Analysis of the Tendency for the Proportion of DLX^D to Vary according to the Proportion of Labelled Cells in Blood Cultures of Mules and Hinnies

Type of hybrid	Regression coefficient	SS due to regression	χ^2	P
Mules	−0.5341	352.4	0.429	>0.5
Hinnies	−1.033	1,531	1.86	>0.1
All*	−1.189	3,777	4.6	<0.05

This analysis was conducted using the angular transformation method[11].

* Since the proportion of DLX^D in the hinnies is lower than that of mules and their cell samples contribute chiefly to the class with a relatively high proportion of labelled cells the data regarding both types of hybrid together are not very meaningful.

Received May 7, 1971.

[1] Mukherjee, B. B., and Sinha, A. K., *Proc. US Nat. Acad. Sci.*, **51**, 252 (1964).

[2] Hamerton, J. L., Giannelli, F., Collins, F., Hallett, J., Fryer, A., McGuire, V. M., and Short, R. V., *Nature*, **222**, 1277 (1969).

[3] Mukherjee, B. B., Mukherjee, A. B., and Mukherjee, A. B., *Nature*, **228**, 1321 (1970).

[4] Hamerton, J. L., and Giannelli, F., *Nature*, **228**, 1322 (1970).

[5] Hamerton, J. L., Richardson, B. J., Gee, P. A., Allen, W. R., and Short, R. V. *Nature*, **232**, 312 (1971).

[6] Cohen, M. M., and Rattazzi, M. C., *Proc. US Nat. Acad. Sci.*, **68**, 544 (1971).

[7] Hsu, T. C., and Kellogg, jun., D. S., *J. Nat. Cancer Inst.*, **25**, 221 (1960).

[8] Moorhead, P. S., Howell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).

[9] Giannelli, F., *Human Chromosomes DNA Synthesis, Monographs in Human Genetics*, **5** (edit. by Beckman, L., and Hauge, M.) (Karger, Basel and New York, 1970).

[10] Hook, E. B., and Brustman, L. D., *Genetics*, **64**, 2, Part 2 (abstract, 1970).

[11] Fisher, R. A., and Yates, F., *Statistical Tables for Biological, Agricultural and Medical Research*, fifth ed. (Oliver and Boyd, Edinburgh, 1957).

LETTERS TO NATURE

PHYSICAL SCIENCES

Ivory Coast Microtektites: Fission Track Age and Geomagnetic Reversals

IN this article we report our measurements of the age of Ivory Coast microtektites, and discuss the association of microtektites with geomagnetic reversals. Microtektites are small glassy particles (<1 mm) found in sediment cores from the oceans adjacent to the Australasian and Ivory Coast tektite fields[1]. They resemble tektites in outward form and composition. Comparatively few microtektites have been recovered so far, but Cassidy *et al.*[2] believe that the total mass of Australasian microtektites may be at least 10^{10} kg, which is 10^5 times larger than the estimated mass of the tektites.

Our Ivory Coast microtektites were kindly given to us by Dr B. P. Glass, and we have used the fission track method of dating[3]. In this method the known rate of spontaneous fission of ^{238}U (we have used a fission decay constant of 8.42×10^{-17} yr^{-1}) allows the age of the material to be determined after the uranium content of the material has been found by exposing the material to a known dose of thermal neutrons. Then the age in years of the sample, A, is given by[4]

$$A = (\rho_n/\rho_i)\ 5.01 \times 10^{-8}\ F \qquad (1)$$

where ρ_n and ρ_i are the natural and induced etch pits per unit area, and F is the time-integrated flux of neutrons (usually a few times 10^{15} thermal neutrons cm^{-2}).

To count as many natural fission tracks as possible in our limited sample, each microtektite was mounted individually in epoxy resin on a plastic backing, and polished to uncover successive layers for etching and examination under the microscope. The etchant was hydrofluoric acid (40% by volume) used for 30 s at 21° C. The initial diameters of the microtektites were ~150–400 μm, and each etching and polishing (using 0–1 μm diamond powder on a microcloth) removed a layer about 20 μm thick. The last few layers were usually saved for irradiation by thermal neutrons, and the same counting procedure was used for the irradiated samples.

Fig. 1 shows typical photomicrographs of natural and induced etch pits, and a poorly polished section including several spurious etch pits is shown for comparison. Altogether 26 microtektites were examined, some in greater detail than

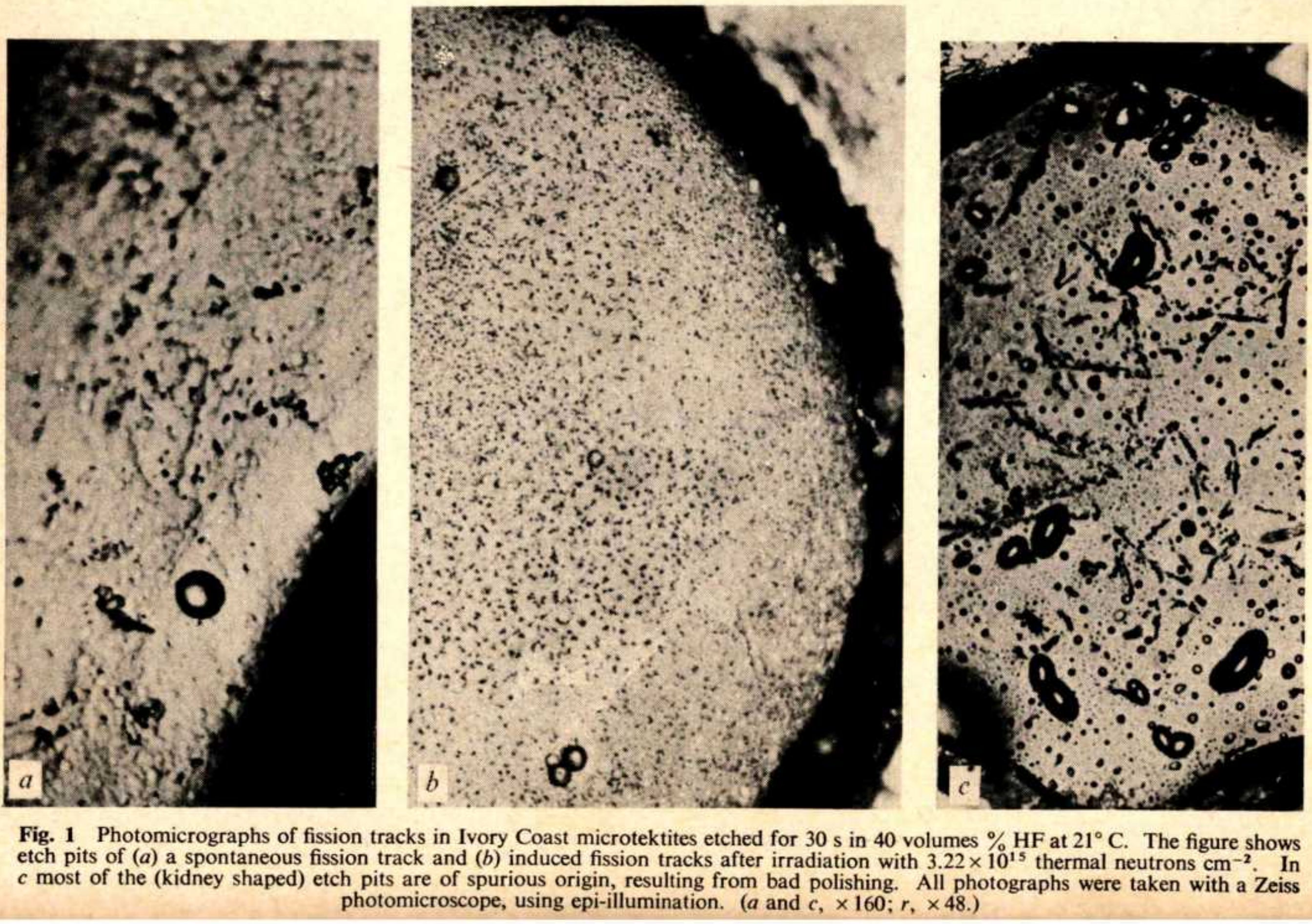


Fig. 1 Photomicrographs of fission tracks in Ivory Coast microtektites etched for 30 s in 40 volumes % HF at 21° C. The figure shows etch pits of (*a*) a spontaneous fission track and (*b*) induced fission tracks after irradiation with 3.22×10^{15} thermal neutrons cm^{-2}. In *c* most of the (kidney shaped) etch pits are of spurious origin, resulting from bad polishing. All photographs were taken with a Zeiss photomicroscope, using epi-illumination. (*a* and *c*, ×160; *r*, ×48.)

Table 1 Ages of Tektites and Microtektites from the Australasian and Ivory Coast Strewn Fields

Strewn field	Ages of tektites (m.y.) K/Ar ages	Ages of tektites (m.y.) Fission track ages	Ages of microtektites (m.y.) Fission track ages Gentner *et al.*[5]	Ages of microtektites (m.y.) Fission track ages Present work	Ages of microtektites (m.y.) Deposition ages
Ivory Coast	1.15 ±0.15*	1.01 ±0.10* 0.86 ±0.06¶	1.09 ±0.20†	0.88 ±0.13‡	~0.8§ −1.0
Australasian	0.72 ±0.10*	0.70 ±0.10* 0.68 ±0.05¶	0.71 ±0.10	——	~0.7§

* Gentner *et al.*[6]

† Based on forty microtektites from the Atlantic Ocean core K9–56 (3° 22.8′ N lat., 15° 37.2′ W long., 2,563 fathoms depth).

‡ Based on twenty-six microtektites from core K9–56. Of the eighteen microtektites which could be successfully analysed (yielding fifty-seven natural tracks), thirteen were irradiated in a reactor for age and U content determinations.

§ Glass[7].

¶ Determined by us. The Ivory Coast tektite age is based on only one specimen (from Ouellé) kindly given to one of us (S. A. D.) by Professor F. Kraut, director, Museum of Natural History, Paris. A total of 1,073 natural tracks was observed over an area of 2.65 cm^2.

others; 57 natural tracks were found (in 18 microtektites) over an area of 40.89 mm^2, and 3,627 induced tracks over an area of 8.40 mm^2.

Table 1 summarizes our results and those of Gentner *et al.*[5], based on the fission track method to date tektites and microtektites from the Australasian and Ivory Coast strewn fields. K–Ar ages of the tektites are also shown, but microtektites are too small for this method. Our ages for the Ivory Coast tektites and microtektites agree among themselves, although they are somewhat lower than those reported by other workers (except for the deposition age[7] of ~0.8 to 1.0 m.y. for the microtektites). The errors shown in Table 1 for our fission track ages are made up of statistical (Poisson) errors, uncertainties in the neutron dose (±5%) and, for the microtektite measurements, uncertainties in the uranium content of the samples which could not be irradiated.

It has already been pointed out that the Australasian microtektites occur within a zone ≈30–50 cm thick associated in time with the last geomagnetic reversal[2,5,8]. In other words, the Australasian microtektites appear to coincide in age with the boundary between the present normal epoch (Brunhes) and the preceding reversed geomagnetic epoch (Matuyama), which is believed to have occurred about 0.7 m.y. ago according to magnetic measurements of lavas and deep-sea sediments[9–11]. There have been four geomagnetic epochs of alternating polarity over the past 3.6 m.y., each lasting ~10^6 yr, interspersed with shorter events (<0.2 m.y.) when the polarity was briefly antiparallel to that of the epoch. We believe that it may be possible to associate the Ivory Coast microtektites with the last such event (Jaramillo) which occurred about 0.9 m.y. ago[12,13]. Ivory Coast microtektites have been found so far in two cores, V19–297 (2° 37′ N lat., 12° 00′ W long.) and K9–56 (3° 23′ N lat., 15° 37′ W long.). The microtektites in V19–297 are concentrated[5] in a thin layer about 15 cm thick that occurs about 40 cm below the Matuyama–Brunhes boundary. The Jaramillo event has been lost in this core, but palaeontological stratigraphy suggests[14] that it coincides with the fall of the microtektites. Our microtektites come from the K9–56 core, and our age of 0.88±0.13 m.y. (as well as the weighted mean of our measurements and those of Gentner *et al.*, 0.94±0.10 m.y.) is in close agreement with the estimates of the age of the Jaramillo event, which range from 0.85 to 0.97 m.y. Unlike the stratigraphic determinations of age in deep-sea sediments (whether palaeomagnetic or palaeontological) which may have been affected by bottom currents and mud-burrowing organisms the fission track determinations are absolute. The age of the Ivory Coast tektite found by us, 0.86±0.06 m.y., is also in good agreement with the date of the Jaramillo event.

Glass and co-workers[2,8] have already suggested that the phenomenon responsible for the Australasian field may also have caused the Matuyama–Brunhes reversal, and similar arguments can be put forward to account for the Jaramillo event. These hypotheses of course do not say whether the mass of microtektites and tektites came from outside the Earth, or whether a comet or meteorite struck the Earth and scooped out material which then fell back to the Earth as a spray of tektites and microtektites. But it seems plausible from what is known of the cause of the geomagnetic field, and its instability, that such comparatively small disturbances could reverse the field direction.

In this context we wish to suggest a possible explanation of the sudden changes in marine fauna which seem to be associated with geomagnetic reversals. Some marine microorganisms become extinct, and others suddenly appear, in sediments at reversal levels[15]. If tektites are produced by cometary impacts, as Urey has suggested, then the gases such as frozen ammonia and methane contained in cometary nuclei might have appreciable ecological effects when rapidly introduced into the atmosphere and oceans. Some organisms thrive on methane, for example, while others do not; ammonia, which would dissolve rapidly in sea water, would have similar effects. Large explosions of methane–air mixtures, sparked by atmospheric lightning, may also have occurred. There needs to be a quantitative examination of whether cometary impact could cause reversals and produce faunal changes over large areas of the globe.

The cometary hypothesis also avoids the difficulties which have appeared in the lunar-origin hypothesis of tektites and microtektites now that lunar samples are available. Although minute glassy particles that look like microtektites have been found on the Moon[16,17], the chemical compositions are not similar[18]. The resemblance of the exceptional lunar sample 12013 (ref. 17) with two untypical Java tektites[18,19] is not firm evidence of affinity.

Finally, we wish to describe in more detail some of the precautions taken in our age measurements of the Ivory Coast microtektites. In determining the age of a material by the fission track method it is important to ensure that no thermal effect that would lower the measured age has occurred in nature. Heating results in the fading of tracks and in shrinkage of the etch-pit diameters of the surviving tracks[20,21]; it can be corrected for by comparing the diameters of the etch pits for natural and freshly induced tracks. These correction factors are obtained by a series of isothermal annealing experiments, and Fig. 2 shows a comparison of the natural and induced fission etch-pit diameters of Ivory Coast microtektites. The *t* test shows no significant difference; so the observed age may be regarded as the true age. The same applies to the Ivory Coast tektite (natural diameter: 4.65±1.24 µm; induced diameter: 4.67±1.09 µm).

The uranium content in p.p.m. by weight is given by

$$C = 9.445 \times 10^7 \rho_i/(D.R_{ap}\cos^2\theta_c.g.f(t).F) \qquad (2)$$

where D is the density of the material (g cm^{-3}), R_{ap} is the apparent range of an average fission fragment, θ_c is known as the critical angle of etching, g is a geometrical factor and $f(t)$ the prolonged etching factor for the material (our unpublished work). $D.R_{ap}$ is taken to be the same as found for a reference glass (U-2) with a known amount of uranium (that is, 2.41 g cm^{-3} × 7.6 µm), and $g=1$ (for 4 π geometry, the contribution of fission fragments from both sides of each etched surface being taken into account). ρ_2 and F are as in equation (1).

The critical angle[3] is defined as the minimum track inclination with the surface that leads to successful etching. We have determined this angle for microtektites using a novel comparison method. A ^{252}Cf spontaneous-fission source was used to bombard first an indochinite and then a microtektite for equal lengths of time under identical ("2π") geometry. The tektite and the microtektite were then etched in identical conditions (30 s at 21° C in 40 volumes % HF). The number of fission-fragment etch pits per unit area can be shown to be in the ratio $(1-\sin\theta_{c,1})/(1-\sin\theta_{c,2})$, where the subscripts 1 and 2 refer to the two materials being compared. The critical angle for the microtektite is found to be 24° 13′, which is in good agreement with that for the indochinite (that is, 25° 21′, determined in a similar manner by comparison with a glass slide of known critical angle). The error in each case is ±30′. This agreement in itself is a confirmation of the chemical similarity of tektites and microtektites. (The critical angle for the soda–lime glass slide, in contrast, is 35° 30′.)

The prolonged etching factor $f(t)$ results from the fact that, as the etching process progresses and new surfaces are revealed, fresh fission tracks are continually encountered and developed. This growth in numbers is not compensated by a corresponding obliteration of tracks which had begun to be developed earlier and whose actual end-points may have been reached in the meantime, for they continue to yield recognizable, although progressively shallower (less acute) etch pits. The function $f(t)$ is defined as the ratio ρ_t/ρ_0, where ρ_t is the track density observed on etching the sample for time t, and ρ_0 is the value for zero etching-time (the density of "etchable tracks" in any surface), obtained by extrapolating the experimental growth curve backwards to $t=0$. Neglecting the factor $f(t)$ in equation (2) would result in an overestimate of the true value of uranium content (whereas in the case of age determination this factor cancels out if both the natural and the irradiated samples are etched in identical conditions).

Fig. 3 shows the growth of observed etch-pit densities with the duration of etching for an irradiated microtektite, an indochinite, and the (soda–lime) reference glass U-2 (all etched in 40 volumes % HF at 21° C—$f(t)$ is temperature-dependent). The value of $f(t)$ for the microtektite is found to be 1.16 for an etching time $t=30$ s. Again, the fact that the growth curves for microtektites and tektites are nearly parallel (whereas that for soda–lime glass is much steeper) demonstrates their kinship.

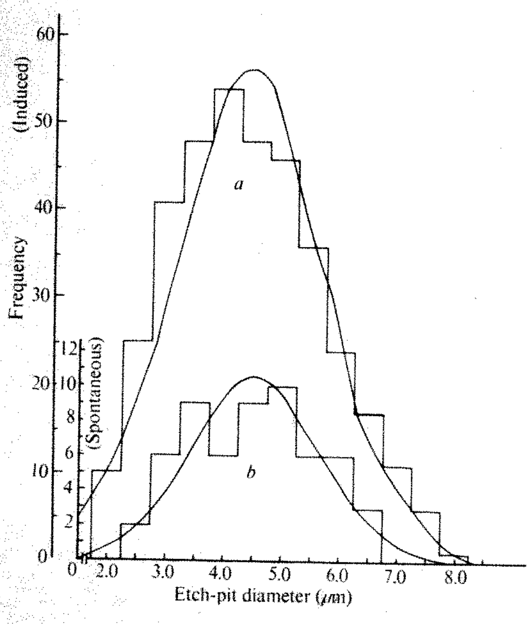


Fig. 2 Comparison of the etch-pit diameters of spontaneous and induced fission tracks in Ivory Coast microtektites etched for 30 s in 40 volumes % HF at 21° C. In the case of elliptical pits, the major axis has been taken instead of the diameter. A Gaussian has been fitted to each histogram. The absence of a significant difference between the two distributions indicates that no thermal lowering of age has occurred in the natural microtektites. *a*, Induced fission (4.41±1.30) μm, 367 tracks; *b*, spontaneous fission (4.52±1.09) μm, 57 tracks.

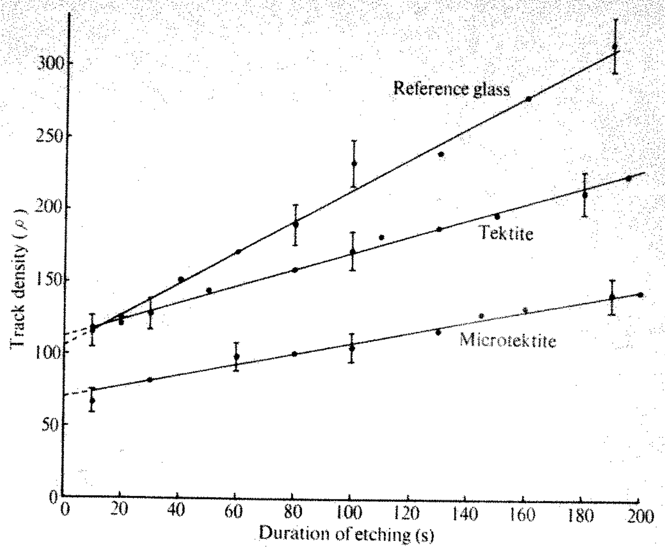


Fig. 3 Prolonged etching factor $f(t)$. The diagram shows the cumulative growth in the number of fission tracks (observed over an arbitrarily chosen area of 0.15 mm^2 in each case) as a function of the duration of etching. Irradiated sections of a microtektite, a tektite and (soda–lime) reference glass have been etched in identical conditions (30 s in 40 volumes % HF at 21° C). The value of $f(t)$ is defined as the ratio of the track density ρ at time t to that at time $t=0$ (see text for discussion); it is found to be 1.16 for the microtektite, 1.15 for the tektite, and 1.41 for reference glass (at $t=30$ s in the etching conditions used). The representative error bars shown indicate (Poisson) standard deviations of actual numbers observed.

The mean value of uranium content, based on thirteen irradiated microtektites, is found to be 0.43 p.p.m. by weight (corresponding to 0.81×10^4 tracks cm^{-2} induced by a fluence of 1×10^{15} neutrons cm^{-2}), with a range of 0.37 to 0.51 p.p.m. between individual samples. This may be compared with an average of 0.23 p.p.m. (and a range of 0.12 to 0.48 p.p.m.) calculated from the results of Gentner *et al.*[5]. Incidentally, our determination of the Ivory Coast tektite uranium content, that is, 1.3 p.p.m., compares well with the value reported by Rybach and Adams[22], namely, 1.10 p.p.m. (mean of twenty samples).

In conclusion, the fact that the ages of the Ivory Coast and Australasian microtektites and tektites seem to coincide with the dates of the last two geomagnetic reversals raises the important question of a causal relationship. If a cometary impact with the Earth resulted in the production of tektites and microtektites, and in the reversal of the geomagnetic field, an interesting corollary is the possible effect of the released gases on the ecological balance on land and sea areas.

We thank the Global Ocean Floor Analysis and Research Group (USNOO), the Science Research Council and the staff of HERALD reactor at AWRE, Aldermaston, and Dr D. Storzer, Dr G. A. Wagner, Professors Sir E. C. Bullard, H. C. Urey and J. H. Fremlin, Dr M. H. Dodson and Dr C. R. Sladden. One of us (H. A. K.) thanks the Pakistan AEC and the Colombo Plan authorities for support.

S. A. DURRANI
H. A. KHAN

Department of Physics,
University of Birmingham,
Birmingham B15 2TT

Received May 10; revised June 25, 1971.

[1] Glass, B., *Nature*, **214**, 372 (1967).
[2] Cassidy, W. A., Glass, B., and Heezen, B. C., *J. Geophys. Res.*, **74**, 1008 (1969).
[3] Fleischer, R. L., Price, P. B., and Walker, R. M., *Ann. Rev. Nucl. Sci.*, **15**, 1 (1965).
[4] Durrani, S. A., and Hancock, D. A., *Earth Planet. Sci. Lett.*, **8**, 157 (1970).

[5] Gentner, W., Glass, B. P., Storzer, D., and Wagner, G. A., *Science*, **168**, 359 (1970).
[6] Gentner, W., Storzer, D., and Wagner, G. A., *Naturwissenschaften*, **56**, 255 (1969).
[7] Glass, B. P., *Geochim. Cosmochim. Acta*, **33**, 1135 (1969).
[8] Glass, B., and Heezen, B. C., *Nature*, **214**, 372 (1967).
[9] Bullard, E. C., *Phil. Trans. Roy. Soc.*, **A263**, 481 (1968).
[10] Cox, A., Doell, R. R., and Dalrymple, G. B., *Nature*, **198**, 1049 (1963).
[11] Ninkovich, D., Opdyke, N., Heezen, B. C., and Foster, J. H., *Earth Planet. Sci. Lett.*, **1**, 476 (1966).
[12] Cox, A., *Science*, **163**, 237 (1969).
[13] Cox, A., and Dalrymple, G. B., *J. Geophys. Res.*, **72**, 2603 (1967).
[14] Ericson, D. B., and Wollin, G., *Science*, **162**, 1227 (1968).
[15] Ericson, D. B., Ewing, M., and Wollin, G., *Science*, **139**, 727 (1963).
[16] Lunar Sample Preliminary Examination Team, *Science*, **165**, 1211 (1969).
[17] Lunar Sample Preliminary Examination Team, *Science*, **167**, 1325 (1970).
[18] Durrani, S. A., *Phys. Earth Planet. Interiors*, **4**, 251 (1971).
[19] O'Keefe, J. A., *Science*, **168**, 1209 (1970).
[20] Storzer, D., and Wagner, G. A., *Earth Planet. Sci. Lett.*, **5**, 463 (1969).
[21] Durrani, S. A., and Khan, H. A., *Earth Planet. Sci. Lett.*, **9**, 431 (1970).
[22] Rybach, L., and Adams, J. A. S., *Geochim. Cosmochim. Acta*, **33**, 1101 (1969).

Measurements of the Gegenschein from Space

THE zodiacal light, which is produced by light scattered from small interplanetary particles, gradually declines in brightness with increasing angle of elongation, ε, from the Sun. As the anti-solar point (ε = 180°) is approached, the surface brightness again increases slightly to produce the counter-glow or gegenschein. This diffuse feature can be best observed against the sky background when it lies outside the region of the Milky Way. Fig. 1 depicts the sky background brightness at the anti-solar point as a function of time throughout the year. The integrated light from stars predominates during the two Milky Way traverses. Observations of the gegenschein are made best away from the Milky Way in February, March and April, or in August, September and October.

Ground-based observations are weakened by atmospheric extinction and diluted by the upper atmosphere airglow and light scattered in the Earth's lower atmosphere. Measurements of the gegenschein from above the Earth's atmosphere avoid the difficulties of these atmospheric effects. Accordingly, an experiment was designed for the wheel of an orbiting solar observatory (OSO–6) to measure the brightness and polarization of the sky. The photometer–polarimeter, which was built by Ball Brothers Research Corporation includes a Sun-shielded objective, a collimator, quartz rotators, a quarter-wave plate, a double-cut prism, interference filters and a field lens, all bringing the light beam through a 2° diameter field stop to an EMR 641 E-type photomultiplier. Although the instrument was designed to measure polarization, we are concerned here only with photometric data at 5000 Å. The equipment is operable only during satellite daytime and scattered light enters the photometer when the Sun (or the sunlit Earth) is at an elongation 90° or less with respect to the line of sight. The gegenschein region, however, is free of photometric contamination from this effect.

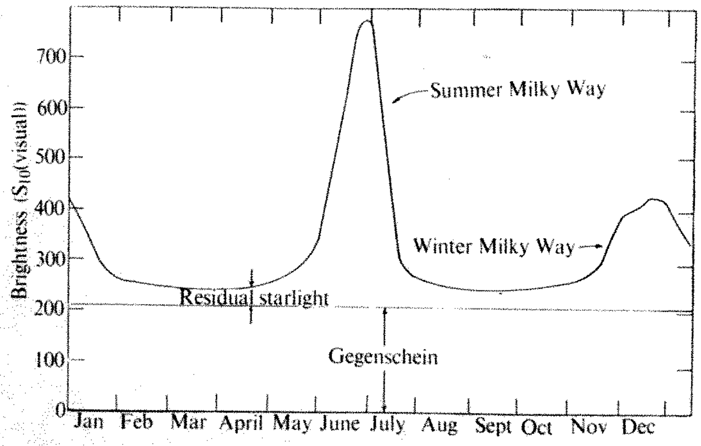


Fig. 1 Sky brightness at the anti-solar point as a function of time as it would appear to an observer above the Earth's atmosphere. Ordinates give surface brightness in units of tenth magnitude stars per square degree, S_{10} (visual). The contributions of the gegenschein are assumed constant, but the starlight (plus galactic light) contributions exhibit maxima during Milky Way traverses.

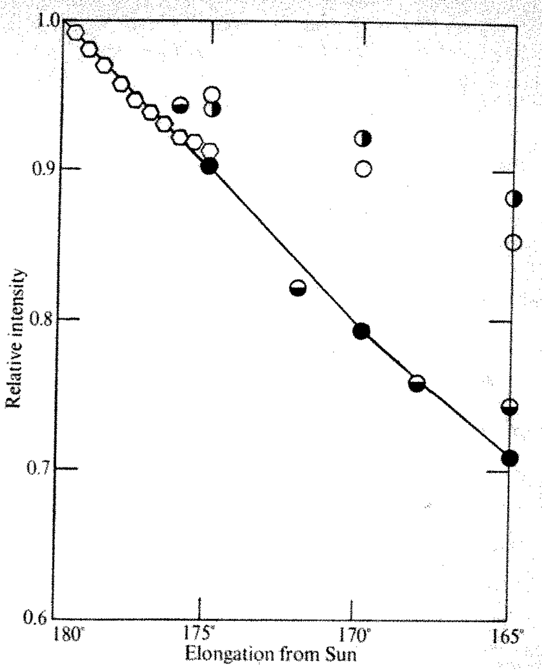


Fig. 2 Relative intensity distribution in the gegenschein. Comparison of the brightness distribution of the gegenschein as measured by ground observers with OSO-6 measurements (●) at 5000 Å. ○, ref. 2; ○, ref. 4; ◑, ref. 3; ◒, ref. 1.

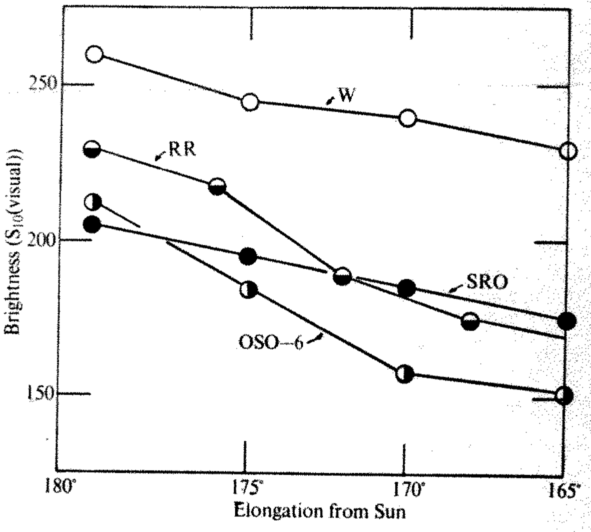


Fig. 3 Absolute gegenschein brightness comparison of the absolute surface brightness in the gegenschein from OSO-6 observations and ground-based observers (W: ref. 3; RR: ref. 1; SRO: ref. 4). Preliminary calibration of the space observations.

Comparison of measurements made towards the same point in the celestial sphere at two different points in the orbit, plus comparison of the space-based and ground-based observations of the Milky Way, indicate that the instrument is operating well and yielding trustworthy information.

In Fig. 2 we compare the relative brightness of space measurements of the gegenschein within 15° of the anti-Sun position with some corresponding ground-based results. The present space measurements are in accord with the data of Roach and Rees[1] and the recent studies of Roosen[2] in suggesting a fairly steep photometric gradient from the anti-Sun position. Weinberg[3] and Smith, Roach and Owen[4] find a less steep gradient. Fig. 3 shows a comparison of the absolute brightness of the gegenschein as measured from space in comparison with several published ground-based observations. The space observations are based on a preliminary absolute calibration.

As further material becomes available we hope to extend the discussion to ±25° from the anti-solar position and improve the absolute calibration. Also, the photometry will be extended to other colours (4000 Å and 6100 Å) and the polarization data evaluated. The exact variation of the polarization at elongations between 150° and 180° is of special interest in assessing the nature of the scattering particles.

We thank R. Wilson, H. Zimmerman, and Wm. Dagle of Ball Brothers Research Corporation for the assembly of the polarimeter–photometer. This work was supported by a NASA grant to Rutgers University.

A. ROUY*
B. CARROLL

Rutgers University,
Newark, New Jersey

L. H. ALLER

University of California,
Los Angeles

F. E. ROACH
L. SMITH

Battelle Northwest Laboratories
and Rutgers University

Received June 21, 1971.

* Deceased March 2, 1970.

[1] Roach, F. E., and Rees, M. H., *Airglow and Aurorae* (Pergamon, London, 1956).
[2] Roosen, R. G., *Icarus*, **13**, 184 (1970).
[3] Weinberg, J. L., *Ann. d'Astrophys.*, **27**, 218 (1964).
[4] Smith, L., Roach, F. E., and Owen, R. W., *Planet. Space Sci.*, **13**, 207 (1965).

Martian Blue Haze Clearings and Flash Phenomena

THE relative lack of contrast in the blue ($\lambda < 0.44$ μm) in examinations of the surface markings of Mars has long been known, but occasionally and unpredictably, detailed blue structure can be seen briefly during blue haze clearings. There is some controversy about these phenomena[1,2], although in our opinion they are well established. A blue obscuring haze may be attributed to scattering of light by suspended particles[3–7] of diameter less than 0.4 μm, in the martian atmosphere so that blue haze clearings would result whenever meteorological conditions caused a partial precipitation of particles, but the mechanisms for the removal remain as obscure. We propose a simple meteorological mechanism which is consistent with available data from martian oppositions and flash observations..

There is evidence[8] of significant water on Mars. Although the occurrence of liquid water would be a transitory, nonequilibrium possibility, Shorn[8] speculates that the triple point pressure may be exceeded occasionally. Fine martian atmospheric dust is assumed to influence the haze. Essentially, our clearing mechanism is the removal of available atmospheric dust by the condensation of water vapour on dust nuclei during highest humidity, resulting in precipitation, clearing the air[9,10].

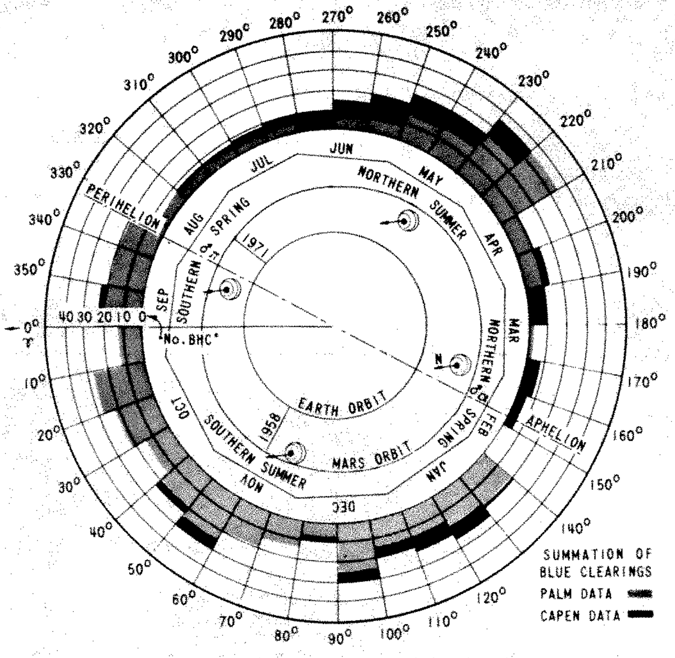


Fig. 1 Histogram chart of martian blue haze clearings (BHC), plotted against martian heliocentric longitude. All seasons shown are martian but months are terrestrial. *The scale shows the number of BHC per 10° sector. The Palm data are opposition data; Capen data are general data for the period October 1966 through March 1968. Earth months correlate with opposition (Palm) data. Arrows on Mars indicate approximate North Pole orientation. It must be kept in mind that this histogram is, in part, a function of the positions of the terminal points of the interval over which one averages in presenting the discrete histogram representation of what is really a smooth curve.

Spectra of water vapour on Mars during 1968–69, taken as Mars was rapidly approaching the Earth[8], show a shift of the martian H_2O lines relative to the telluric water vapour signature and show much more water on Mars than had recently been found[11,12]. Humidity can exceed 50% over large areas, which is conducive to occasional precipitation on martian aerosols and changes the atmospheric conditions radically, explaining even abrupt blue haze clearings. Possibly fogs settle on Mars, and rain and snow may originate from reported, heavier, grey and yellow clouds[10].

Consider the orbits of Mars and Earth as being fixed in space (Fig. 1). All months referenced are Earth months. The orbital perihelion of Mars lies in the August sector and the aphelion in the February sector. Mars may, of course, be anywhere in its orbit during August or February, but Earth's near circular orbit provides for closest oppositions around August or September, as for 1971. At close oppositions, Mars is in its northern hemisphere autumn. For oppositions during February and March, Mars is in the middle of the northern hemisphere spring. These are important considerations because most of the data on both blue haze clearings and flares[13] have been taken during oppositions.

With one polar cap partially or completely dissipated during either martian summer, the humidity will then be highest and conducive to possible precipitation. Blue haze clearings (opposition data) have predominated during martian summers, (both northern and southern) which are favourable seasons for precipitation.

Earth weather probably prejudiced blue haze data, influencing the deficiency during February and March as shown in Fig. 1. Conversely in northern skies it is best to look at Mars when the cool air contributes to greater clarity even at larger distance.

For an April opposition, Mars is not far from its northern summer solstice, favouring increased precipitation in its northern hemisphere, but if this is indeed a factor it implies that opposition blue haze clearings in March and February should be more frequent than are indicated. Another effect, however, is the occurrence of aphelion in the late February sector implying a colder planet overall, with lower humidity due to increased condensation of volatiles. Further correlations of locations of clearings with seasons are indicated.

During our summer months of June, July and August the monthly integrated averages from opposition data[14] of blue haze clearings have been almost a constant. Capen's data[15], however, show a progressive decrease for this period. This total number (approximately 100) has also been low. Most of these data were obtained during oppositions when the warming martian southern hemisphere was emerging from winter, thermal gradients were increasing, with winds carrying dust. The humidity may still be too low for significant precipitation, producing regional atmospheric hazes while northern terrestrial weather hinders observations.

From September to February, the average opposition blue haze clearings per unit time initially increases and is then approximately constant.

The centre of the season of more frequent blue clearings lags the martian southern solstice by about three terrestrial weeks. The duration of this clearing season, however, is almost triple that of the season which centres 3 weeks after the northern summer solstice. This, too, is explicable by increased temperatures over more martian surfaces. This follows from the fact that in the vicinity of northern summer solstice the polar axis inclination favours higher polar surface temperatures, but is thermally opposed by being in the orbital aphelion, whereas in the vicinity of southern summer solstice, polar inclination and orbital eccentricity coordinate to produce higher temperatures, possible precipitation and clearings. Furthermore, while the line of solstices is near to the line of apsides it misses coincidence with it by a few months, causing a long warm period even though the southern summer is shortest. Solar power to Mars is over 40% greater at perihelion than at aphelion because a longer season of more frequent clearings in the austral summer part of the martian orbit is anticipated and supported by the available data (Fig. 1). Apparently orbital eccentricity has greater influence than the inclination of the polar axis.

Recent observations[15] show that, excepting early February, blue clearings have occurred throughout the year. Since Mars presents its smallest image to astronomers during February, atmospheric blurring could easily mask detail. Although a blue deficiency of Mars was observed from Earth during NASA Mariners 6 and 7, none was detected in photographs from the fly-bys with red, blue, and green filters[16]. This indicates a terrestrial, faint image, or space contained effect. The high contrast and definition of the Mariner images at close range may have made any obscuration undetectable. Qualitative martian colour contrasts could hardly be established from these unprecedented series of photographs. Opik[6] feels that Leighton's conclusion of non-existence of the blue haze based on Mariner photographs (which show limb haze) is not justified[10]. Blue light photographs taken at Flagstaff, Arizona and Bloemfontein, South Africa, with equivalent refractors, Kodak film and developers consistently show more blue details in the Flagstaff photographs[10]. Flashes of light have been reported on Mars[16,17] which could, it seems, be possible ground water since no volcanic activity is known.

With possible precipitation on Mars, a new perspective is gained in the Mars blue haze clearings and known soil erosion. White and blue clouds, possibly composed of water, have long been seen which are conducive to rapid atmospheric clearing. Evidence indicates that Earth's summer haze, with appropriate martian conditions could possibly block much blue light from all, or part, of Mars.

There are four factors involved; the inclination of the martian axis of rotation, the eccentricity and orientation of the martian line of apsides, the occurrence of precipitation and dust and terrestrial seeing conditions. These can be combined to explain the distribution of reported opposition blue haze clearings. Photographs or vidicon observations from an orbiting (or lunar based) telescope, or stratoscope, could test our hypothesis by viewing Mars simultaneously from Earth and Earth orbit at times when the surface was obscured in the blue from the ground. Similar tests would be made early in a February when no clearings have been reported. These observations should show how terrestrial weather influences blue haze clearings.

We also speculate that numerous light flashes reported on Mars, coinciding primarily with the respective summer hemispheres at the times of observation, may be reflexions from temporary surface waters formed by precipitation.

E. H. WELLS
D. P. HALE

Space Sciences Laboratory,
Marshall Space Flight Center,
Alabama 35812

Received April 7; revised June 10, 1971.

[1] Leighton *et al.*, *Science*, **166**, 49 (1969).
[2] Opik, E. I., *Irish Astron. J.*, **9**, 138 (1970).
[3] *Planets and Satellites* (edit. by Kuiper, G. P., and Middlehurst, B. M.), 383, 434, 554, 555, 557, 562 and 596 (1961).
[4] Hess, S. L., *Meteorology*, **7**, 1 (1950).
[5] Schatzman, E., *CR Acad. Sci.*, **232**, 652 (1951).
[6] Opik, E. I., *J. Geophys. Res.*, **65**, 3057 (1960).
[7] Van der Hulst, *Light Scattering by Small Particles*, 443 (Wiley, New York, 1962).
[8] Schorn, R. A., *et al.*, *Icarus*, **11**, 286 (1969).
[9] Kviv, Z., *Bull. Astron. Inst. Czechoslovakia*, **2**, 1 (1961).
[10] Slipher, E. C., *Mars*, 30 (Sky Publishing Company, 1962).
[11] Spinrad, H., Munch, G., and Kaplan, L. D., *Astrophys. J.*, **137**, 1319 (1963).
[12] Kaplan, L. D., Munch, G., and Spinrad, H., *Astrophys. J.*, **139**, 1 (1964).
[13] Newburn, R., *Mars Scientific Model I*, JPL report 606-1, 4.1, 11 (1968).
[14] Palm, A., and Basu, B., *Icarus*, **4**, 116 (1965).
[15] Capen, C. F., *Icarus*, **12**, 118 (1970).
[16] Saheki, T., *Sky and Telescope*, 144 (February 1955).
[17] Pskovskiy, Yurii Pavlovich, Appendix in NASA translation of ref. 8.
[18] Katterfel'd, G. N., NASA Technical Translation F-140 from *Priroda*, **8**, 103 (1966).

Permeabilities of Precambrian Onverwacht Cherts and Other Low Permeability Rocks

QUANTITATIVE estimates of rock permeability are of importance in any branch of science or engineering where flow of fluids through rocks is considered. Although there is a large amount of data available for rocks with relatively high permeabilities (exceeding 1 mdarcy), very few data are available for rocks such as shales, cherts, dense carbonates and evaporites which may have permeabilities of less than 1 mdarcy. Permeability measurements on Precambrian cherts help to interpret the results of organic geochemical studies made on these ancient rocks.

The search for geological evidence about the origin and evolution of life on Earth has led to detailed searches for morphological and molecular fossils in Early Precambrian sedimentary rocks, most of which have extremely low permeabilities[1,2]. The oldest rocks considered so far are the Onverwacht Group of the Swaziland Sequence exposed in the Eastern Transvaal of South Africa. The age of this group is at least 3.2×10^9 yr. Fossil-like microstructures as well as polymeric organic matter (commonly called kerogen) have been found in carbonaceous cherts of the Onverwacht Group[3], but the biogenic nature of the microstructures is uncertain[4]. The presence of several kinds of molecular fossils in these rocks (*n*-alkanes, isoprenoid hydrocarbons, fatty acids, porphyrins, amino-acids) has also been reported[5–7]. But the significance of these microstructures and molecular fossils for the origin and evolution of life on Earth is still in question.

Nagy[8] has recently determined the permeability and porosity of a single sample of Onverwacht chert to be 5.7×10^{-7} mdarcy and 0.5% respectively. He showed that even at this low permeability there is the possibility of contamination of these rocks with organic compounds dissolved in fluids which have flowed through for several thousand million years. This finding has wide implications for the significance of organic compounds found in small concentrations in low permeability rocks of all geological ages.

In January 1968 we began to develop a novel liquid permeameter for measuring very low permeabilities in rocks. Permeabilities and porosities of four samples of chert from four different formations of the Onverwacht Group were measured. For comparison the same measurements were made on a sample of Precambrian Keewatin chert from Canada, a sample of Precambrian Bitter Springs limestone from Australia, and samples of Castile gypsum, Rustler dolomite, and Bone Springs limestone from west Texas.

Permeabilities were determined by a new technique described in detail elsewhere (S. K. Sanyal, R. M. Pirnie, III, G. O. Chen, and S. S. Marsden, jun., to be published). The sample is held in a Hassler sleeve which can be maintained at confining pressures as high as 10,000 pounds inch^{-2} with a hydraulic pump. Upstream pressures are maintained by a "thermal pump" which utilizes the thermal expansion of a liquid to create pressures $\leqslant 1{,}000$ pounds inch^{-2}. Pressures are measured with a low displacement, diaphragm-type transducer and permeabilities deduced indirectly from the pressure decline over a period of time.

For the permeability measurements reported here, hydraulic oil was used in both the hydraulic and thermal pumps by contrast with Nagy's choice[8]—a 2,000 p.p.m. NaCl brine. Theoretically permeability of a rock is independent of the flowing fluid but in practice this independence is not strictly valid[9]. Extremely low permeability rocks such as those studied have a higher permeability to air than to water[10]. This difference may be due to dehydration of minerals in contact with flowing air (causing an increase in permeability), or the swelling of clay minerals in contact with flowing water which would cause a decrease in permeability. The second effect can be minimized by use of strong brine or a non-interacting fluid such as hydraulic oil.

Permeability measurements were made at confining pressures between 1,500 and 1,800 pounds inch^{-2}. An increase in confining pressure on a rock generally reduces its permeability[9,11,12] and this effect is particularly evident for rocks of very low permeability[13]. Nagy[8] did not specify the confining pressures used and this to some extent limits a comparison of the results. Permeabilities were measured at various orientations to bedding because in the presence of small scale structures (laminations, banding, fractures, solution channels and so on), rock permeability depends strongly on the directions in which the fluid flows.

Porosity, φ, of a sample was calculated from its bulk volume, V_B, its dry weight, W_0, and its weight after it was saturated with oil under vacuum, W_s, by the relation

$$\varphi = \frac{W_s - W_0}{\rho_0 V_B}$$

where ρ_0 is the density of the oil.

Permeability and porosity values of the samples studied as well as directions of permeability measurements and confining pressures are listed in Table 1. For Onverwacht cherts, permeabilities vary from a value which is too small to be measured by our technique (that is less than 10^{-6} mdarcy) to 2.09×10^{-2} mdarcy, and porosities vary from 0.03 to 0.72%. The chert sample from the Hooggenoeg Formation had a very low permeability which probably approaches the value obtained by Nagy[8] (5.7×10^{-7} mdarcy). The other Onverwacht chert samples had permeabilities greater by several orders of magnitude. The Keewatin chert from Canada had a permeability of 1.1×10^{-4} mdarcy which is similar to the chert permeability given by Davis[14] (1.9×10^{-4} mdarcy). Permeabilities to air of two Precambrian cherts have been obtained by Smith *et al.*[15]; Gunflint chert from Canada had air permeabilities of 1.9×10^{-1} and 2.0×10^{-2} mdarcy and porosities of 0.44 and 0.55%, and Bitter Springs chert had values of 2.2×10^{-1} and 1.7×10^{-1} mdarcy and 0.62 and 1.15%. Thomas *et al.*[10] have shown that air permeabilities are generally slightly higher than liquid permeabilities in tight rocks. Therefore Gunflint and Bitter Springs cherts seem to be about as permeable as the most permeable Onverwacht cherts. Bitter Springs limestone, with a liquid permeability of 4.0×10^{-2} mdarcy and a porosity of 0.24%, is about as permeable and porous as samples of chert from the same formation.

With one exception, too few measurements were obtained to determine whether there was any relationship between perme-

Table 1 Permeability and Porosity Measurements

Sample No.	Name	Age	Porosity (%)	Permeability (mdarcy)	Direction of permeability	Confining pressure (pound inch^{-2})
Onverwacht cherts						
5–6	Theespruit Formation	Precambrian	0.72	2.09×10^{-2}	Perpendicular to laminations	1,500
5–10	Hooggenoeg Formation	Precambrian	0.03	$< 10^{-6}$	No specific orientation	1,700
5–18	Kromberg Formation	Precambrian	0.12	1.16×10^{-3}	Parallel to laminations	1,600
5–23	Swartkoppic Formation	Precambrian	0.44	1.23×10^{-2}	50° to laminations	1,600
Other rocks						
20	Keewatin chert	Precambrian	0.10	1.1×10^{-4}	No specific orientation	1,600
8–9	Bitter Springs limestone	Precambrian	0.24	4.05×10^{-2}	No specific orientation	1,400
3–2–1 (A)	Castile gypsum	Permian	4.80	1.3×10^{-4}	Perpendicular to laminations	1,600
3–2–2 (A)	Castile gypsum	Permian	3.42	1.6×10^{-4}	Perpendicular to laminations	1,500
3–2–1 (B)	Castile gypsum	Permian	6.43	9.17×10^{-2}	Parallel to laminations	1,800
3–2–2 (B)	Castile gypsum	Permian	4.60	3.10×10^{-2}	Parallel to laminations	1,600
3–1–1	Rustler dolomite	Permian	1.37	4.39×10^{-2}	Parallel to laminations	1,500
3–1–2	Rustler dolomite	Permian	0.41	9.35×10^{-2}	Parallel to laminations	1,500
3–4	Bone Springs limestone	Permian	0.44	4.90×10^{-4}	No specific orientation	1,400

abilities and direction of flow within the rocks. Castile gypsum had permeabilities two to three orders of magnitude higher parallel to the laminations than perpendicular to the laminations. Three out of four samples of Onverwacht chert had measured permeabilities much higher than the single value reported by Nagy[8]. The relationship between measured permeabilities and true permeabilities during geological time is not known but cementation, compaction and metamorphism may have caused significant permeability reduction during the geological past. On the other hand, earth movements may have increased the effective *in situ* permeabilities of these rocks by inducing fracturing which may not be evident in small laboratory samples.

It is quite likely that fluids have been able to flow through these rocks by means of both intergranular and fracture channels. Our data suggest that flow of fluids in these rocks may have been even higher than estimated by Nagy[8], and consequently many of the Onverwacht cherts may have been host rocks rather than source rocks for some of the organic compounds they now contain.

S. K. SANYAL

Department of Petroleum Engineering,
Stanford University,
Stanford, California 94305

K. A. KVENVOLDEN

Exobiology Division,
NASA-Ames Research Center,
Moffett Field, California 94035

S. S. MARSDEN, JUN.

Department of Petroleum Engineering,
Stanford University,
Stanford, California 94305

Received June 15, 1971.

[1] Barghoorn, E. S., and Schopf, J. W., *Science*, **152**, 758 (1966).
[2] Schopf, J. W., and Barghoorn, E. S., *Science*, **156**, 508 (1967).
[3] Engel, A. E. J., Nagy, B., Nagy, L. A., Engel, C. G., Kremp, G. O. W., and Drew, C. M., *Science*, **161**, 1005 (1968).
[4] Nagy, B., and Nagy, L. A., *Nature*, **223**, 1226 (1969).
[5] MacLeod, W. D., jun. *J. Gas Chromatog.*, **6**, 591 (1968).
[6] Han, J., and Calvin, M., *Nature*, **224**, 576 (1969).
[7] Kvenvolden, K. A., and Hodgson, G. W., *Geochim. Cosmochim. Acta*, **33**, 1195 (1969).
[8] Nagy, B., *Geochim. Cosmochim. Acta*, **34**, 525 (1970).
[9] Afinogenov, Yu. S., *Sniiggims, Novosibirsk*, **6**, 34 (1969).
[10] Thomas, L. K., Katz, D. L., and Tek, M. R., *Trans. AIME*, **243**, II-174 (1968).
[11] McLatchie, A. S., Hemstock, R. A., and Young, J. W., *Trans. AIME*, **213**, 386 (1958).
[12] Fatt, I., and Davis, D. H., *Trans. AIME*, **195**, 329 (1952).
[13] Vairogs, J., Hearn, C. L., Dareing, D. W., and Rhoades, V. W., *Proc. 45th Annual Fall Meeting of Soc. Petrol. Engineers of AIME* (1970).
[14] Davis, S. N., *Flow Through Porous Media*, 53 (Academic Press, New York, 1969).
[15] Smith, J. W., Schopf, J. W., and Kaplan, I. R., *Geochim. Cosmochim. Acta*, **34**, 659 (1970).

Conversion of Oleic Acid to Saturated Fatty Acids in Severn Estuary Sediments

THE upper, biologically active, layers of Recent aquatic sediments contain considerable quantities of saturated and unsaturated long chain fatty acids[1-3], presumably from the living and dead organisms found there[4,5]. Although fatty acids are major constituents of organisms, in the upper layers of sediments, their concentration is of the same order of magnitude as that of sterols[6,7], usually minor constituents of organisms. The ratio of fatty acids to total organic carbon in marine sediment is at least ten times lower than in plankton[1], again indicating that fatty acids are subject to rapid changes in the sediment. Palmitic acid ($C_{16:0}$) is the most abundant saturated fatty acid in organisms, and oleic acid ($C_{18:1}$) the most widely distributed and plentiful of all fatty acids[4,5]. The lower, biologically less active, layers of sediments, contain little or no unsaturated fatty acid; palmitic acid is the most abundant of the fatty acids[1,2,8]. This difference between the upper and lower layers is unlikely to be the consequence simply of hydrogenation of the carbon–carbon double bands, for in this case the proportion of stearic acid ($C_{18:0}$) in the lower layers and the concentrations of saturated fatty acids would be larger. These reactions could occur by chemical and/or biological means, perhaps through the large number of anaerobes present in upper layers of sediments[9,10].

Our studies were designed to discover the fate of oleic acid in an aquatic sediment. The basis of our experiments was to add 9,10-^{3}H, 1-^{14}C-oleic acid to the upper layers of the Recent sediments of the Severn Estuary and then to isolate the labelled products of short term diagenesis. The ratios of ^{3}H to ^{14}C measured for the various saturated fatty acids obtained are compared with the $^{3}H/^{14}C$ ratio for the original oleic acid. We hope to extend the methods described here to other substances of geochemical and environmental chemical interest.

If oleic acid present in sediments is converted into saturated acids (of chain length C_{12} and above), two biochemical pathways could be operative: (1) the simple hydrogenation of unsaturated carbon–carbon bonds, followed by loss of C_2 units to give the fatty acids of shorter chain length (the degradative pathway); (2) total breakdown of oleic acid by β-oxidation to give C_2 units (probably as acetyl-CoA) followed by a resynthesis through the fatty acid synthetase complex[11] (the resynthesis pathway).

If the oleic acid introduced is labelled in the carboxylic carbon with ^{14}C and in the 9 or 10 positions with ^{3}H (to give 9,10-^{3}H, 1-^{14}C-oleic acid), the isotopic labels will be incorporated differently into the products of the two pathways outlined here.

In the case of the degradative pathway, which involves the simple loss of C_2 units without total breakdown, the ^{14}C will be lost from the molecule (for all acids except stearic), whereas all the ^{3}H will be retained. Thus, for fatty acids of length C_{16} down to C_{10}, the ratio of ^{3}H to ^{14}C becomes infinitely large, whereas for stearic acid the ratio stays the same as that of the added oleic acid. The ^{14}C removed from the long chain acids will appear in acetyl-CoA (as 1-^{14}C-acetyl-CoA), which will have a ratio of ^{3}H to ^{14}C of 0 to 1.

In the case of the resynthesis pathway, the various enzymatic reactions undergone during β-oxidation and resynthesis from the resulting acetyl-CoA will lead to loss of ^{3}H relative to ^{14}C. The first turn of the β-oxidation spiral will remove all the ^{14}C from the molecule as 1-^{14}C-acetyl-CoA. All the ^{3}H from C-9 will be lost (as $NAD^{3}H$ or $FAD^{3}H$) as this carbon becomes the terminal carboxylic carbon. The fate of the ^{3}H on C-10 may depend on the stereospecificity of the enzymes involved in the reduction step. Enzymes from various sources[12] have been shown to react stereospecifically with fatty acids and it is likely that the β-oxidation enzymes behave similarly, but their stereospecificity is unknown and either all or none of the ^{3}H could be lost from C-10 of the degraded fatty acid. The acetyl-CoA produced through the β-oxidation spiral could therefore contain anything between zero and 0.5 of the amount of the ^{3}H originally present in the oleic acid (all as 2-^{3}H-acetyl-CoA), and all of the ^{14}C. This acetyl-CoA is most likely to be incorporated into long chain fatty acids by the fatty acid synthetase complex which is present in many organisms, including *Escherichia coli* and yeast[11]. All the acetyl-CoA units to be utilized, except one, are first converted to malonyl-CoA before incorporation into the growing fatty acid. The overall effect is to lose approximately 0.67 of the ^{3}H present as 2-^{3}H-acetyl-CoA but to retain all the ^{14}C. In the case of palmitic acid, the overall result should be a ratio of ^{3}H to ^{14}C of 8.34 : 1, based on acetyl-CoA having an

Table 1 Radioactivity in Saturated Long Chain Fatty Acids from Sediments Incubated with 9,10-^{3}H, 1-^{14}C-Oleic Acid (Corrected for Experimental Losses)

	Yields of radioactivity (Ci 10^{-9})									
	A		*B*		*C*		*D*		*E*	
Acid	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Stearic	146.6	5.2	71.2	2.95	12.2	0.41	56.4	2.7	18.9	0.5
Palmitic	167.6	3.8	107.6	4.7	13.7	0.40	70.8	1.07	12.8	0.44
Myristic	80.4	0.7	161.4	3.9	10.3	0.19	46.6	0.27	20.8	0.10
Lauric	74.6	0.2	*	*	4.4	0.03	15.4	0.02	*	*

* Not determined.

100 μCi of 9,10-^{3}H-oleic acid and between 2.5 and 10 μCi of 1-^{14}C-oleic acid were injected into the sediment in each experiment. All the results have been normalized to an initial ratio of ^{3}H to ^{14}C for oleic acid of 20 : 1 (100 μCi of ^{3}H). The conditions of extraction and purification of the acids, and the corrections for losses in these procedures, are described in the text. The conditions of incubation were as follows: *A*, laboratory core, incubated for 6 days at 18–20° C; *B*, laboratory core, incubated for 9 days at 18–20° C; *C*, incubated *in situ* for 7 days, August 1970. *D*, incubated *in situ* for 17 days, September–October 1970; *E*, incubated *in situ* for 38 days, September–October 1970. The statistical errors in the ^{3}H values caused by variation in count rates were in all cases less than ±1%. The ^{14}C values will be subject to larger errors because of the lower count rates, and where the quoted yields are less than 10^{-10} Ci the error may be greater than ±5%.

initial ratio of ^{3}H to ^{14}C of 20 to 1. Experimental verification of this expected ratio comes from the work of Wakil[13] with the synthetase complex from avian liver, and other studies[14] in rat adipose tissue. Overall, the resynthesis pathway, starting from oleic acid with a ratio of ^{3}H to ^{14}C of 20 to 1 should generate palmitic acid with a ratio of ^{3}H to ^{14}C between 0 to 1 and 4.17 to 1, the exact value depending on the ^{3}H loss during β-oxidation. The theoretical ratios for lauric, myristic and palmitic acids formed via the resynthesis pathway are very similar.

Thus the two pathways, degradative and resynthetic, should give end products with very different ratios of ^{3}H to ^{14}C. Except for stearic acid, the degradative pathway causes a total loss of ^{14}C, whereas the resynthesis pathway causes an almost total loss of ^{3}H relative to ^{14}C. We are following only the flow of label and not the total flow of material through each pathway. From the actual ratios of ^{3}H to ^{14}C observed in the saturated fatty acids extracted from the sediments injected with labelled oleic acid, it is possible to calculate what proportion of the label has come through the degradative pathway compared with that arising by resynthesis.

Incubations of oleic acid in aquatic sediments were carried out either *in situ* in the mud of the Severn Estuary (National Grid Reference ST534832) at a depth of 10 cm below the surface, or in cores (15 × 3 cm) which had been removed from areas adjacent to the *in situ* sites, and then transferred to the laboratory. The area of study was chosen near the high water mark on a large expanse of mud flats. A thin layer of grey silt covered a 20 cm layer of black thixotropic mud which contained sulphide and elemental sulphur. The sites were covered by the tide for approximately 4 h in each 24 h, and the temperature varied between 5° and 25° C; the laboratory cores were incubated at 18°–20° C. Labelled oleic acid (from the Radiochemical Centre, Amersham, Buckinghamshire) was prepared by mixing aliquots of 1-^{14}C and 9,10-^{3}H-oleic acid (no additional unlabelled acid was added) to give a ratio of ^{3}H to ^{14}C of between 10 to 1 and 40 to 1. The resulting oleic acid (up to 100 μCi of ^{3}H) was injected (as a 'Tween 80' suspension) so as to cause as little disturbance of the sediment as possible. This represents the addition of a maximum of 0.2 μmol of free oleic acid.

After incubation for up to 38 days, the cores were removed and extracted for free fatty acids using a modification of the Dole extraction procedure[15], or with a 3 to 1 mixture of toluene/methanol. Carrier fatty acids were added (3 μmol for each acid) and the extract methylated with BF_3/methanol[16]. The methyl esters of the saturated acids were then separated from other material by preparative thin-layer chromatography on silver nitrate-impregnated silica gel[17], and finally separated into individual esters by preparative gas chromatography. The peaks corresponding to the even chain length saturated acids were collected directly in 10 ml. aliquots of toluene scintillator (NE 220: Nuclear Enterprises Ltd, Edinburgh) and the samples assayed for radioactivity using a Nuclear Chicago mark I liquid scintillation spectrometer. Quench corrections were applied[18].

Control experiments have shown that the isolation procedures used (Dole extraction, methylation, thin-layer chromatography) result in approximately 70% recovery of U-^{14}C-palmitic acid before preparative gas–liquid chromatographic analysis. Toluene–methanol extraction is less efficient giving 25% recovery of label before gas–liquid chromatography. Similar recoveries have been obtained for 9,10-^{3}H-oleic acid. Table 1 shows the amounts of radioactivity (in nCi) in the saturated fatty acid fraction estimated as being present in sediments after various periods of incubation with 9,10-^{3}H, 1-^{14}C-oleic acid. The figures listed are estimates calculated using these measured efficiencies of recovery of the saturated fatty acids.

The most striking feature of Table 1 is the low conversion of oleic acid into saturated fatty acids observed for all the incubations. The combined long chain saturated fatty acid fraction extracted from the mud was never found to contain more than 3% of the label from the added oleic acid, even though the labelled oleic acid rapidly disappeared (for example in experiment *B* of Table 1, only 5% remained after 9 days incubation). Thus, most of the label of the starting oleic acid does not appear in the free saturated fatty acid component of the mud. The usual fate of oleic acid added to this particular sediment is probably breakdown to acetyl units, which may then be oxidized to CO_2 and H_2O or incorporated into other compounds such as the carbohydrate constituents of cell walls.

Table 2 shows the ratios obtained after various incubation times, with 9,10-^{3}H, 1-^{14}C-oleic acid. There is a definite trend in the ^{3}H to ^{14}C ratio, which increases with decreasing chain length. We have already stated that acids produced by the degradative pathway would have a ratio of ^{3}H to ^{14}C of infinity, whereas those formed by the resynthesis pathway would have ratios of ^{3}H to ^{14}C between 0 to 1 and 4.2 to 1, starting with that for oleic acid—20 to 1. A combination of the pathways would give intermediate ratios. A qualitative

Table 2 Ratios of ^{3}H to ^{14}C measured in Saturated Fatty Acids from Severn Estuary Sediments after Injection of 9,10-^{3}H, 1-^{14}C-Oleic Acid

Acid	*A*	*B*	*C*	*D*	*E*
Stearic	29 : 1	24 : 1	30 : 1	21 : 1	38 : 1
Palmitic	44 : 1	23 : 1	34 : 1	66 : 1	29 : 1
Myristic	112 : 1	41 : 1	54 : 1	170 : 1	215 : 1 *
Lauric	440 : 1 *	—	142 : 1 *	627 : 1 *	

Procedures for incubation and isolation of the acids are given in the text. The ratios of ^{3}H to ^{14}C used in the injected oleic acid varied between 10 to 1 and 40 to 1; all the results have been normalized to a starting ratio for oleic acid of 20 to 1. The conditions of incubation (*A–E*) are given beneath Table 1.

* These values are subject to errors of greater than ±5%.

picture can be readily inferred from the data in Table 2. Lauric acid and myristic acid, which have high ratios of ^3H to ^{14}C, seem to arise chiefly through the degradative pathway, while palmitic acid, which has a ratio nearer that of oleic acid, seems to be formed through both pathways. The position with stearic is more complex, as a ratio of ^3H to ^{14}C greater than that of oleic acid is impossible according to the scheme outlined above. Some of the stearic acid, however, may be formed through a recycling pathway involving palmitic acid:

$$\text{oleic acid} \rightarrow \text{stearic acid} \rightleftharpoons \text{palmitic acid}$$

A ratio of ^3H to ^{14}C greater than that of oleic acid would then be obtained. Such recycling of metabolites is well known in biochemistry[13,19], and it is quite likely that recycling is also occurring here.

Table 3 Calculated Fractions of Labelled Saturated Fatty Acids derived by the Degradative Pathway

Acid	*A*	*B*	*C*	*D*	*E*
Palmitic	0.67 (0.69)	0.48 (0.53)	0.60 (0.63)	0.76 (0.77)	0.56 (0.60)
Myristic	0.84 (0.85)	0.65 (0.66)	0.72 (0.73)	0.90	0.92
Lauric	0.96	—	0.88	0.97	

The observed ratios of ^3H to ^{14}C used to calculate these results are taken from Table 2, and the conditions of incubation (*A*–*E*) are given in the footnote to Table 1. The values in this table have been calculated using the formula

$$x = \frac{c_{res}t_{obs} - c_{obs}t_{res}}{c_{obs}\,(t_{deg} - t_{res}) + t_{obs}(c_{res} - c_{deg})}$$

where x is the fraction of acid formed through the degradative pathway. t_{res} to c_{res} is the theoretical ratio of ^3H to ^{14}C obtained for an acid formed through the resynthesis pathway. t_{deg} to c_{deg} is the theoretical ratio ^3H to ^{14}C through the degradative pathway. t_{obs} to c_{obs} is the observed ratio of ^3H to ^{14}C in the purified methyl esters of the long chain fatty acids. The first figure of each value is that assuming minimal loss of ^3H through the resynthesis pathway; that in parentheses is assuming total loss of ^3H. Where only one figure is given, the two results differ by less than 0.01. Stearic acid has been omitted from this table because recycling between stearic and palmitic acids cannot be estimated.

The results in Table 3 provide an approximate order for the proportion of individual labelled saturated fatty acids formed from labelled oleic acid by the degradative pathway:

$$\text{lauric} > \text{myristic} \gg \text{palmitic}$$

A significant portion of the labelled palmitic acid is formed on the resynthesis pathway, which is probably a reflexion of the fact that the fatty acid synthetases of many organisms produce predominantly palmitic acid[12]. Thus shorter chain acids (lauric and myristic acids) formed through the resynthesis pathway would have been produced by partial degradation of palmitic acid, with concomitant dilution of label by the cellular pool of palmitic acid.

Several assumptions have been made in this treatment of the results, and these will be discussed briefly. It has been assumed that the major pathways for the transition of label from oleic acid to the saturated acids involve either removal of a small number of C_2 units by means of β-oxidation (degradative pathway), or total breakdown to acetyl-CoA followed by resynthesis (resynthesis pathway). These are the principal pathways of fatty acid metabolism in most organisms[20], and we have assumed that other pathways, such as α-oxidation[21,22], are of little importance. Our calculations of the proportions of flow of label through the two pathways also require the assumption that any acid which has been formed by the resynthesis pathway is not then subsequently totally broken down again. If this occurs, our estimates of the flow through the resynthesis pathway will be too low, for the label will be lost from the system. During the whole incubation period the ratio of ^3H to ^{14}C for oleic acid should remain constant, and any variation will cause errors in the calculations of the fractions of labelled acids derived by the two pathways. The oleic acid fractions recovered after the incubations had ratios of ^3H to ^{14}C within 10% of the starting ratios.

The composition and abundance of biolipids and geolipids present in Recent sediments vary markedly with depth. Measurement of these differences provides one approach to the problem of studying the path of carbon during diagenesis. Farrington and Quinn[23] measured fatty acids at various depths in Recent sediment from Narragansett Bay, Rhode Island. They concluded that $C_{16:0}$ is the predominant acid at the depths studied (surface to 52 cm) and that the concentrations of the unsaturated fatty acids $C_{16:1}$ and $C_{18:1}$ decrease more rapidly with depth than do the saturated fatty acids. Brogden[24] has studied the anaerobic decomposition of zooplankton in a mixture of sediment and seawater which had been sterilized and inoculated with bacteria from marine sediments. The quantities of $C_{16:1}$ and $C_{18:1}$ acids fell rapidly over the period of the experiment, whereas the concentration of saturated fatty acids increased. Brogden suggested hydrogenation of the unsaturated fatty acids.

The novel approach described here involves direct introduction of radiolabelled biolipids into a Recent sediment. It provides specific and detailed information on the fate of a labelled compound introduced into the sediment. The method involves only minor disturbance of the ecosystem and allows estimates to be made of the fluxes through various biochemical pathways. This work substantiates the current view[1,23] that oleic acid rapidly disappears after burial in a Recent aquatic sediment. We have shown that some of the atoms of oleic acid reappear in the saturated normal fatty acid fraction, although the conversion is small. It is probable that most of the oleic acid is metabolized by degradation of the carbon skeleton through β-oxidation to acetyl-CoA, followed by conversion to CO_2 and H_2O or incorporation into non-lipid cellular components. We have calculated the proportions of the various saturated acids which have been produced from labelled oleic acid by the pathways we have considered, namely the degradative and resynthesis pathways (Table 3). The majority of the label was incorporated through the degradative pathway with retention of part or all of the original carbon skeleton of the oleic acid. The proportion is highest for lauric and myristic acids. But there are difficulties in deriving detailed inferences from information of this sort. The two pathways must differ markedly in their capability for dilution of label. Thus, the degradative pathway is unlikely to be subject to a large dilution of label and the radioactivity incorporated can be used to estimate the rate of carbon flow in this pathway. In contrast, in the resynthesis pathway the label passes through the acetyl-CoA pool of the cell. Acetyl-CoA is a key metabolite in most cells, and several pathways lead into and away from this pool. Calculations using the flow of label through the resynthesis pathway probably underestimate the total carbon flow from acetyl-CoA to long-chain fatty acids.

Our experiments certainly show that oleic acid present in a Recent sediment can be converted into saturated fatty acids with retention of carbon skeleton over a period of a few days. The process is almost certainly effected microbiologically. Whether this represents a process which contributes significantly to the content of the saturated fatty acid fraction found in Ancient sediments is uncertain. The rapid loss of oleic acid during the short periods of our experiments, coupled with the slight incorporation into saturated fatty acids through both pathways, suggests, however, that the saturated fatty acids found in compacted Recent and Ancient sediments are not derived directly from the fatty acids originally contributed to surface layers, but are derived from a number of precursors through many metabolic pathways.

On occasions the steady process of sedimentation may be interrupted by the sudden deposition of large quantities of sediment as a result of major floods or earthquakes, and in this case the sediment might be buried complete with a greater abundance of biolipids and their immediate products of alteration. Another mechanism whereby fatty acids might escape direct biological alteration could be encapsulation within a seed or nut[25]. Our prime concern in this study has been to investigate the processes of hydrogenation which are believed to occur early in the formation of a sediment. In deeper layers, as lithification proceeds, free water is gradually removed and bacterial activity ceases. As the temperature and pressure rise, slow maturation processes begin. Hydrogen transfer is a part of these slow maturation processes. Thus radiolabelled cholesterol, when intimately mixed with a sample of pulverized Green River shale (Eocene) and heated in a sealed tube at 200° for 1,000 h, afforded various radiolabelled products which included 5α and 5β-cholestane (unpublished results). The relationships between biolipids incorporated into a freshly forming sediment and those geolipids remaining after the initial period of biological and chemical diagenesis probably now encompass the following: (1) complete retention of structure as in the odd-numbered alkanes originating in the surface waxes of plant debris; (2) partial retention of structure, as in the saturated fatty acids formed by the degradative pathway in the present study, and partial or complete retention of stereochemistry as in isoprenoid acids derived, presumably, from phytol[26]; (3) complete breakdown and resynthesis as in the saturated fatty acids formed by the resynthesis pathway.

This study indicates that hydrogenation processes play a part in the early diagenesis of fatty acids. We are investigating the fate of several unsaturated biolipids for which saturated geolipid analogues are encountered in Ancient sediments. It seems likely that saturated geolipids may have a dual origin—biologically effected hydrogenation during early diagenesis, and thermally and catalytically induced hydrogen transfer processes during maturation. These hydrogenation processes are the only ones tenable unless biological sources can be found for fully saturated geolipids such as steranes, triterpanes and tetraterpanes and related compounds[27,28].

We thank Drs G. Ware, R. Denton, V. Collins, L. Morris and B. Nicholls and Mr F. J. H. Mackereth for advice, equipment and facilities. We thank Mrs Pat Muir for collecting samples and for the microbiological studies and Mr D. Morgan and Miss J. Hay for technical assistance. We thank the SRC, NERC and NASA for financial support. P. J. E. is a Beit Memorial research fellow.

M. M. RHEAD
G. EGLINTON
G. H. DRAFFAN *

Organic Geochemistry Unit,
School of Chemistry, and

P. J. ENGLAND

Biochemistry Department,
University of Bristol,
Bristol 8

Received March 15; revised June 4, 1971.

---

* Present address: Clinical Pharmacology Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12.

[1] Parker, P. L., and Leo, R. F., *Science*, **148**, 373 (1965).
[2] Peterson, D. H., thesis, Univ. Washington (1967).
[3] Parker, P. L., in *Organic Geochemistry* (edit. by Eglinton, G., and Murphy, M. T. J.) (Springer, Berlin and Heidelberg, 1969).
[4] Shorland, F. B., in *Chemical Plant Taxonomy* (edit. by Swain, T.) (Academic Press, London and New York, 1963).
[5] Gunstone, F. D., *An Introduction to the Chemistry and Biochemistry of Fatty Acids and their Glycerides* (Chapman and Hall, London, 1967).
[6] Parker, P. L., *Contributions in Marine Science*, **4** (1967).
[7] Schwendinger, R. B., and Erdman, J. G., *Science*, **144**, 1575 (1964).
[8] Rosenfeld, W. D., *Arch. Biochem. Biophys.*, **16**, 263 (1948).
[9] Oppenheimer, C. H., *Geochim. Cosmochim. Acta*, **19**, 244 (1960).
[10] Zobell, C. E., and Upham, H. C., *Bull. Scripps Inst. Oceanog.*, **5**, 239 (1944).
[11] Lynen, F., *Biochem. J.*, **102**, 381 (1967).
[12] Morris, L. J., *Biochem. J.*, **118**, 681 (1970).
[13] Wakil, S. J., *J. Lipid Res.*, **2**, 1 (1960).
[14] Rognstad, R., and Katz, J., *Arch. Biochem. Biophys.*, **127**, 437 (1968).
[15] Dole, V. P., and Meinertz, H., *J. Biol. Chem.*, **235**, 2595 (1960).
[16] Metcalfe, L. D., Schmitz, A. A., and Belka, J. R., *Anal. Chem.*, **38**, 514 (1966).
[17] Morris, L. J., *Chem. Ind.*, 1238 (1962).
[18] Williams, M. A., Cope, G. H., Jackson, J. L., and Hill, P., *Biochem. J.*, **118**, 379 (1970).
[19] Denton, R. M., and Randle, P. J., *Biochem. J.*, **104**, 423 (1967).
[20] Mahler, H. R., and Cordes, E. H., *Biological Chemistry* (Harper and Row, New York, 1966).
[21] Mead, J. F., and Levis, G. M., *J. Biol. Chem.*, **238**, 1634 (1963).
[22] Hajra, A. K., and Radin, N. S., *J. Lipid Res.*, **4**, 270 (1963).
[23] Farrington, J. W., and Quinn, J. G., *Nature*, **230**, 67 (1971).
[24] Brogden, W. B., thesis, Univ. Florida (1968).
[25] Godwin, H., *The History of the British Flora* (Cambridge University Press, Cambridge, 1956).
[26] Cox, R. E., Maxwell, J. R., Eglinton, G., Pillinger, C. T., Ackman, R. G., and Hooper, S. N., *Chem. Commun.*, 1639 (1970).
[27] Henderson, W., Wollrab, V., and Eglinton, G., in *Advances in Organic Geochemistry* (edit. by Schenck, P. A., and Havenear, I.) (Pergamon, London, 1968).
[28] Streibl, M., and Herout, V., in *Organic Geochemistry* (edit. by Eglinton, G., and Murphy, M. T. J.) (Springer, Berlin and Heidelberg, 1969).

Late Quaternary Stratigraphy and Radiocarbon Chronology of Water Level Fluctuations in Lake Keilambete, Victoria

WHEN compared with the northern hemisphere, the southern hemisphere has provided little palaeoclimatic information on the past 30,000 yr. A systematic study of lakes is under way to help establish a reliable palaeoclimatic sequence covering this period in south-east Australia and to explore possible correlations with worldwide climatically controlled events. This is a preliminary report of the stratigraphic and radiocarbon analysis of deposits from Lake Keilambete, located within the western Victorian volcanic province near Kerang 190 km west-south-west of Melbourne (Fig. 1).

The lake occupies the crater of a maar—a volcanic eruption crater lying below the general level of the surrounding country

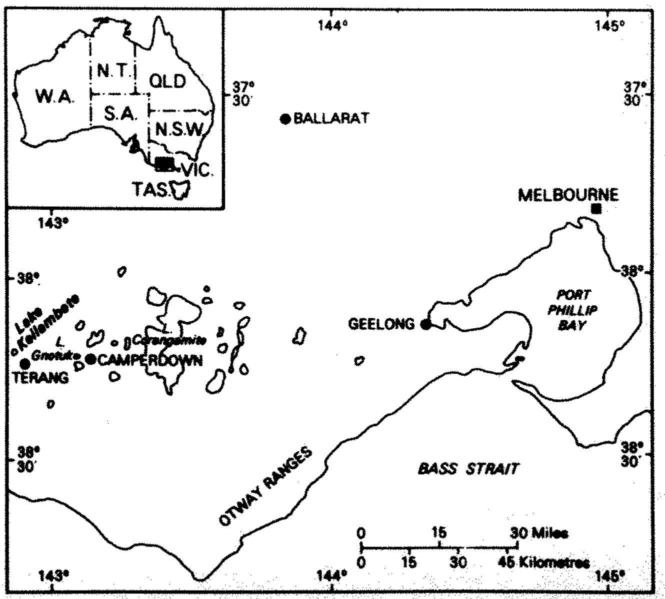


Fig. 1 Diagram showing location of Lake Keilambete.

and surrounded by a low tuff ring. The crater forms an internal drainage basin 1.8 km in diameter; it has a catchment area of 4.2 km^2 and is controlled by a relatively simple hydrologic system. Lake level responds sensitively to changes in precipitation or evaporation and lake sediments offer a means of reconstructing the sequence of climatic oscillations in late Quaternary time.

The lake derives water from three sources: direct precipitation, runoff from the crater slopes, and a small (almost negligible) ground water increment. Water is lost by evaporation alone as a thick clay effectively prevents water loss through the lake floor. During former high water phases, overflow may have occurred through the lowest point of the crater rim.

Throughout the period of European settlement, the crater has remained filled with water but has not overflowed. Compared with the low salinities of the contributing waters, the high salinity of the lake (60,000 p.p.m.) indicates that it has remained a closed basin for a long time. Lake stratigraphic data are available from two sources. First, around the margins of the lake, partly lithified lake deposits unconformably overlie Miocene calcarenite, the local bedrock of the region. Near the water's edge approximately 6 m of coarse, bedded and rounded detritus alternates with fine white marls. The coarse detritus is reworked volcanic fragments and contains lacustrine mollusca (*Coxiella* and *Limnea*). It is interpreted as a shallow lake deposit. The marls resemble carbonates forming in the lake today. Second, a series of ten cores taken from the shoreline out towards the deepest part of the lake provides a record of the recent lake deposits.

Table 1 Radiocarbon Dates from Lake Keilambete*

Lab. No.	Description	^{14}C age (yr BP†)
N-517	11 to 21 cm, core 4	610±110
N-518	21 to 33 cm, core 4	935±110
N-390	Tree submerged by lake waters and overlain by lacustrine sediments	1,890±115
N-519	55 to 65 cm, core 4	1,970±110
N-520-1	79 to 90 cm, core 4	2,410±120
N-521-1	102 to 112 cm, core 4	2,600±110
N-522-1	130 to 140 cm, core 4	2,970±120
N-523-1	165 to 175 cm, core 4	3,580±125
N-524-1	190 to 200 cm, core 4	4,200±125
N-525-1	235 to 245 cm, core 4	5,250±135
N-526-1	290 to 300 cm, core 4	6,440±145
N-527-1	325 to 345 cm, core 4	7,850±165
N-528	395 to 412 cm, core 4	14,300±300
ANU-199	Carbonate from upper marl below disconformity	20,340±500
N-567	Carbonate from upper marl band interbedded in reworked volcanic agglomerate	21,600±650
N-566	Carbonate from marl near base of lake sequence	29,100±1,250

* Data from refs. 1 and 2.

† Except where specified, all dates were obtained from total organic carbon.

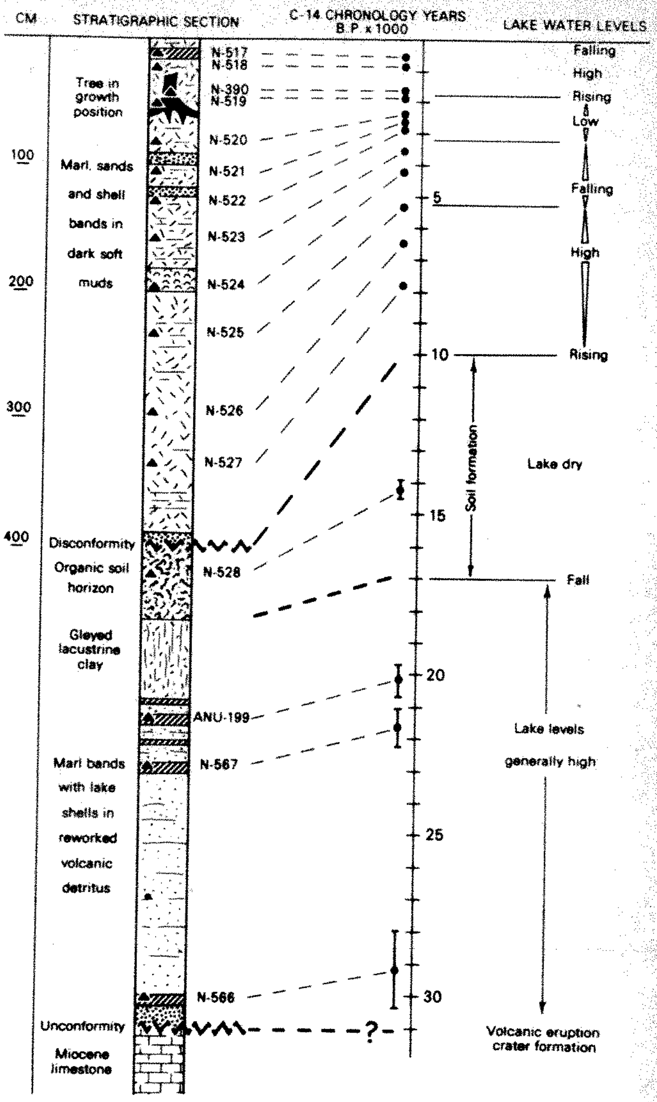


Fig. 2 Generalized stratigraphic section through sediments of Lake Keilambete showing ^{14}C chronology and sequence of reconstructed water level oscillations. Standard age deviations are shown only when values exceed ±250 radiocarbon years.

The stratigraphic section (Fig. 2), generalized from both outcrop and core sequences, reveals a basal unit of volcanic agglomerate and marl representing earliest lake deposits on which a soil horizon has formed. This is disconformably overlain by a 4 m sequence of unconsolidated, dark, organic-rich muds which alternate with pale carbonate bands. This upper unit was deposited since the last major drying, represented by the soil horizon below 400 cm.

Within the upper unit several sandy horizons in the otherwise fine textured deposits denote important facies variations related to changes in water level. In present day lake sediments, sands similar to those at 100, 125 and 400 cm (Fig. 2) are restricted to water depths of less than 3 m while clays are characteristic of deeper water. Assuming that the depth control of texture found in the relatively simple hydrodynamic system of the lake today also applied in the past, alternations of coarse and fine sediments in the cores can be directly related to past water level oscillations. Moreover, the occurrence of organic matter throughout the upper unit permits the facies variations to be accurately dated, thereby establishing the age of the oscillations and of the hydrologic changes which controlled them.

Radiocarbon dates representing 10 cm vertical thicknesses were obtained from core samples throughout the upper lake muds, from organic material in the buried soil horizon and from lake marls in the lower lithified unit. Additional ^{14}C analyses of carbonates from the upper lake muds[1] will be discussed elsewhere. The radiocarbon dates (Table 1) form a consistent series. Moreover, the ^{14}C ages of organic remains in the upper muds are consistent with ages based on estimated rates of deposition. They show no measurable initial error due to incorporation of old carbon into the organic content. The basal lacustrine sediments indicate that lake-full conditions existed from about 30,000 BP until after 18,000 BP. Some time between 15,000 and 18,000 BP the lake dried and remained in this state for several thousand years, long enough to permit soil development on older lake sediments. Ephemeral swampy conditions probably existed at this time.

The age of the next water level rise, estimated by extrapolating the ^{14}C ages to the base of the cores (assuming approximately constant rates of deposition), began about 10,000 BP.

The lake then remained deep for several thousand years and may even have overflowed. About 5,500 BP a change in the hydrologic environment is reflected in an increase in carbonate production and the eventual coarsening of bottom sediments, culminating in the deposition of shallow facies at 3,100 BP. At 3,100 BP water level rose slightly and then fell again, maintaining this low position until about 2,000 BP. Trees found in positions of growth near and below present water level and dated at 1,890 BP (N-390) were submerged by rising waters at this time. The trees belong to the last period when water level was below that of today. The lake rose and remained deep until after European settlement. In the late nineteenth century it began to fall and in 1969 stood at its lowest level for the past 1,900 yr.

In nearby Lake Gnotuk, submerged trees have been dated at 1,865 ± 85 BP (GX-0149, E. D. Gill, personal communication). Synchronous oscillations in lakes 20 km apart provide strong evidence that the controlling factor was climate rather than local hydrologic variations. Furthermore, the major drying between 18,000 and 15,000 BP is in close agreement with evidence documented from lakes in different hydrologic settings in north Victoria and south-west New South Wales recorded by J. M. B.[3]. The rise at about 10,000 BP, reflecting an increase in precipitation or decrease in temperature, cannot yet be correlated accurately with events elsewhere in Australia, although Kershaw[4] suggests a wet period in north Queensland at this time.

Effects of a Post-Glacial thermal maximum are weakly represented by a slow fall in water level and a corresponding rise in carbonate production from 5,500 to 3,100 BP. The magnitude and implied rates of these changes suggest conditions little different from those in the area today. The data therefore do not support the hypothesis of widespread Post-Glacial aridity so widely claimed in Australia[5–7]. Although temperatures from 5,500 to 3,100 BP may have been slightly higher than in the preceding interval, the degree of aridity would almost certainly not have been sufficient to cause the vegetation destruction and widespread landscape instability which are sometimes attributed to events of this time.

Conditions moderated at 3,100 BP when a slight rise of lake level occurred, but the major rise marking the beginning of the next relatively wet period did not occur until 1,900 BP.

Oscillations in Lake Keilambete can be matched in other lakes in a variety of settings in south-east Australia supporting the interpretations of the water level changes discussed here. The sequence of palaeoclimatic conditions probably has general application through south-east Australia and it may help provide a reliable basis for climatic correlations within Australia and between different continents in both hemispheres.

We thank Mr J. N. Jennings and Mr C. D. Ollier who read and criticized the manuscript, and Mr Keith Mitchell for drawing the figures.

J. M. BOWLER

Department of Biogeography and Geomorphology,
Australian National University,
Canberra, ACT

TATSUJI HAMADA

Institute of Physical and Chemical Research,
Yamato-Machi Saitama Prefecture,
Japan

Received June 14, 1971.

---

[1] Yamasaki, F., Hamada, T., and Hamada, C., *Radiocarbon*, **12**, 559 (1970).
[2] Polach, H. A., Lovering, J. F., and Bowler, J. M., *Radiocarbon*, **12**, 1 (1970).
[3] Bowler, J. M., in *Aboriginal Man and Environment in Australia* (edit. by Mulvaney, D. J., and Golson, J.), 47 (Australian National University, 1971).
[4] Kershaw, A. P., *New Phytol.*, **69**, 785 (1970).
[5] Brown, W. R., *Proc. Linn. Soc. New South Wales*, **70**, 5 (1945).
[6] Downes, R. G., *Austral. J. Agric. Res.*, **5**, 448 (1954).
[7] Gill, E. D., *Austral. J. Sci.*, **17**, 204 (1955).

Asbestos Fibres in Beverages and Drinking Water

THE hazards of inhaling asbestos fibres[1–4], the high incidence of gastrointestinal cancer[1,2,5–7] and abdominal neoplasms[3,8,9] among asbestos workers and the fact that asbestos fibres may penetrate the mucosa of the stomach and the intestine of animals[10,11] are known and so it is important to study the distribution of asbestos fibres in the environment. Asbestos fibres have already been found in beer[12], and the occurrence of fibres 0.5 nm in length and 0.01 nm in diameter suggests that such material may find its way through filtering systems into city drinking water supplies, and this we have been able to confirm.

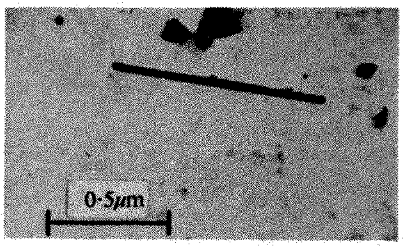


Fig. 1 Electron micrograph of asbestos fibre isolated from beer (enlarged 31,000 times).

The asbestos fibres in 400 ml. samples of beverages and water were concentrated by centrifuging in excess of 16,000*g*, drying at 100° C and ashing at 500° C[12]. The ash was taken up in 1 ml. of distilled water and agitated in an ultrasonic vibrator to disperse the fibres and ash particles[12]. Five µl. of this dispersion was evaporated with the aid of a heat lamp on 200 or 400 mesh carbon coated copper grids. Using a Siemens Elmiscop IA electron microscope at 80 kV and a magnification of 20,000, the number of fibres in each of five or ten grid holes was counted, and corrected for dilution. Precautions were taken to prevent contamination of samples from extraneous airborne fibres and no fibres were detected in distilled water

Table 1 Asbestos Fibres in Beverages and Water

Sample	Source*	No. of fibres × 10^6 l.$^{-1}$
Beer	Canadian 1	4.3
Beer	Canadian 2	6.6
Beer	USA 1	2.0
Beer	USA 2	1.1
Sherry	Canadian	4.1
Sherry	Spanish	2.0
Sherry	South African	2.6
Port	Canadian	2.1
Vermouth	French	1.8
Vermouth	Italian	11.7
Soft drink	Ginger ale	12.2
Soft drink	Tonic water I	1.7
Soft drink	Tonic water II	1.7
Soft drink	Orange	2.5
Tap water	Ottawa, Ottawa river (F)	2.0
Tap water	Toronto, Lake Ontario (F)	4.4
Tap water	Montreal, St Lawrence river (F)	2.4
Tap water	Hull, Quebec, Ottawa river (NF)	9.5
Tap water	Beauport, Quebec, St Lawrence river (6 km below Quebec City) (NF)	8.1
Tap water	Drummondville, Eastern Townships, Quebec, St Francois river (F)	2.9
Tap water	Asbestos, Eastern Townships, Quebec, Nicolet river (F)	5.9
Tap water	Thetford Mines, Eastern Townships, Quebec, Lac à la Truite (NF)	172.7
Melted snow	Ottawa, top 30 cm (2–3 weeks precipitation)	33.5
River water	Ottawa river, at Ottawa	9.5

* F, Filtration plant used; NF, no filtration plant used.

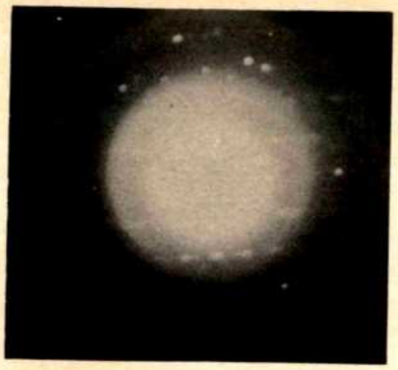


Fig. 2 X-ray diffraction pattern of asbestos fibre shown in Fig. 1.

blanks which were treated in the same manner as the liquid samples.

Twelve brands of Canadian beer, wine, sherry and port, six of American beer, six of European wine, vermouth and sherry and two of South African sherry were examined and all were found to contain asbestos fibres. Counts made on some of these brands are reported in Table 1. The fibres in Canadian beer (Fig. 1), sherry and water were identified as being chrysotile asbestos by comparing their electron diffraction pattern (Fig. 2) with that of pure chrysotile asbestos. Some of the European samples contained fibres of amphibole asbestos. Many asbestos fibres were hidden in clumps of debris such as mineral matter in water and diatomaceous earth which is used as a filtering aid in beer. As a result, the number of fibres reported in Table 1 must be considered a minimum estimate of those actually present. Most of the fibres found in Canadian beer and in filtered city water were less than 1 µm in length while most samples of wine, some American beer and unfiltered melted snow or river water also contained a considerable number of asbestos fibres ranging from 1 to 15 µm in length.

Tap water from three major Canadian cities, Ottawa, Toronto and Montreal, combined from 2.0 to 4.4 million fibres per litre. All of these cities have filtration systems and Ottawa tap water, drawn from the Ottawa river, had considerably fewer fibres than unfiltered river water. Many of Canada's major asbestos mines are located in the Eastern Townships of Quebec and filtered tap water from towns in this area did not differ greatly from that in the larger cities but unfiltered tap water in Thetford Mines, drawn from a small lake in the heart of an asbestos mining area, was exceptionally high in fibre content. The high level of asbestos fibres in melted snow in the Ottawa area gives some indication of one source of asbestos in surface waters.

The question as to the hazard of orally ingested asbestos has not yet been resolved. The amounts by weight of the asbestos found in these samples were exceedingly small—about 1 µg per 10 million fibres—obtained from counts on 1 mg samples of fibres of approximately the same size as those found in the beverages and water. Further work is required to determine the extent to which the fibres penetrate the walls of the digestive tract, the degree to which they may be transported to other organs of the body and the biological effects of their residence in various tissues. Studies of this nature are now in progress.

We thank Mr D. Campbell and Mr G. Bergeron for technical assistance and the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, for obtaining the water samples.

H. M. Cunningham
R. Pontefract

Food and Drug Directorate,
Department of Health and Welfare,
Ottawa

Received May 3; revised June 14, 1971.

[1] Mancuso, T. F., and Coulter, E. J., *Arch. Environ. Health*, **6**, 210 (1963).
[2] Selikoff, I. J., Churg, J., and Hammond, E. C., *J. Amer. Med. Assoc.*, **188**, 142 (1964).
[3] Hourihane, D. O., *Ann. NY Acad. Sci.*, **132**, 647 (1965).
[4] Wagner, J. C., *Ann. NY Acad. Sci.*, **132**, 575 (1965).
[5] Enterline, P. E., and Kendrick, M. A., *Arch. Environ. Health*, **15**, 181 (1967).
[6] Knox, J. F., Holmes, S., Doll, R., and Hill, I. D., *Brit. J. Indust. Med.*, **25**, 293 (1968).
[7] Newhouse, M. L., and Wagner, J. C., *Brit. J. Indust. Med.*, **26**, 302 (1969).
[8] Keal, E. E., *Lancet*, ii, 1211 (1960).
[9] Hinson, K. F. W., *Brit. J. Dis. Chest*, **59**, 121 (1965).
[10] Smith, W. E., Miller, L., Elsasser, R. E., and Hubert, D. D., *Ann. NY Acad. Sci.*, **132**, 456 (1965).
[11] Westlake, G. E., Spjut, H. J., and Smith, M. N., *Lab. Invest.*, **14**, 2029 (1965).
[12] Biles, B., and Emerson, T. R., *Nature*, **219**, 93 (1968).

BIOLOGICAL SCIENCES

Molecular Conformation of Deoxyuridine

THE structure determination of deoxyuridine was undertaken as part of a series of conformational studies of nucleic acid components. Such studies give information about the preferred conformation of nucleosides and nucleotides[1,2], and may clarify the conformational differences and changes that occur in nucleic acids. The conformational parameters of interest in deoxyuridine are the pucker of the sugar ring, the torsion angle of the sugar relative to the base about the glycosidic bond and the orientation of the C(5′)–O(5′) bond about the C(4′)–C(5′) bond.

In ribose and deoxyribose nucleosides, the most common type of pucker of the sugar is either C(2′) endo or C(3′) endo. In the deoxynucleosides the C(2′) endo pucker is by far the most common. Since one of the nucleosides with a C(3′) exo pucker is thymidine[3] it was of particular interest to know whether deoxyuridine would also have this type of pucker. Even the same nucleoside in different environments can have a different type of pucker[4], but these differences have only been observed in ribose nucleosides. There is therefore a need for further studies of deoxy nucleosides and nucleotides.

Crystals of deoxyuridine were obtained from aqueous solutions. The unit cell is monoclinic with dimensions $a=7.91$ Å, $b=6.71$ Å, $c=18.77$ Å, $\beta=96.6°$ and the space group is $P2_1$. There are four molecules in the unit cell and therefore two molecules in the asymmetric unit. Intensity data were collected with a Hilger and Watts linear diffractometer using molybdenum Kα radiation. The structure was solved by Patterson interpretation methods. The first step was the determination of the orientation of the plane of the pyrimidine bases in the unit cell using the $I(\theta,\varphi)$ function of Tollin and Cochran[5]. The orientation of the pyrimidine base within the plane was determined by means of a rotation function[6], and the positions of the molecules relative to the 2_1 axis were found using the $Q(X_0Z_0)$ function of Tollin[7]. Full details of the structure determination will be given elsewhere. The R factor at the present stage of refinement is 0.08.

The conformation of both molecules in the asymmetric unit is very similar. The pucker of both sugars is C(2′) endo, or C(2′) endo–C(3′) exo with respect to the three-atom plane C(1′)–O(1′)–C(4′). Displacements of the C(2′) atoms in molecules I and II relative to this plane are 0.508 and 0.383 Å respectively, and of atoms C(3′) they are 0.111 and 0.210 Å respectively. Thus the sugar pucker in deoxyuridine is like that in the majority of deoxynucleosides and differs from that in thymidine.

The glycosidic torsion angle, φ_{CN}, defined by Donohue and Trueblood[8], is $-28°$ in molecule I and $-24°$ in molecule II.

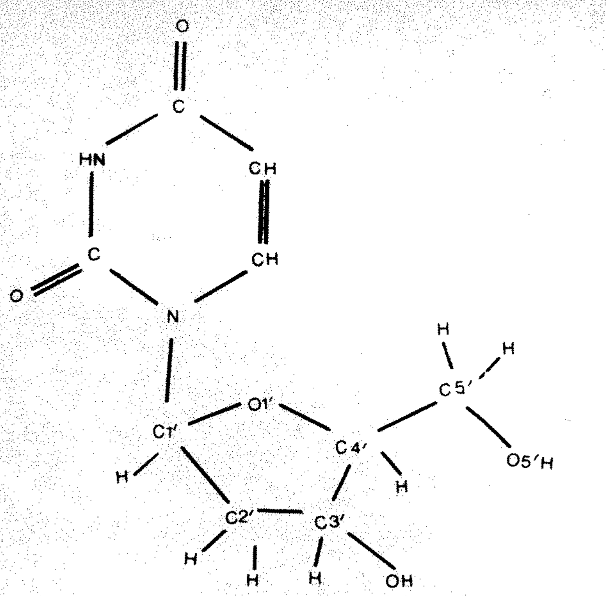


Fig. 1 The chemical structure of deoxyuridine.

Thus both molecules are in the *anti* conformation. A survey of nucleosides and nucleotides shows that the preferred conformations are such that in general the φ_{CN} value is larger for a C(2′) endo pucker than for a C(3′) endo pucker[1,2,4]. The values of φ_{CN} in deoxyuridine, however, are smaller than those found previously for C(2′) endo puckers and indicate that the preferred ranges of φ_{CN} values overlap.

In both molecules the orientation of the C(5′)–O(5′) bond is *trans* with respect to the C(4′)–O(4′) bond, and *gauche* with respect to the C(4′)–C(3′) bond. This *trans–gauche* conformation also occurs in fluorodeoxyuridine[9] and iodouridine[10], and energetically is just as favourable as the more common *gauche–gauche* conformation[11]. Indeed, the conformation of the C(5′)–O(5′) bond in those deoxynucleosides which contain uracil shows the three types of preferred conformations for this bond, as shown in Table 1, which summarizes the conformational parameter in these nucleosides.

Table 1 Conformational Parameters of the Nucleosides

Nucleoside	φ_{CN}	Sugar conformation	C(5′)–O(5′) conformation
Fluorodeoxyuridine[9]	−59°	C(2′) endo	*trans-gauche*
Bromodeoxyuridine[12]	−47°	C(2′) endo	*gauche-trans*
Iododeoxyuridine[13]	−63°	C(2′) endo	*gauche-gauche*
Thymidine[3]	−39°	C(3′) exo	*gauche-trans*
Deoxyuridine I	−28°	C(2′) endo	*trans-gauche*
Deoxyuridine II	−24°	C(2′) endo	*trans-gauche*

We thank Dr P. Tollin, Dr D. W. Young and Mr A. R. I. Munns for discussion and advice, and Mr J. Low for assistance with the data collection. We also thank the Science Research Council for the diffractometer and the Medical Research Council for financial support.

Asadur Rahman
H. R. Wilson

Carnegie Laboratory of Physics,
University of Dundee

Received March 19, 1971.

[1] Sundaralingam, M., *Biopolymers*, **7**, 821 (1969).
[2] Arnott, S., and Hukins, D. W. L., *Nature*, **224**, 886 (1969).
[3] Young, D. W., Tollin, P., and Wilson, H. R., *Acta Cryst.*, **B25**, 1423 (1969).
[4] Wilson, H. R., *Nature*, **225**, 545 (1970).
[5] Tollin, P., and Cochran, W., *Acta Cryst.*, **17**, 1322 (1964).
[6] Tollin, P., *Crystallographic Computing* (edit. by Ahmed, F. R.), 90 (Munksgaard, Copenhagen, 1970).
[7] Tollin, P., *Acta Cryst.*, **21**, 613 (1966).
[8] Donohue, J., and Trueblood, K. M., *J. Mol. Biol.*, **2**, 363 (1960).
[9] Harris, D. R., and MacIntyre, W. M., *Biophys. J.*, **4**, 203 (1964).
[10] Rahman, A., and Wilson, H. R., *Acta Cryst.*, **B26**, 1765 (1970).
[11] Wilson, H. R., and Rahman, A., *J. Mol. Biol.*, **56**, 129 (1971).
[12] Iball, J., Morgan, C. H., and Wilson, H. R., *Proc. Roy. Soc.*, A, **295**, 320 (1966).
[13] Camerman, N., and Trotter, J., *Acta Cryst.*, **18**, 203 (1965).

Pole Figures of the Orientation of Apatite in Bones

The orientation of the apatite and collagen in bone was first considered in this work because of its significance in the possible piezoelectric effect in bone. Bone is a polycrystalline material composed chiefly of apatite and collagen, and crystals of a component must be both piezoelectric and orientated in order to produce an overall piezoelectric effect. Orientation studies are also important for an understanding of the mechanical properties and anisotropic characteristics of bone as a structural material. Biologically, bone is a tissue composed of lamellae, blood, bone cells and fluids in a continual state of change. Within this biological system, however, could be a three dimensional network of apatite and collagen on a much smaller scale, which would maintain the mechanical properties of the bone at a constant level, irrespective of the normal sequence of biological changes. This work shows that such a highly organized three dimensional mesh of apatite crystals does exist, and that the exact structure of the mesh in a particular bone may be related to the function of that bone.

Other workers found that apatite crystals were orientated along the length of long bones. Clark and Iball[1], using the film method, reported that the apatite crystals in rat femur were aligned parallel to the axis of the shaft, and also in a transverse plane, tangential to the bone surface.

In this work, preliminary orientation studies of rat bone were made using the film method. Arcing of the 002 powder ring when the X-ray beam was normal to the length of the bone indicated that some apatite crystals were orientated along the length of the bone. When the X-ray beam was parallel to the length of the bone the patterns seemed inconclusive. A more accurate method of determining orientation was required to determine accurate pole figures which would demonstrate the three dimensional arrangement of apatite crystals in bone. A Schulz texture goniometer was used and the pole figures were plotted by computer. This method was originally developed for orientation studies in polymers[2].

The pole of a set of planes in a crystal is the point where the normal to the planes intersects an enclosing sphere. A pole figure of a particular set of planes is a stereographic projection of such a sphere, on which equal intensities of all poles are contoured. Thus the peaks on a pole figure represent the directions of maximum distribution of the poles of a set of crystal planes. Suitable planes to use were determined by preliminary film and diffractometer work. Planes with a θ Bragg angle well isolated from other peaks give optimum results. In the case of apatite, 002 is a good reflexion and, as it is a basal plane, it has the further advantage that it is comparatively easy to interpret. The prismatic plane (310) was also used because two reflexions incorporating all three indices are necessary to determine completely the orientation of a crystal.

The intensity of the diffracted beam was measured by a proportional counter and recorded on a chart recorder, and was proportional to the distribution of poles in that direction. To produce a pole figure, 720 intensities were read off the chart and the levels of equal intensity contoured by a computer plotter.

Pole figures of the shaft of a bovine femur and of the frontal bone of a bovine skull are shown in Fig. 1*a* and *b* respectively. The orientation of the bone specimen with respect to the pole

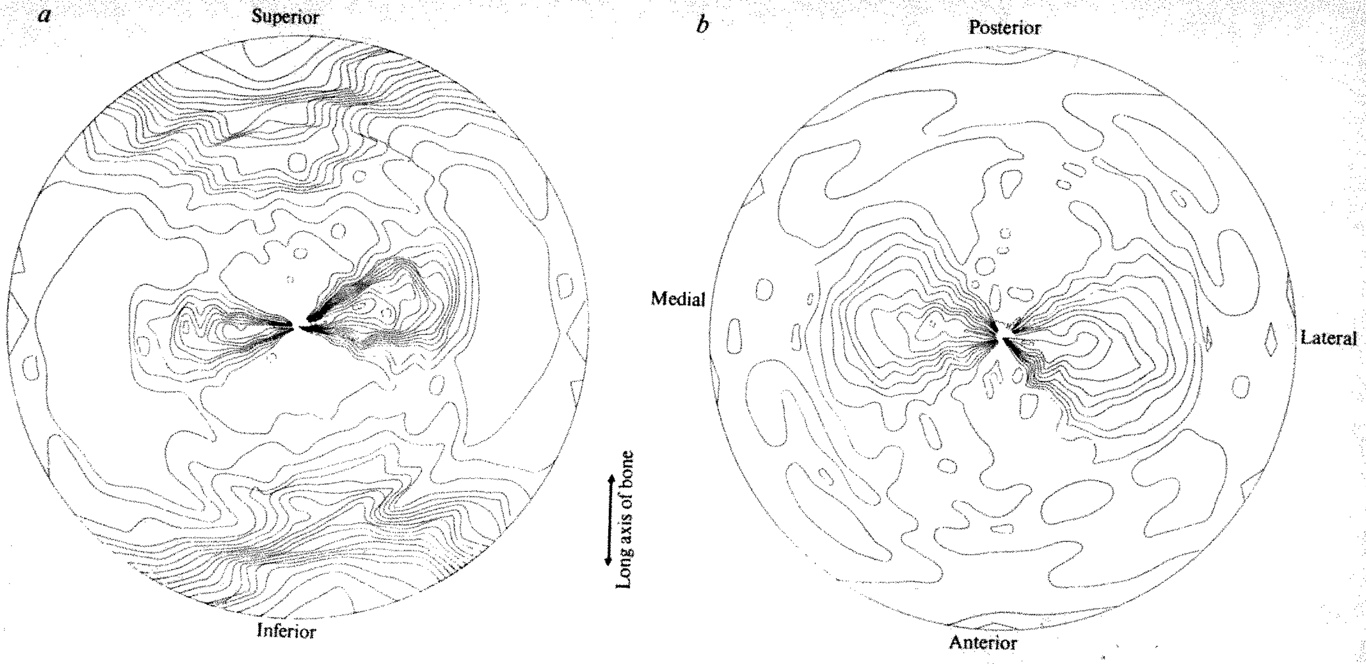


Fig. 1 *a*, 002 pole figure of the apatite crystals in a longitudinal section of the shaft of a bovine femur; *b*, 002 pole figure of the apatite crystals in a tangential section of the frontal bone of a bovine skull.

figure is shown on its periphery. The high peaks of intensity at the north and south poles of the femur 002 pole figure indicate that the long axis of the shaft of the femur is the main direction of orientation. The results also reveal an orientation which is perpendicular to the length of the bone and radial to the long axis. Further, the distribution about this direction is in a characteristic figure-of-eight configuration, which recurs in all the pole figures so far plotted. By contrast, the main orientation direction in the frontal bone of a bovine skull is normal to the surface of the bone. Again, the distribution of intensities about this direction is in the form of a figure-of-eight. There is a considerably smaller distribution in a direction roughly posterior–anterior in the plane of the bone.

Histological slides were prepared from pieces of the femur and skull bones taken as closely as possible to the specimens used for the pole figures. The osteons were orientated along the length of the femur, and tangentially to the surface of the frontal bone of the skull. There is thus a correlation between the osteon orientation and the pole figures for apatite.

In each pole figure the osteon direction is roughly north–south. Thus in the femur the distribution of apatite crystals along the length of the osteons seems to be dominant, but there is also a comparatively strong orientation in a direction perpendicular to the osteon axis. In the skull, the orientation of the apatite is dominantly perpendicular to the osteon axis. This indicates an adaptability of the apatite distribution to the differing mechanical roles of the two bones.

We thank Professor J. M. Zarek for suggesting the topic of this work and for helpful advice, and M. J. Dickens for preparing the histological slides. The work was supported by the Medical Research Council.

J. P. Nightingale
D. Lewis

Department of Mechanical Engineering,
University of Surrey,
Guildford,
Surrey

Received March 11, 1971.

[1] Clarke, S. M., and Iball, J., *Prog. Biophys.*, 7, 226 (1957).
[2] Lewis, D., Wheeler, E. J., Maddams, W. F., and Preedy, J. E., *J. Appl. Crystallog.*, 4, 55 (1971).

Non-surgical Assessment of Cardiac Function

Cardiac catheterization to evaluate myocardial or valvular function should not be used in the seriously ill patient and is difficult in the infant[1]. Furthermore, its use is justified for neither periodic screening examinations nor for routine monitoring at surgery. The clinical value of continuous monitoring of a parameter closely related to myocardial efficiency during and immediately after surgery cannot be over-emphasized. The social implications of the ability to screen for valvular disorders and decreased myocardial efficiency before infarction are more difficult to predict.

The close proximity of the mediastinal vessels, especially the aortic arch, to the oesophagus has stimulated the development of an ultrasonic Doppler shift oesophageal probe as a non-surgical technique for estimating aortic arch blood velocity in the conscious patient. The probe consists of an oesophageal tube near the tip of which is inserted a small 'Perspex' mount carrying a piezo-electric disk of 5.0 mm radius. Both faces of the disk are coated with conducting paint and a scratch is made across the diameter of one face, thus electrically separating the crystal into transmitter and receiver. The intact surface on the opposite face forms the common earth electrode. Wires are soldered directly to these three surfaces using low melting point solder (flux 362, alloy, TLC, Multicare Solders Ltd) before passing through the lumen of the oesophageal tube to a Parks 805 flowmeter. Ultrasound is generated continuously by the transmitter part of the crystal which is excited by an rf oscillator to produce a beam of power $\simeq 30$ mW cm^{-2}. Ultrasonic waves are scattered from moving particles such as blood cells in the path of the beam. The frequency of the back-scattered ultrasound detected by the receiver crystal is Doppler shifted by an amount (Δf) related to the particle velocity (v) by

$$\Delta f = \frac{2fv \cos \varphi}{c}$$

where f is the frequency of the emitted ultrasound, φ the angle between the ultrasonic beam and the path of the particle and c the velocity of the ultrasound in the intervening tissue.

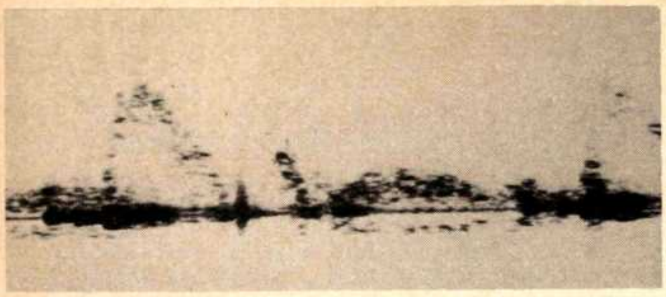


Fig. 1 Spectral analysis of Doppler shift signals from the descending thoracic aorta. The sonagram shows frequency as ordinate against time as abscissa with blackening of trace proportional to amplitude of each frequency component. The second peak represents reverse flow and the high amplitude low frequency components are due to arterial wall movements.

Using an excitation frequency of 5 MHz, the Doppler shift frequencies at each instant are detected as a range of audio signals corresponding to the range of particle velocities in the path of the beam. The problems of analysing such a spectrum of signals have been discussed[2]. To some extent the problems are reduced by the bluntness of the velocity profile in the upper thoracic aorta[3], so that the maximum frequency envelope of the sonagram of signals originating from the aortic arch approximates to the mean velocity across the spectrum (Fig. 1).

Alternatively, if the audio signals are fed into a zero-crossing frequency meter, an uncalibrated velocity-time pulse can be displayed on an oscilloscope screen or on a chart recorder. Although the trace is uncalibrated, turbulence caused by a stenotic aortic valve can be detected and percentage regurgitation can be estimated directly from the records. In addition, beat to beat changes in the flow pattern and peak velocity and acceleration can be of considerable value to the surgeon by giving immediate warning of deteriorating cardiac efficiency. The accurate measurement of the flow velocity and its quantitative relationship to myocardial function is the subject of our current work.

The work was supported by a grant (to C. D. S.) from the Medical Research Council, and the Wellcome Trust as part of a general study on pulsatile flow (R. G. G.).

C. D. SIDE
R. G. GOSLING

Department of Physics,
Guy's Hospital Medical School,
*London SE*1

Received February 18, 1971.

[1] Mendel, D., *A Practice of Cardiac Catheterisation*, 5 (Blackwell, Oxford, 1968).

[2] Gosling, R. G., King, D. H., Newman, D. L., and Woodcock, J. P., *J. Ultrasonics*, UIC Papers, 16 (1969).

[3] Schultz, D., Tunstall-Pedoe, D., Lee, G. de J., Gunning, A., and Bellhouse, B., in *Circulatory and Mass Transport*, CIBA Conf. 172 (Churchill, London, 1969).

Lithium attenuates Drug-induced Hyperactivity in Rats

LITHIUM salts have been used in psychiatry for more than 20 yr, especially for treating manic behaviour[1], and recently there has been a resurgence of interest in these compounds both as a specific treatment of mania and as prophylactics against manic-depressive illnesses in general[2,3].

Few systematic behavioural studies, however, seem to have been carried out on animals in controlled experimental situations[4-7], possibly because effects on behaviour of non-toxic doses are difficult to demonstrate in 'normal' animals. If rats are given some combinations of dexamphetamine with amylobarbitone or chlordiazepoxide they show a characteristic and prolonged hyperactivity in novel environments, which cannot be induced by any dose of the separate drugs[8,9]. The hyperactivity consists of persistent and apparently stereotyped walking in a maze, and is elicited with great reliability. Because one aspect of mania is increased motor activity, we thought that the hyperactivity induced by the mixture could provide a suitable baseline for testing behavioural effects of lithium in animals. Chronic as well as acute treatments of lithium were given; usually several days are needed for lithium to exert therapeutic effects in manic patients.

We used naive hooded female rats approximately 120 days old at the beginning of the experiments which had been reared in standard conditions of lighting, temperature and humidity and had not been handled since weaning. In chronic experiments rats were injected with either 3 mequiv./kg of lithium chloride (LiCl, isotonic solution), or with the same amount of isotonic saline intraperitoneally daily, always at the same time in the morning, for 15 days. On the fifteenth day, the rats pretreated with LiCl received their last dose (chronic lithium group); those pretreated with saline were divided into two sub-groups: one received its usual morning injection of saline, and the other was given a single injection of 3 mequiv./kg LiCl (acute lithium group). Three hours later all rats were injected subcutaneously with either a mixture of 12.5 mg/kg chlordiazepoxide and 1.18 mg/kg dexamphetamine, dissolved in saline, or with saline (2 ml./kg) alone. Thirty-five minutes after this injection the rats were placed singly in symmetrical Y-shaped mazes, and their behaviour was scored for 5 min, as: (1) the number of times the rats entered the arms of the maze with all four feet across the entrance ("entries"); (2) the number of rears; (3) number of faecal boluses deposited, and the presence or absence of urine in the maze. The observers also rated the ataxia of the rats' walking, using a six point scale. At the end of the Y-maze trial, the rats were anaesthetized with pentobarbitone, blood was withdrawn from the descending aorta, and lithium content was assayed with a flame-photometer (EEL) using a standard method[10].

In further acute experiments the same sequence of operations was followed as that on the last day of the chronic experiment except that: (1) in the morning all rats were injected with 2 mequiv./kg of LiCl (instead of 3 as in the chronic experiments) or the same amount of NaCl; (2) during the second part of the experiment, apart from rats which received subcutaneous injections of saline or of the drug mixture, other groups were given one or other of the constituent drugs, that is, either 12.5

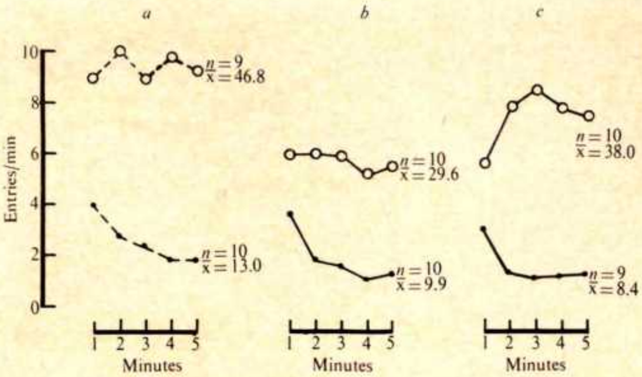


Fig. 1 Effects of acute and chronic LiCl (3 mequiv./kg) on hyperactivity induced in rats by a mixture of 12.5 mg/kg chlordiazepoxide and 1.18 mg/kg dexamphetamine. Group (*a*) received fifteen daily injections of saline; (*b*) fourteen injections of saline and one of LiCl; and (*c*) fifteen injections of LiCl. The rats were tested in a Y-maze either with the mixture (○) or with saline (●). The single injections of LiCl (*b*) significantly reduced the hyperactivity; chronic pretreatment with LiCl (*c*) had very little effect. No significant differences were found among saline controls.

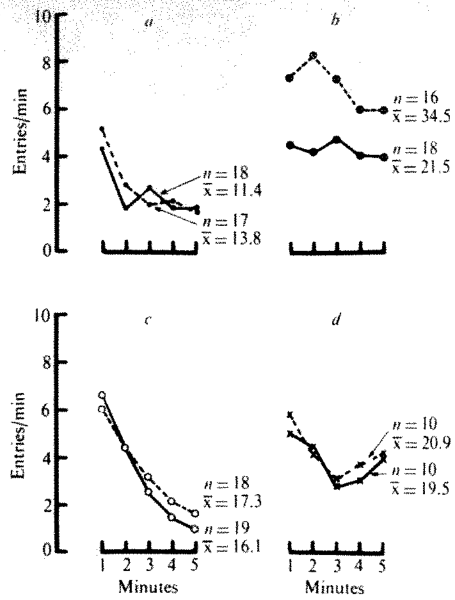


Fig. 2 Effects of a single dose of 2 mequiv./kg LiCl on spontaneous activity of rats under the influence of (*a*) saline, (*b*) a mixture of 12.5 mg/kg chlordiazepoxide and 1.18 mg/kg dexamphetamine, (*c*) 12.5 mg/kg chlordiazepoxide and (*d*) 1.18 mg/kg dexamphetamine. As in Fig. 1, a single injection of lithium had no significant effects on the number of entries into the arms of a Y-maze by rats unless they were made hyperactive by the drug mixture (*b*). ——, Rats given 2 mequiv./kg isotonic LiCl 3–5 h before testing; - - -, rats given the same amount of saline.

mg/kg chlordiazepoxide alone, or 1.18 mg/kg dexamphetamine. Otherwise the same experimental procedures were followed throughout.

In the chronic experiments it was found (Fig. 1) that the animals which had been pretreated with saline for 15 days and had then received the drug mixture showed a near four-fold increase in the number of entries as compared with their own controls ($P < 0.001$, Fig. 1*a*). The rats pretreated with saline which were given the single dose of LiCl and then the mixture on the test day (Fig. 1*b*) were less stimulated (Student's *t* test, $P < 0.05$, two tailed). In the chronic lithium group the reduction in the drug mixture-induced hyperactivity was negligible (Fig. 1*c*). There were no significant differences among the three saline control groups. Thus only acute lithium treatment significantly reduced the hyperactivity.

Because animals tested with 3 mequiv./kg showed some signs of toxicity, for example, diarrhoea, the dose was reduced to 2 mequiv./kg for the next acute experiments (Fig. 2). The mixture again produced a marked, almost three-fold, increase in activity ($P < 0.001$, Fig. 2*b*) and, as before, lithium treated animals which were tested under the influence of the mixture were less active ($P < 0.01$) than mixture animals pretreated with saline. The single lithium pretreatment did not, however, alter the activity of the saline controls (Fig. 2*a*). It is clear that acute pretreatment with this dose (2 mequiv./kg) of lithium chloride, as in the previous experiment with 3 mequiv./kg, mitigated though did not completely abolish this particular form of drug-mixture induced hyperactivity; in both cases the number of entries was reduced by approximately a third. The higher levels of activity with the mixture alone in the chronic experiments may have been due to handling; some forms of handling have previously been found to have a similar effect[11].

Mixture treated animals were hyperactive in spite of motor incoordination and flaccidity which were reflected in high ataxia ratings made by the observers. Lithium did not increase ataxia ratings in any of the groups. It therefore seems unlikely that the reductions in activity with lithium in the mixture groups can be explained by muscle weakness, although this is frequently reported as a side effect in lithium therapy. As expected, lithium-treated animals urinated more than their controls during testing although the differences were not statistically significant.

Plasma lithium levels after acute administration of 3 mequiv./kg LiCl were 1.06 ± 0.258 mequiv./l. (mean ± s.d.) in the mixture and 0.83 ± 0.099 in the saline groups; the corresponding levels in the chronic groups were 0.69 ± 0.166 and 0.84 ± 0.106. Thus after acute administration plasma levels with the mixture were higher than with saline ($P < 0.05$), and considerably higher than with the mixture after chronic lithium pretreatment ($P < 0.01$).

The mean plasma lithium levels after acute 2 mequiv./kg pretreatment were: 0.49 ± 0.089 (mean ± s.d.) with saline; 0.52 ± 0.178 with chlordiazepoxide and 0.52 ± 0.144 with dexamphetamine, and 0.67 ± 0.197 with the mixture. The separate drugs did not increase plasma levels but, as before, the levels were increased by the mixture ($P > 0.05$). The results with acute lithium therefore seem to indicate that in the two doses used lithium does not have any marked depressant effect on the spontaneous activity of rats unless the animals have been made hyperactive. Our results are consistent with a study in which lithium inhibited a form of behaviour induced by a combination of desmethylimipramine and Ro 4-1284 (a reserpine-like drug) but it did not inhibit excitation and increased stereotyped behaviour induced by amphetamine alone[5].

Evidence has been accumulating that acute or short term lithium treatment reduces the amount of noradrenaline available for interaction at receptor sites[12,13]. The biochemical effects of chlordiazepoxide–dexamphetamine mixtures are not yet established, but in addition to the well known sympathomimetic actions of dexamphetamine, chlordiazepoxide has been found to lower noradrenaline turnover in the brain[14]. Thus it is possible that the mixture-induced hyperactivity depends on noradrenergic mechanisms which are affected by acute lithium treatment. This suggestion is supported by the finding that chronic lithium does not markedly alter hyperactivity induced by the mixture nor does it affect noradrenaline turnover in the brain[15–17]. Patients have been reported to excrete much less lithium during manic episodes[18,19]; in our experiments the animals which would have been hyperactive with the drug mixture showed higher concentrations of lithium in the plasma; this may suggest that they also excreted less lithium than animals which were not given the mixture. To that extent results from our acute experiments reflect the clinical situation. In humans, however, lithium usually reduces manic behaviour only after at least several days of treatment, whereas in our experiments chronic lithium was relatively ineffective in mitigating the mixture induced hyperactivity, nor did the mixture increase plasma levels.

We are now testing the effectiveness of lithium against various forms of hyperactivity induced by other means, including by different drugs, by brain lesions and by behavioural manipulations, in order to investigate further the generality of our findings and the possible mechanisms involved.

We thank Drs D. H. Jenkinson and A. V. Juorio and Professors M. Maizels, H. McIlwain and H. O. Schild for valuable comments and discussion and D. U'Prichard, D. I. Carruthers-Jones and Terri Miller for help with experiments. The work was supported by a grant from the National Institute of Mental Health, US Public Health Service, and the Smith Kline and French Foundation. C. C. holds an MRC postgraduate award. P. E. H.-R. holds a University of London postgraduate studentship.

CHRISTINA COX
P. E. HARRISON-READ
HANNAH STEINBERG
M. TOMKIEWICZ

Department of Pharmacology,
University College London,
*Gower Street, London, WC*1

Received April 17, 1971.

[1] Cade, J. F. J., *Med. J. Austral.*, **36**, 349 (1949).
[2] Schou, M., *J. Psychiat. Res.*, **6**, 67 (1968).

[3] Gatozzi, A. A., *US Government Printing Office, National Clearinghouse for Mental Health Information Publication* No. 5033 (1970).
[4] Davenport, V. D., *Amer. J. Physiol.*, **163**, 633 (1950).
[5] Matussek, N., and Linsmayer, M., *Life Sci.*, **7**, 371 (1968).
[6] Sheard, M. H., *Nature*, **228**, 284 (1970).
[7] Harrison-Read, P. E., and Steinberg, H., *Nature New Biology*, **232**, 120 (1971).
[8] Rushton, R., and Steinberg, H., *Brit. J. Pharmacol.*, **20**, 145 (1963).
[9] Rushton, R., and Steinberg, H., *Nature*, **211**, 1312 (1966).
[10] Helm, H. J. v.d., and Andriesse, D., *Clin. Chim. Acta*, **6**, 747 (1961).
[11] Rushton, R., Steinberg, H., and Tomkiewicz, M., *Nature*, **220**, 885 (1968).
[12] Corrodi, H., Fuxe, K., Hokfelt, T., and Schou, M., *Psychopharmacologia*, **11**, 345 (1967).
[13] Schildkraut, J. J., Logue, M. A., and Dodge, G. A., *Psychopharmacologia*, **14**, 135 (1969).
[14] Taylor, K. M., and Laverty, R., *Europ. J. Pharmacol.*, **8**, 296 (1969).
[15] Corrodi, H., Fuxe, K., and Schou, M., *Life Sci.*, **8**, 643 (1969).
[16] Ho, A. K. S., Loh, H. H., Craves, F., Hitzemann, R. J., and Gershon, S., *Europ. J. Pharmacol.*, **10**, 72 (1970).
[17] Bliss, E. L., and Ailion, J., *Brain Res.*, **24**, 305 (1970).
[18] Trautner, E. M., Morris, R., Noack, C. H., and Gershon, S., *Med. J. Austral.*, **42**, 280 (1955).
[19] Gershon, S., and Yuwiler, A., *J. Neuropsychiat.*, **1**, 229 (1960).

Procoagulant Synthesis by Cultured Fibroblasts triggered by Cell Adhesion

SYNTHESIS of a potent procoagulant by cells in tissue culture has been demonstrated but its identity is obscure[1]. Activity is low at the beginning of culture of normal human fibroblasts and increases several hundred-fold after 12–24 h of incubation at 37° C. This increase is readily detected by the one-stage assay for factor VIII (antihaemophilic globulin) but can also be demonstrated by the two-stage factor VIII assay based on the thromboplastin generation test. Studies in progress to characterize this procoagulant have demonstrated a marked increase of factor XI as well as factor VIII activity and evolution of factor XI activity during contact activation with celite. Increase in activity detectable in the one-stage assay for factor IX and XII was much less. The procoagulant was incapable of clotting a fibrinogen solution and no change in assays for factor V and VII was observed during culture.

These data suggest a relationship of this procoagulant to first-stage coagulation factors, particularly factors VIII and XI. But identification (by *in vitro* coagulation tests) of specific coagulation factors within cells is necessarily complicated by the presence of other procoagulant substances such as phospholipid and tissue factor. Evaluation of the latter is so far incomplete. *In vivo* infusion studies and coagulation factor identification by immunological methods as well as by further *in vitro* testing will be reported in detail elsewhere.

In previous studies considerable variability in procoagulant production was observed between cultures of various cell types[1]. Suspension cultures of leucocytes generated procoagulant levels much lower than observed in monolayer cultures. Furthermore, the increase in procoagulant activity in monolayer cultures of several cell types was independent of cell doubling time and paralleled the period during which cells were becoming adherent to the flask. Attention was thus drawn to the process of cell adhesion as a possible triggering mechanism for procoagulant synthesis.

Procoagulant production was studied in cultures of human foreskin fibroblasts prepared in fresh medium at various cell concentrations. Maximum levels were achieved with a cell concentration of 2×10^5/ml. By contrast, cultures established at a concentration of 4×10^5/ml. yielded procoagulant levels of approximately 50% of cultures established at the lower cell concentration. Cells in a concentration of 4×10^5/ml. formed a virtual monolayer from the outset. There was little surface on which the potentially adherent cells could spread.

To investigate further the possible relationship between cell adhesion and procoagulant synthesis, cell cultures were prepared in a concentration of 2×10^5/ml. in disposable plastic culture flasks (Falcon) and either allowed to remain at rest or placed on a mechanical shaking device to prevent cell adhesion. All cultures were carried out at 37° C. The tilt-top mixer (Lab-Tek aliquot shakers, model 4651, Ames Co., Elkhart, Indiana) rotated through an angle of 80 degrees at a frequency of 20 c.p.m. Periodic observation of cultures by inverted microscopy revealed virtually complete adherence of cells in stationary culture after 4–6 h of incubation. Thereafter rare cells were observed in suspension and haemocytometer cell counts performed on the medium were virtually zero. Conversely, in agitated cultures few cells became adherent. Cultures removed from the mixing device after 12 h of incubation and placed at rest promptly formed monolayers and were indistinguishable from initially stationary cultures. The number of cells declined after 24 h on the shaker, resulting in a less dense monolayer when culture flasks were placed at rest.

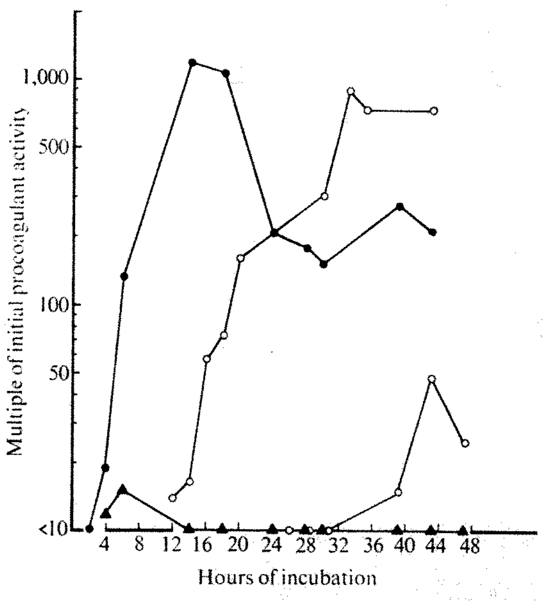


Fig. 1 Effect of shaking on procoagulant production by cultured fibroblasts. ●, Stationary cultures; ▲, cultures incubated on shaker; ○, cultures removed from shaker at 12 and 24 h.

Cells in stationary and rotated flasks were collected at intervals; the plastic flask was opened with a heating element and the monolayer was mechanically dislodged from the floor of the flask. Cell clumps were broken and cells were distributed evenly in the overlying medium by repeated passage through a Pasteur pipette and finally frozen and thawed three times in preparation for analysis. The one-stage assay for factor VIII based on the activated partial thromboplastin time was used in these experiments because it is a sensitive indicator of procoagulant production. Results of assays are presented in Fig. 1. In each of seven experiments the increase of procoagulant activity was prompt and dramatic in stationary cultures. By contrast, cultures incubated on the mixing device failed to generate procoagulant activity. Cultures removed from the mixer after 12 h of incubation and placed at rest promptly began to synthesize procoagulant as cells were permitted to become adherent to the floor of the flask. Ultimate levels equalled those achieved in initially stationary cultures. Cultures agitated for 24 h retained a definite but lesser ability to generate procoagulant when removed from the shaker and placed at rest.

These experiments demonstrate a correlation between cell adhesion and synthesis of procoagulant which is potentially important in elucidating the process of haemostasis regardless of whether the procoagulant is a specific coagulation factor, for example, factor VIII, or tissue thromboplastin. Local endothelial damage might lead to a number of surface-related events which could enhance blood coagulation locally. For example, the making and breaking of cell surface contacts are necessarily involved in cell infiltration, phagocytosis, migration and proliferation.

The mechanism by which adhesion triggers procoagulant production is obscure. The pertinent cell membrane receptor or metabolic activity responsible for procoagulant synthesis is not defined. It is unlikely, however, that the *in vivo* counterpart of this phenomenon (if such exists) would be restricted to fibroblasts. We postulate that many cell types may synthesize procoagulant *in vivo* and that such synthesis may be mediated by adhesive events occurring at cell surfaces. Perhaps enhanced adhesive activity is the common denominator uniting the host of pathological states characterized by elevated circulating levels of factor VIII. Furthermore, the association of diminished levels of factor VIII and a defect in platelet adhesiveness is well known in von Willebrand's disease. This raises the possibility that the low level of factor VIII observed in this disease is the result of a defective triggering mechanism. Alternatively, the chemical configuration on the cell which permits adhesion may be identical to factor VIII.

LEO R. ZACHARSKI

Research Service,
Veterans Administration Center,
White River Junction,
Vermont

O. ROSS MCINTYRE

Department of Medicine,
Dartmouth Medical School,
Hanover,
New Hampshire

Received January 4; revised March 17, 1971.

[1] Zacharski, L. R., Bowie, E. J. W., Titus, J. L., and Owen, C. A., *Mayo Clin. Proc.*, **44**, 784 (1969).

Mode of Action of Pollen in Breaking Resistance of *Vicia faba* to *Botrytis cinerea*

THE discovery that pollen can transform the mildly virulent fungus *Botrytis cinerea* into a destructive pathogen of broad bean and strawberry[1] poses fundamental and practical problems. One concerns the nature of the stimulants from pollen[1] because *B. cinerea* is normally unable to spread from limited lesions in broad bean leaves even in the presence of fungal nutrients[2]. Another is the mode of action of the stimulants, and particularly their interaction with wyerone acid metabolism. Wyerone acid[3] has been identified as an antifungal product of infected bean (*Vicia faba*), and its formation, activity and degradation have been suggested as important factors controlling development of *Botrytis* in *Vicia*[3,5]. If pollen extracts prevent accumulation of wyerone acid or render the fungus insensitive to the acid, support would be provided for this suggestion.

A suspension of 2×10^6 *V. faba* pollen grains/ml. of sterile distilled water was frozen at −20° C, thawed and shaken for 15 min at 21° C. This procedure was repeated and after centrifugation the supernatant was dialysed for 12 h against sterile water at 5° C. The dialysate was concentrated by rotary evaporation to the original volume of suspension. This pollen extract (*p*H 5.3) was stored at −20° C. Drops (10 µl.) of suspension of 5×10^5 conidia of *B. cinerea*/ml. sterile water or pollen extract were placed on upper surfaces of detached bean leaves. Within 5 days at 17° C in moist chambers, conidia in pollen extract caused complete degradation of the leaves, whereas those in water caused some brown flecking beneath infection droplets. From similar leaves, 5 mm diameter disks bearing infection sites were excised 82 h after inoculation, and wyerone acid was extracted, separated and estimated by solvent partition, paper chromatography and ultraviolet spectrophotometry[3]. Green disks bearing brown lesions caused by spores in water contained 56 µg of wyerone acid/g fresh weight. Black disks from spreading lesions caused by spores in pollen extract contained 97 µg/g fresh weight, so pollen extract had not prevented accumulation of wyerone acid.

Table 1 Germination and Growth of *Botrytis cinerea* in Presence and Absence of 20 µg/ml. Wyerone Acid

Test solution	Germination %	Germ-tube length (µm)
Water alone	95	50–100
Bean extract	100	500–600
Bean extract + wyerone acid	0	0
Pollen extract	100	500–600
Pollen extract + wyerone acid	100	500–600

Spores of *B. cinerea* were suspended (10^5/ml.) in water alone, and in pollen and bean[4] extracts in the presence and absence of 20 µg/ml. wyerone acid. Droplets (20 µl.) of suspensions were incubated on slides for 20 h at 20° C. Results (Table 1) show that bean and pollen extracts are stimulatory to germ tube growth in the absence of wyerone acid, but that pollen extract also apparently renders spores insensitive to wyerone acid. Separate tests showed that wyerone acid was not lost during incubation with pollen extract. The lowest concentration of wyerone acid which prevented all spore germination in pollen extract was between 500 and 1,000 µg/ml. This contrasts with the concentration of 18 µg/ml. which had a similar effect in bean extract[3].

We conclude that pollen enables *B. cinerea* to overcome the inhibitory action of wyerone acid in infected broad bean, and that wyerone acid is an important factor in normal lesion limitation. This raises questions about the mode of action of pollen in stimulating infections of strawberry fruit[1], rye leaves[6] and wheat heads[7], and also about normal mechanisms of resistance in these plants.

J. W. M. acknowledges the receipt of a research studentship from the Ministry of Agriculture.

J. W. MANSFIELD
B. J. DEVERALL[*]

Department of Botany,
Imperial College,
London SW7

Received December 11, 1970; revised March 18, 1971.

[*] Present address: ARC Unit, Wye College (University of London), Ashford, Kent.

[1] Chou, M. C., and Preece, T. F., *Ann. Appl. Biol.*, **62**, 11 (1968).
[2] Purkayastha, R. P., and Deverall, B. J., *Ann. Appl. Biol.*, **56**, 139 (1965).
[3] Letcher, R. M., Widdowson, D. A., Deverall, B. J., and Mansfield, J. W., *Phytochemistry*, **9**, 249 (1970).
[4] Deverall, B. J., *Ann. Appl. Biol.*, **59**, 375 (1967).
[5] Deverall, B. J., and Vessey, J. C., *Ann. Appl. Biol.*, **63**, 449 (1969).
[6] Fokkema, N. J., *Neth. J. Plant. Pathol.*, **74**, 159 (1968).
[7] Strange, R. N., and Smith, H., *J. Gen. Microbiol.*, **59**, ii (1969).

Coated Microvesicles in Neurosecretory Terminals of Posterior Pituitary Glands shed their Coats to become Smooth "Synaptic" Vesicles

WE have suggested that microvesicles ("synaptic vesicles") within neurosecretory terminals of posterior pituitary glands are by-products of exocytosis formed by micropinocytosis-like activity serving to recapture excess membrane, of neurosecretory granules, incorporated in the cell surface. Our view arose from the discovery of exocytotic figures in electron micrographs of these terminals and the presence, in some such figures, of indications of membrane vesiculation: caveolae with microvesicles of similar size near them[1,2]. The suggestion harmonized not only with the theoretical requirement for membrane recapture after exocytosis but with the familiar characteristics of the "synaptic vesicle" population: localization in terminal regions where hormone is released; aggregates against the cell membrane, the so called "synaptoid" formations; and increased number after stimulation. The scheme, however, was not entirely satisfactory since classical "synaptic vesicles"[3] have smooth membranes while those apparently formed by membrane vesiculation were often seen to be coated, as were the adjacent caveolae[2]. While this coating supported the interpretation of such figures as micropinocytosis-like activity (coating is believed to represent the mechanism inducing membrane vesiculation[4,5]) it required us to postulate that coated vesicles are somehow transformed into the smooth "synaptic" type[2]. We here present evidence for this transformation.

Neurohypophyses of unanaesthetized rats were exposed following decapitation[2] and fixed, according to Karnovsky[6], during electrical stimulation. This yielded many microvesicles with readily discernible coats, some complete but others apparently partial. Such microvesicles can be seen mingled with smooth "synaptic" vesicles and electron-dense neurosecretory granules in Fig. 1. A coated "caveola" is present in the plasmalemma of the fibre toward the left of this illustration.

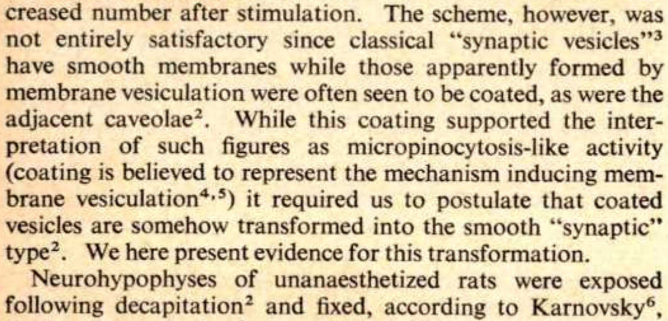


Fig. 1 Neurosecretory terminals in the posterior pituitary gland of the rat fixed during stimulation. The gland, exposed by decapitation, was stimulated electrically (28 V, 1 ms, 20 Hz) through electrodes applied to the stalk and fixed, after 2 min, without stopping stimulation, by flooding with a mixture of glutaraldehyde and paraformaldehyde at room temperature, post-fixed in osmic acid, extracted in block with uranyl acetate according to Karnovsky[6], and embedded in 'Vestopal'. This is a general view at medium magnification (× 86,000) showing, principally, terminal regions of two neurosecretory fibres abutting basement membrane. The terminals contain a fully coated microvesicle (long arrow), two half-coated microvesicles (shorter arrows), and a number of vesicles with lesser amounts of coating or none at all. A coated caveola suggestive of micropinocytotic activity is evident in the plasmalemma of the terminal toward the left.

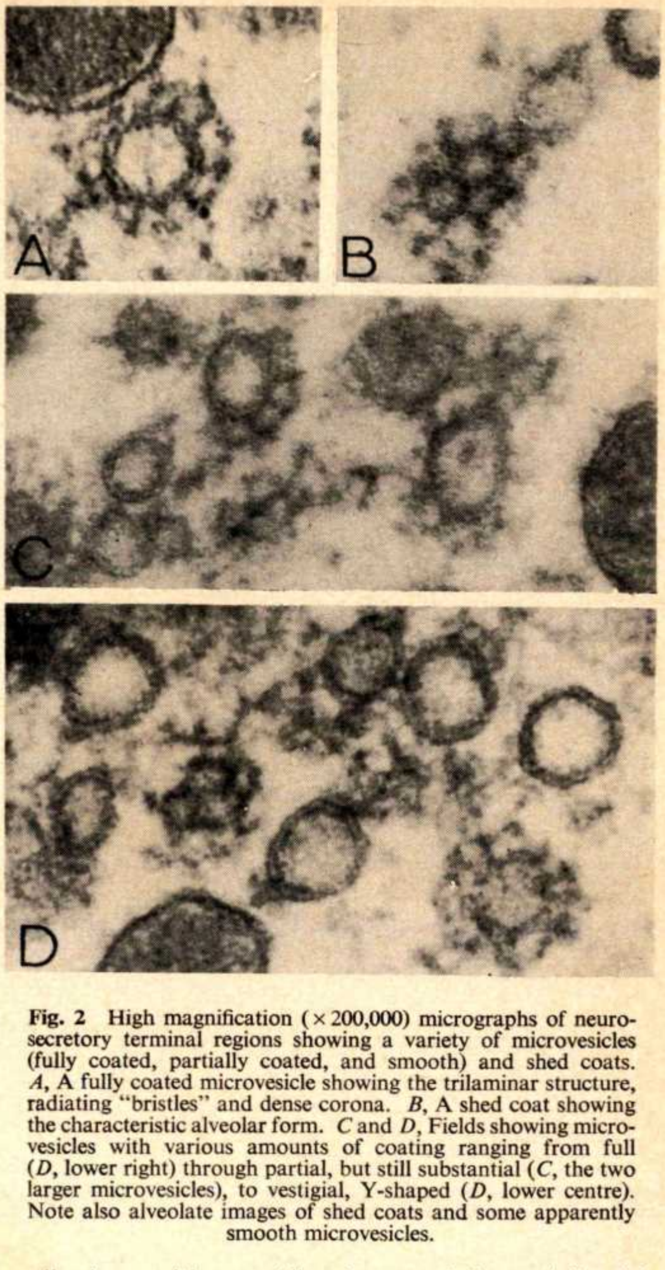


Fig. 2 High magnification (× 200,000) micrographs of neurosecretory terminal regions showing a variety of microvesicles (fully coated, partially coated, and smooth) and shed coats. *A*, A fully coated microvesicle showing the trilaminar structure, radiating "bristles" and dense corona. *B*, A shed coat showing the characteristic alveolar form. *C* and *D*, Fields showing microvesicles with various amounts of coating ranging from full (*D*, lower right) through partial, but still substantial (*C*, the two larger microvesicles), to vestigial, Y-shaped (*D*, lower centre). Note also alveolate images of shed coats and some apparently smooth microvesicles.

The appearance of the fully coated vesicle at higher magnification (Fig. 2*A*) conforms to the pattern found in other tissues[5]: a trilaminar vesicle resembling the smooth "synaptic" vesicle except for an investment of rather geometrical appearance suggestive of the spokes of a wheel or a corona of radiating bristles, commonly about ten in number, whose apices are joined and interstices filled by fainter electron densities. From their analysis of corresponding images from conventional neurones Kanaseki and Kadota[5] suggested they result from the superimposition of the walls of a honeycomb structure (or "basket") investing the vesicle.

Examples of partially coated and smooth vesicles at equally high magnification are presented in Fig. 2*C* and *D*. Scattered among them are faint alveolate figures in which no vesicle can be discerned. These were abundant in our material and with practice easily recognized. Their appearance, in favourable sections (Fig. 2*B*), corresponds closely with that depicted by Kanaseki and Kadota[5] and, following these authors, we take them to be shed coats. The model of the coated vesicle constructed by Kanaseki and Kadota has a surface composed of twelve pentagons and twenty hexagons. It follows that the figure depicted in Fig. 2*B*, with about ten alveolae, represents a large area of coating. Shedding of such large pieces would result in partially-coated vesicles with extensive denuded areas, but coating could be shed in smaller or larger pieces. Still greater loss of coating, by whatever means, seems likely to account for vesicles with only a few spokes or bristles (Fig. 2*D*, centre), and shedding of these vestiges would yield smooth vesicles. The sequence explains how smooth "synaptic" vesicles, devoid of the coating stigmatic of membrane vesiculation[4,5], can yet be of micropinocytotic origin.

Although coated vesicles are abundant in our material, it is understandable that they have hitherto received so little attention[2] in this much studied gland, for coats are not prominent without special staining such as uranyl extraction in block, and when reduced to a few spikes are inconspicuous although often recognizable by characteristic thorn shapes or Y shapes (Fig. 2*D*, centre). Moreover, the relative abundance of coated forms in glands fixed during stimulation suggests they are short-lived and refractory to capture by conventional methods.

The presence of so many coated vesicles in glands fixed during stimulation considered along with the evidence they shed their coats to become smooth "synaptic" vesicles, greatly strengthens our hypothesis on the micropinocytotic origin of these long-enigmatic structures. Further support of a quite different sort is provided in the following companion report (see next communication[7]).

This work was supported by grants from the US Public Health Service.

W. W. DOUGLAS
J. NAGASAWA
R. A. SCHULZ

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut 06510

Received April 6; revised June 22, 1971.

[1] Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **227**, 407 (1970).
[2] Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Mem. Soc. Endocrinol.* (in the press).
[3] Palay, S. L., in *Ultrastructure and Cellular Chemistry of Neural Tissue* (edit. by Waelsch, H.), 31 (Hoeber, New York, 1957).
[4] Roth, T. F., and Porter, K. R., *J. Cell. Biol.*, **20**, 313 (1964).
[5] Kanaseki, T., and Kadota, K., *J. Cell. Biol.*, **42**, 202 (1969).
[6] Karnovsky, M. H., *J. Cell. Biol.*, **35**, 213 (1967).
[7] Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **232**, 341 (1971).

Micropinocytotic Origin of Coated and Smooth Microvesicles ("Synaptic Vesicles") in Neurosecretory Terminals of Posterior Pituitary Glands demonstrated by Incorporation of Horseradish Peroxidase

ELECTRON microscopic evidence[1-3] has led us to believe that smooth microvesicles ("synaptic vesicles") within neurosecretory terminals of posterior pituitary glands arise from coated microvesicles formed by invagination and vesiculation of the cell surface following exocytosis (see preceding communication)[3].

This view implies that microvesicles of both sorts have been open, at some time, to the extracellular environment, and it should be possible to test this by determining whether they engulf marker substances that do not traverse the intact cell membrane. One such substance, horseradish peroxidase, has been used more than any other to demonstrate micropinocytosis in various cells[4-6]. We here report the presence of horseradish peroxidase in both coated and smooth "synaptic" microvesicles of neurohypophyseal terminals of rats and hamsters (the species in which exocytosis has been demonstrated[1,2]) after injecting this substance intravenously or introducing it to isolated glands incubated with excess K to induce secretion[7].

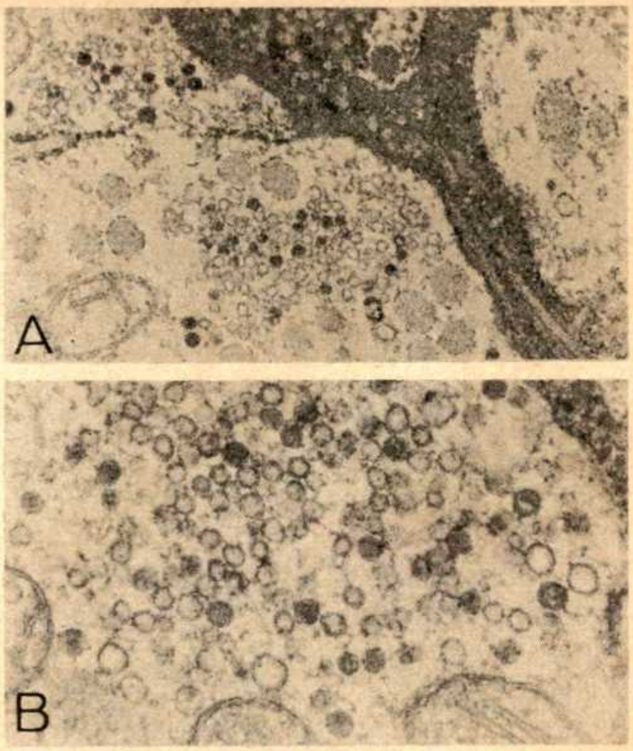


Fig. 1 Incorporation of horseradish peroxidase (HRP) into microvesicles within neurosecretory terminals of posterior pituitary glands of hamster and rat. *A*, Hamster (♂, 90 g), urethane anaesthesia (0.3 ml., 33% w/v given intraperitoneally). HRP 100 mg in 1 ml. Locke by jugular vein. Animal decapitated 10 min later and posterior pituitary fixed with a mixture of glutaraldehyde (3.4%) and paraformaldehyde (1%) in cacodylate buffer and processed for demonstration of HRP according to Karnovsky[4]. Note electron-dense reaction product indicative of HRP is present in numerous microvesicles as well as perineuronal spaces (× 38,000). *B*, Rat (♂, 190 g), unanaesthetized. The posterior pituitary was removed after decapitation and incubated in "high K Locke's solution"[7] containing HRP (100 mg/ml.) for 15 min at 37° C. Many microvesicles contain electron-dense reaction product (× 60,000).

The presence of horseradish peroxidase in many members of the microvesicle population in the two species is illustrated in Fig. 1 where the electron density of the reaction product has rendered these vesicles dark. No reaction product was found in microvesicles in control experiments where horseradish peroxidase was omitted. High power micrographs show most of the labelled microvesicles are smooth "synaptic vesicles" (Fig. 2*E*), but others are coated (Fig. 2*A*–*D*). As noted before[2], the coated vesicles commonly lie close under the plasmalemma in association with coated caveolae. This, taken together with the incorporation of horseradish peroxidase, leaves little doubt they are the immediate product of membrane vesiculation. The presence of horseradish peroxidase in smooth microvesicles establishes that they too are of micropinocytotic origin. But they are unlikely to arise directly by plasmalemmal vesiculation since coating is believed to be essential for the membrane buckling inducing vesiculation[8,9] and no caveolae were observed without coating in these or previous experiments[3] employing uranyl extraction which renders coats readily visible. The smooth vesicles more probably arise from the coated forms by the shedding of coats described in the preceding report[3].

With the micropinocytotic origin of many coated and smooth microvesicles now established, there remain two important questions. First: do all microvesicles in neurohypophyseal terminals arise in this way? This is feasible, for each exocytotic event involving one neurosecretory granule would require formation of some ten to twenty-five microvesicles if the cell surface is not to expand[1,2]. Failure to detect reaction product

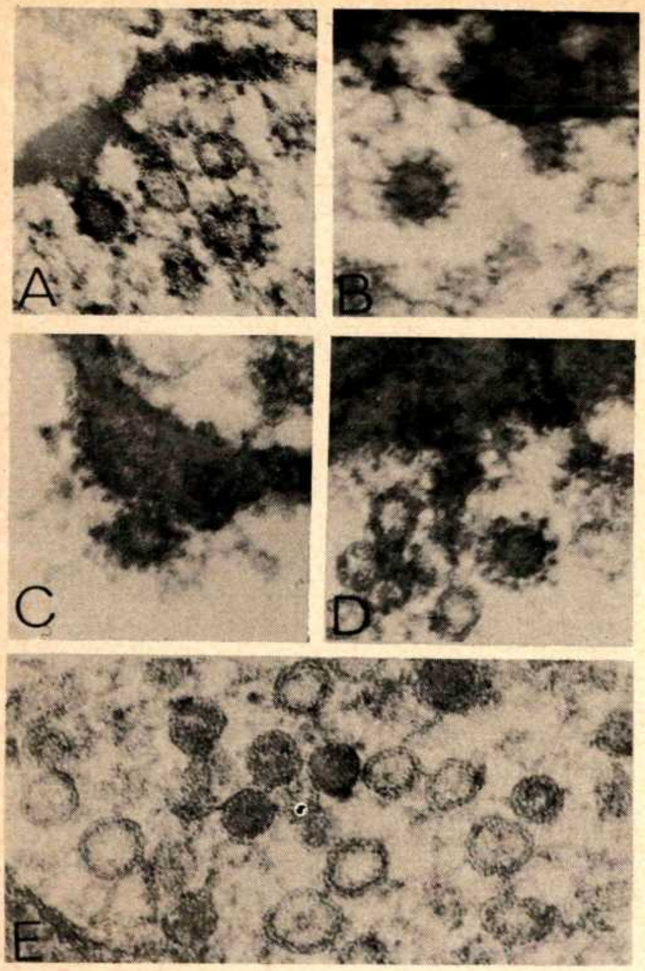


Fig. 2 Terminal portions of neurosecretory fibres showing evidence of micropinocytosis; coated caveolae and adjacent coated microvesicles containing horseradish peroxidase (HRP). (All × 128,000.) *A* and *B*, Hamsters. Pituitaries were removed and fixed 5 min after HRP (100 mg) had been given intravenously. Note coated caveolae in plasmalemma in both, and, in *B*, a coated microvesicle containing reaction product. *C* and *D*, Rats. Pituitaries were incubated *in vitro* (15 and 2.5 min respectively) in high K Locke containing HRP (100 mg/ml.). In *D*, a caveolus and adjacent coated microvesicle is filled with reaction product. In *C*, a coated microvesicle filled with reaction product lies close under a coated depression in the plasmalemma. This may correspond with the images previously interpreted[1,2] as micropinocytosis occurring within an exocytotic pit. *E*, Horseradish peroxidase in smooth microvesicles of the classical "synaptic vesicle" type. Same preparation as 1*B* but higher magnification (× 128,000).

in many microvesicles does not necessarily mean their origin is different: some were probably present before horseradish peroxidase was introduced, and others could have formed without admitting this bulky molecule; moreover, it is unlikely that histochemical detection is 100% efficient. Nevertheless, it is evident that alternative suggestions[10,11] concerning the origin and significance of microvesicles could still apply to some of the population.

The second question concerns the fate of the microvesicles. To this we can only repeat our previous conjecture that they are disposed of somehow, possibly by lysosomes[2]. They are not likely to be re-used for packaging of hormone since this takes place in the distant perikarya and not in the neurosecretory terminals[12]. But, as we pointed out at a symposium in Bristol last year[2], a similar tightly coupled exocytosis–micropinocytosis sequence could be of functional significance in ordinary nerve endings where local synthesis and packaging (of neurohumour) do occur[13] and allow for recycling of true synaptic vesicles. Support for this conjecture has since been provided by Gray and Willis[14] who, like us[3], have been inspired by Kanaseki and Kadota's work[9] and have obtained evidence, in ordinary nerves, that coated vesicles, probably of micropinocytotic origin, can give rise to smooth synaptic vesicles. But clearly our "recycling" hypothesis could only apply if neurohumours, like the neurohormones, are released by exocytosis. Electron microscopy has left this question unanswered since in ordinary nerve endings the caveolae, devoid of electron-dense material, could as well represent micropinocytosis as exocytosis[6]. Nevertheless, some electrophysiological[15] and biochemical[13] evidence favours exocytosis, and from ontogenetic and ionic parallels it has been argued that secretory mechanisms in ordinary neurones and neurosecretory fibres are probably similar[16].

This work was supported by grants from the US Public Health Service.

J. NAGASAWA
W. W. DOUGLAS
R. A. SCHULZ

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut 06510

Received April 6; revised June 22, 1971.

[1] Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **227**, 407 (1970).
[2] Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Mem. Soc. Endocrinol.* (in the press).
[3] Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Nature*, **232**, 340 (1971).
[4] Karnovsky, M. J., *J. Cell. Biol.*, **35**, 213 (1967).
[5] Friend, D. S., and Farquhar, M. G., *J. Cell. Biol.*, **35**, 357 (1967).
[6] Zacks, S. I., and Saito, A., *J. Histochem. Cytochem.*, **17**, 161 (1969).
[7] Douglas, W. W., and Poisner, A. M., *J. Physiol.*, **172**, 1 (1964).
[8] Roth, T. F., and Porter, K. R., *J. Cell. Biol.*, **20**, 313 (1964).
[9] Kanaseki, T., and Kadota, K., *J. Cell. Biol.*, **42**, 202 (1969).
[10] Bargmann, W., in *Handbuch der Experimentellen Pharmakologie* (edit. by Berde, B.), 1 (Springer, Berlin, 1969).
[11] Herlant, M., in *Neurosecretion* (edit. by Stutinsky, F.), 20 (Springer, Berlin, 1966).
[12] Sachs, H., in *Handbook of Neurochemistry*, **4**, 373 (Plenum Press, New York, 1970).
[13] Geffen, L. B., and Livett, B. G., *Physiol. Rev.*, **51**, 98 (1971).
[14] Gray, E. G., and Willis, R. A., *Brain Res.*, **24**, 14 (1970).
[15] Katz, B., *The Release of Neural Transmitter Substances* (Thomas, Springfield, Illinois, 1969).
[16] Douglas, W. W., *Brit. J. Pharmacol.*, **34**, 451 (1968).

Changes in ATP and Creatine Phosphate Storage in Skeletal Muscle of Rats trained at 900 and 7,600 Feet

THE hydrolysis of ATP provides the energy source for muscular contraction. ATP is either stored in the muscle or produced by phosphorylation of ADP by creatine phosphate or glycolytic processes, or through oxidative processes in the mitochondria. The processes of adaptation which facilitate the glycolytic or oxidative production of ATP have been studied in trained and acutely exercised animals. After work has been done by the muscle, the oxidative[1] and glycolytic[2,3] capacities of its fibres increase. Christensen[4] has shown that neither glycolysis nor oxidative processes take place during short anaerobic bursts of activity. The energy for such limited activities is provided by the hydrolysis of stored ATP and phosphorylation of ADP by creatine phosphate by the Lohman reaction. Previous exercise studies using endurance types of training have not shown any adaptation of either ATP or creatine phosphate[5–7], although electrical stimulation[8] and dwelling at high altitude[9] have produced increases in creatine phosphate.

Seventy Sprague-Dawley albino rats, 35 days old, were tested for their ability to run on a motor driven treadmill. Fifty-four animals were selected and placed into six groups of nine rats each. Three groups, sedentary, sprint trained and endurance trained, each lived in large dormitory cages, 28 inches by 13 inches by 8 inches, kept in ambient conditions

900 feet above sea level. Three other groups were housed in altitude cages of equal dimensions. These cages were maintained at a simulated altitude of 7,600 feet. The sprint and endurance trained rats at simulated altitude ran on a treadmill housed in a hypoxic atmosphere at ambient pressures, such that the inhaled oxygen pressure was equivalent to that at 7,600 feet. All the groups were maintained for 63 days.

Endurance trained animals were run with progressively increased work loads until they were running for 30 min at an average speed of 34 m min^{-1} and 30 m min^{-1} in the ambient and altitude conditions respectively. Throughout the training period the altitude endurance group ran at about 88% of the ambient group's speed. Sprint trained rats were all run at identical speeds regardless of the altitude condition. These rats were run for six to eight intervals of 15 s each with 1 min of rest between each interval. For the final 3 weeks of the study all rats ran at 81.1 m min^{-1}. The rats were run daily; they were run six days per week, beginning with the thirty-fifth day.

At the completion of the training period the rats were anaesthetized with sodium pentobarbitol, and the gastrocnemius–plantaris (as one unit) and the soleus were removed. The muscles were immediately frozen in isopentane chilled to liquid nitrogen temperature. All of the muscles were pulverized in a cold room, deproteinized in cold perchloric acid (30 ml./0.5 g frozen tissue) and analysed for ATP and creatine phosphate[10]. Data were analysed using a two-way analysis of variance for groups with uneven *n* values[11]. The Scheffe *post hoc* test[12] was used to determine significant differences between means.

Table 1 Molar Quantities of Measured Phosphate Compounds of the Soleus

	ATP	Creatine phosphate
Ambient control $N=8$	2.48	6.50
Ambient sprint $N=9$	2.15	5.03
Ambient endurance $N=4$	2.61	7.18
Altitude control $N=9$	2.28	6.28
Altitude sprint $N=7$	2.58	6.04
Altitude endurance $N=8$	2.20	5.99

Quantities in μmol/g of tissue (wet).

Table 2 Molar Quantities of Measured Phosphate Compounds of Gastrocnemius–Plantaris

	ATP	Creatine phosphate
Ambient control $N=8$	3.13±0.638	7.89±0.589
Ambient sprint $N=9$	3.77±0.754	8.60±2.104*
Ambient endurance $N=3$	3.45±1.006	7.49±1.966
Altitude control $N=9$	3.51±0.484	8.59±1.561
Altitude sprint $N=7$	3.68±0.711	10.82±1.876†
Altitude endurance $N=8$	3.64±1.136	8.78±1.965‡

Quantities in μmol/g of tissue (wet) ±s.d.

* Greater than ambient endurance ($P<0.05$).

† Greater than all other groups ($P<0.05$).

‡ Greater than ambient endurance ($P<0.05$).

Table 1 summarizes the results of the biochemical analyses of the soleus. Because of the muscle's small size all the soleus from each group was analysed as one sample and no statistical analyses were performed. There was little difference in the quantities of ATP or creatine phosphate between the groups. Table 2 summarizes the values for the gastrocnemius-plantaris. ATP values were not affected by either training or altitude ($P>0.05$). Sprint training affected creatine phosphate stores. The ambient sprint group had more creatine phosphate than the ambient endurance group ($P<0.05$). The altitude sprint group had more creatine phosphate than either the altitude control or endurance groups and also more than the ambient sprint group ($P<0.05$). Although the two control groups were not different ($P>0.05$), both of the altitude activity groups had greater quantities of creatine phosphate than their respective ambient counterparts. All of the gastrocnemius-plantaris values exceeded those of the soleus.

Changes in the storage of creatine phosphate did occur as a result of sprint training in the gastrocnemius-plantaris muscles which have a large area of white fibres[13,14]. Although the low altitude alone had no effect, the increase in creatine phosphate was potentiated by altitude for the running groups. This would suggest that even at 7,600 feet there is a hypoxaemic state which is greater than that even in the sprinting muscle at sea level.

The difference between the soleus and the gastrocnemius–plantaris would be the expected relationship between red and white muscle, red muscle being lower in the high energy phosphates. The fact that the quantities in the soleus were not affected by sprinting would suggest that red muscle is not used during this type of activity. Some fibres of the gastrocnemius–plantaris, however, were affected by endurance running at altitude. It remains to be seen whether the fibres which increased their creatine phosphate stores as a result of altitude endurance training were red, white or intermediate. It is assumed that the white fibres did adapt to short, anaerobic stresses by increasing the quantities of creatine phosphate available for phosphorylation of ADP.

This work was supported by the Wisconsin Heart Association.

JAMES B. GALE

Departments of Health Sciences and Physical Education,
Sonoma State College,
Rohnert Park, California 94928

FRANCIS J. NAGLE

Departments of Physiology and Physical Education,
University of Wisconsin,
Madison, Wisconsin 53706

Received August 27, 1970; revised March 27, 1971.

[1] Holloszy, J. O., *J. Biol. Chem.*, **242**, 2278 (1967).

[2] Barnard, J., and Peter, J. B., *J. Appl. Physiol.*, **27**, 691 (1969).

[3] Peter, J. B., Jefferson, R. N., and Lamb, D. R., *Science*, **160**, 200 (1968).

[4] Christensen, E. H., Hedman, R., and Salten, B., *Acta Physiol. Scand.*, **50**, 269 (1960).

[5] Harris, J. W., *Res. Quart.*, **38**, 598 (1967).

[6] Rulcker, C., Lundberg, P., and Lamek, N., *Acta Vet. Scand.*, **8**, 189 (1967).

[7] Procter, H. A., and Best, C. H., *Amer. J. Physiol.*, **100**, 506 (1932).

[8] Palladin, A., and Ferdmann, D., *Z. Physiol. Chem.*, **174**, 284 (1928).

[9] Tappan, D. V., Reynafarje, D. B., Potter, V., and Hurtado, A., *Amer. J. Physiol.*, **190**, 93 (1957).

[10] Lamprecht, W., and Trautschold, I., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. V.) (Academic Press, New York, 1963).

[11] Edwards, A. L., *Experimental Design in Psychological Research* (Holt, Rinehart, and Winston, New York, 1950).

[12] Hays, W. L., *Statistics* (Holt, Rinehart, and Winston, New York, 1963).

[13] Romanul, F. C. A., *Arch. Neurol.*, **11**, 355 (1964).

[14] Stein, J. M., and Padykula, H. A., *Amer. J. Anat.*, **110**, 103 (1962).

Fertilization of Denuded Rabbit Eggs *in vitro* by Sperm recovered from the Uterus or Vagina

RECENTLY shed eggs, are surrounded by a sticky mass of follicular cells known as the cumulus oophorus. The follicular cells, which are closely attached to the zona pellucida and are arranged in a compact radial pattern, are called the corona radiata. It has been postulated that the follicular cells in the cumulus oophorus can protect the egg against polyspermic fertilization, and can improve the chance of fertilization by providing a large target for the sperm. Further, due to the radial arrangement of the corona radiata, these follicular cells can act in orientating the sperm towards eggs[1]. After the dissolution of cumulus oophorus by treatment with hyaluronidase *in vitro*, however, rabbit eggs can be fertilized when transferred into the tubes of mated rabbits[2,3]. After the dissolution of cumulus oophorus and removal of the corona radiata by manual shaking, the denuded eggs can also be fertilized *in vitro*[4] or *in vivo*[5,6]. A comparison of the proportion of eggs fertilized *in vitro* between denuded eggs and those with follicular cells intact has not been made. In addition, it has been speculated that the progesterone present in the follicular fluid or synthesized by the granulosa cells may be necessary for the "acrosome reaction", capacitation of sperm, and the fertilization of eggs *in vitro*[7]. The experiment described here demonstrates (1) that denuded rabbit eggs lacking follicular cells (or progesterone) can be fertilized equally as well as intact eggs with cumulus oophorus, and (2) that sperm recovered from the vagina can fertilize rabbit eggs *in vitro* as well as those recovered from the uterus.

Table 1 Fertilization of Denuded Rabbit Eggs *in vitro* by Sperm recovered from the Uterus or the Vagina

Collection site and No. of sperm (millions/ml.)	Denuded eggs			Eggs with follicular cells intact		
	Total	No. fertilized	Percentage fertilized	Total	No. fertilized	Percentage fertilized
Uterus (0.06–0.17)	48	16	33	92	27	29
Vagina (0.23–4.2)	45	24	53	30	14	47
Ejaculate (0.97–5.1)	41	0	0	32	0	0

Mature female rabbits were injected either subcutaneously or intramuscularly with 170 IU of PMS to induce growth of a large number of follicles and 72 h later they were injected intravenously with 90 IU of HCG to induce ovulation. Approximately 15 h after injection of HCG the Fallopian tubes were removed and flushed with a tissue culture medium (TC 199) to recover the eggs. Immediately after recovery, one group of eggs in the mucous clot was placed in a watch glass containing 0.5 ml. of 0.1% hyaluronidase in TC 199 and the other group was placed in another dish containing no hyaluronidase. After the dissolution of the cumulus (about 10 min), the hyaluronidase-treated eggs were placed in a small test tube (4 cm long) and denuded by being shaken vigorously by hand for about 5 min. The denuded eggs were then placed in another dish containing TC 199. The procedures for fertilizing rabbit eggs are essentially similar to those reported previously[8].

For the recovery of capacitated sperm, one female was mated consecutively by three fertile bucks. It was anaesthetized with nembutal 14–18 h after mating. After making a medial incision the uterine horn or vagina was flushed with 2–3 ml. of a freshly prepared medium[9]. About 1 ml. of uterine or vaginal washing (containing strongly motile sperm) was placed in a 1.5 ml. Carrel flask. The denuded eggs were placed into one flask prepared in this way, and the intact eggs with cumulus clot were placed into another. The flasks were closed with a rubber bulb and attached to a gentle rocking device in an incubator at 38° C. After 3–4 h the eggs were picked up and subcultured in a 6 ml. capacity Carrel flask containing 4 ml. of TC 199 incorporating 20% heated rabbit serum. After incubation for another 18–20 h, the eggs were taken out, mounted on a slide and examined. They were then fixed with neutral formalin, stained for the assessment of fertilization[2,8], and examined again. Usually a second maturation chromosome pattern or a group of small nuclei can be seen in the unfertilized eggs. Most of the fertilized eggs are at the four-cell stage. Fragmentation of fertilized and especially unfertilized eggs is fairly common.

The results presented in Table 1 indicate that there is no difference in the proportion of eggs fertilized *in vitro* whether the eggs are denuded or not. The presence of follicular cells (or progesterone) does not appear to play a role, therefore, for the fertilization of rabbit eggs *in vitro*. We have also discovered that the proportion of eggs fertilized by vaginal sperm is significantly higher than by uterine sperm. Whether this is due to a higher concentration of sperm or better vitality of sperm in the vaginal washing is still to be determined.

This work was supported by grants from the National Institute of Child Health and Human Development, US Public Health Service, and the Ford Foundation.

M. C. Chang
A. Hanada
Dorothy M. Hunt

Worcester Foundation for Experimental Biology,
Shrewsbury,
Massachusetts

Received April 13, 1971.

[1] Austin, C. R., *The Mammalian Egg*, 100 (Blackwell Publications, Oxford, 1961).
[2] Chang, M. C., *J. Exp. Zool.*, **121**, 351 (1952).
[3] Dickmann, Z., *J. Anat.*, **98**, 392 (1964).
[4] Bedford, J. M., and Chang, M. C., *Nature*, **193**, 898 (1962).
[5] Chang, M. C., and Bedford, J. M., *Fert. and Ster.*, **13**, 421 (1962).
[6] Harper, M. J. K., *J. Exp. Zool.*, **173**, 47 (1970).
[7] Edwards, R. G., Steptoe, P. C., and Purdy, J. M., *Nature*, **227**, 1304 (1970).
[8] Chang, M. C., *Nature*, **184**, 466 (1959).
[9] Brackett, B. G., *Schering Symposium on Mechanisms Involved in Conception*, 73 (Pergamon Press, Vieweg, 1970).

Role of Skin and Neurohypophyseal Hormones in the Adaptation of the Toad *Bufo viridis* to High Salinities

Many people maintain that ADH is important in the control of water and salt metabolism of amphibia[1]. The toad *Bufo viridis* is one of the species most resistant to dehydration and high ambient salinity, in which situations it has a high plasma salt concentration. In toads adapted to high salinity, the urine sodium chloride concentration increases to about plasma level, whereas active sodium transport, as measured by short circuit current, is almost completely reduced (ref. 2 and our measurements). The animals apparently do not drink and water is passively transferred along the osmotic gradient from the ambient solution into the body. We found that the sensitivity to added hormone was lost from the skins of animals that were adapted to saline solutions above 400 mosm.

We studied the water flux across isolated portions of skin in saline-adapted animals. The skin was carefully dissected and attached to a 'Perspex' conus, the inside facing out, and the preparation was maintained in aerated Ringer solution (NaCl 120 mmol l.$^{-1}$; KCl 2.5 mmol l.$^{-1}$; $CaCl_2$ 2 mmol l.$^{-1}$ at $pH=8$). The inner solution was hypotonic, and water transfer was followed gravimetrically on a 0.1 mg precision Metler balance. The temperature was maintained at 20° ± 1° C. A typical experiment is shown in Fig. 1. When skin was used from animals adapted to high saline, osmotic equilibrium was adjusted with sodium chloride. Synthetic oxytocin ('Syntocinon', Sandoz) was used throughout.

Skin from toads kept in either tap water of 230 mosm solution (containing either sodium chloride or sucrose) was almost impermeable to water; the application of hormone to the external bath (serosal side) stimulated the water flux across the skin. When skin from toads adapted to 400 mosm NaCl solution was tested, water flux occurred as soon as there was a gradient across the skin; the application of hormone to the external bath did not have any further effect.

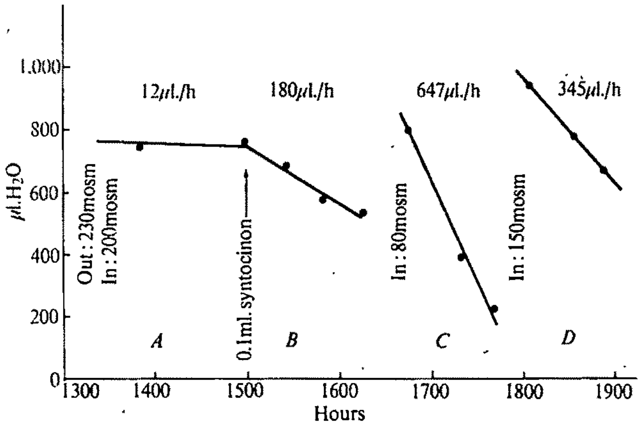


Fig. 1 Results of a typical experiment of the *in vitro* method to follow water flux across the isolated skin. In the first period (*A*) an osmotic gradient was imposed across the skin, with its inside facing outwards. At the arrow, hormone was added to the external medium and weight change followed. In the two successive periods (*C*, *D*) different gradients were imposed. The rate of water flux for each period is indicated above.

Although the solutions used in the latter case were hypertonic to normal Ringer of amphibia, they were definitely not hypertonic to the preparations being studied, which were always in osmotic equilibrium. To check this, however, we tested skin from normal animals in hypertonic solution and skin from 500 mosm NaCl-adapted animals in hypotonic solution. An effect similar to that of ADH was observed in the first case, which was similar to observations[3] on frog urinary bladder. In the second case, the change of the solution did not change the properties of the skin, and water transfer ensued without the addition of hormone.

In animals adapted to 400 mosm NaCl and then brought back to tap water through 230 mosm NaCl, the effect was reversible in that the responsiveness of the skin to the hormone was restored.

The increased permeability of the skin to water, which is maintained without the addition of hormone, seems to be very important to the water metabolism of the toad. To ascertain the amount of hormone in the circulation, we used the *in vitro* assay[4] of hormone on isolated normal bladders. Blood collected from three or four animals was pooled, centrifuged in the cold, and 2 ml. of the plasma was added to 10 ml. of the external solution. No response was observed with plasma from animals that were maintained in either tap water, 230 mosm or 450 mosm NaCl. Hydro-osmotic activity was found, however, in the plasma of animals dehydrated in 2.5% NaCl for 3 h (ref. 5). In another series of experiments the hormonal activity of the hypophysis was investigated semi-quantitatively by using the *in vitro* method with the addition of neurohypophyseal extract. The results showed the existence of almost the same activity in the animals from either tap water, 230 mosm NaCl or 400 mosm NaCl solutions.

It seems that adaptation to high salinity in these toads leads to some fundamental physiological changes in the skin. It is of interest to mention that Dicker and Elliott[6] working on *Rana crancrivora*, a euryhaline species inhabiting brackish mangrove swamps, also found an apparent lack of hydro-osmotic response to neurohypophyseal peptides in the isolated bladder.

We are at present investigating whether the changes in the skin that we found are also reflected at the ultrastructural level.

URI KATZ
JUDITH WEISBERG

Department of Zoology,
Hebrew University of Jerusalem

Received December 14, 1970; revised March 12, 1971.

[1] Deyrup, I. J., in *Physiology of the Amphibia* (edit. by Moore, H. J.) (1964).
[2] Gordon, M. S., *J. Exp. Biol.*, **39**, 261 (1962).
[3] Pipoche, P., Parisi, M., and Bourguet, J., *Biochim. Biophys. Acta*, **193**, 231 (1969).
[4] Bentley, P. J., *J. Endocrinol.*, **17**, 201 (1958).
[5] Bentley, P. J., *J. Endocrinol.*, **43**, 359 (1969).
[6] Dicker, S. E., and Elliott, A. B., *J. Physiol.*, **210**, 137 (1970).

Sterilization of *Aedes aegypti* (L.) as a Result of Chronic Exposure to Low Doses of Beta Radiation

THE application of the sterile male technique for the control of insect populations depends on the release of an adequate number of sterilized males. Whitten and Taylor[1] have suggested that the effectiveness of the method might be enhanced by releasing sterile females along with the sterilized males. In the case of mosquitoes, however, any addition to the number of biting females already present in a native population would be unwelcome.

So far efforts to use the sterilized insect for mosquito population control have generally involved insects that were irradiated during pupal or adult stages. We have succeeded in sterilizing more than 70% of the adults of laboratory populations of *Aedes aegypti* (L.) by exposing them to a total dose of beta radiation estimated to be as small as 90 rad during the early larval stages. This dose is about one-tenth of the X-ray doses which have been used to sterilize the pupal or adult stages[2]. Willard[3] has studied the long term effects of acute X-ray irradiation of the egg, larval and pupal stages of *A. aegypti*, on the population dynamics of that species.

If sterilized males and females are to be used for mosquito population control, it is important that a significant number of viable eggs is not laid as a result of their mating. Twelve groups of 100 newly hatched *A. aegypti* larvae were irradiated for 192 h during instars I to IV, by rearing them at 24±1° C in media containing 0.1, 1.0 and 10 μCi/ml. ^{90}Sr in equilibrium with its daughter product ^{90}Y. The amounts of ^{90}Sr and ^{90}Y accumulated by the larvae were quite variable, so it was not feasible to calculate the dose delivered. The use of sealed $^{90}\mathrm{Sr}+{}^{90}\mathrm{Y}$ plaques placed in the rearing medium will deliver only an external dose which can be calculated. Controls were exposed to natural background radiation only. Hatching and rearing methods were essentially those described by Riordan[4]. After 192 h, instar IV larvae were removed from the radioactive media, rinsed and placed in demineralized water to pupate. The adults were allowed to mate. Males were offered a 50% solution of honey in water. Females were also allowed to feed on guinea-pigs. Blood-feeding from guinea-pigs, and egg laying were observed. The effects on egg laying of mating irradiated males with non-irradiated females, and *vice versa*, were not examined in these experiments.

Larvae reared in 10 μCi ^{90}Sr/ml. did not pupate, while those reared in 0.1 and 1.0 μCi/ml. developed normally and the adults had no gross external morphological abnormalities. The development of the ovaries and the testes, however, had been arrested.

Table 1 Effect of Continuous Beta Irradiation of Instar I to IV Larvae on Feeding and Egg Viability of Adult *A. aegypti*

^{90}Sr concentration (μCi/ml.)	Males		Females	
	Took honey-water	Took blood meal	Laid eggs	Viability of eggs laid
Background	Yes	Yes	Yes	80%
0.1	Yes	Yes	No	—
1.0	—†	No	No	—
10.0*	—	—	—	—

* Larvae failed to pupate and died 25 days after moulting to instar IV.

† Not observed.

Feeding and egg laying are summarized in Table 1. All females were dissected 10 days after those in control groups laid eggs, or when they became moribund. Females irradiated as larvae laid no eggs. In addition, females in the 1.0 μCi ^{90}Sr/ml. treatment groups took no blood. Dissection showed that most of the radiation-treated females had motile sperm in their spermathecae, but their ovaries were atrophied. According to Bugher and Taylor[5], failure to produce eggs is the first indication of abnormality caused by increasing radiation dose. Leahy and Craig[6] have reported an accessory gland substance, transmitted from the male to the female during copulation, to be a stimulant for oviposition in *A. aegypti* and *A. albopictus*. The irradiated females in our experiments may not have received this substance. Smittle[7], studying the effects of gamma radiation on *Culex pipiens quinquefasciatus*, suggested that sterilization of the females was the result of inhibition of egg production. Although some of the irradiated females in our experiments took blood (Table 1), their ovaries were atrophied, and therefore eggs could not be produced.

Our observations, that females reared in 1.0 μCi ^{90}Sr/ml. took no blood meals and laid no eggs, suggest that sterile females might usefully be used in combination with sterile males to control mosquito populations. The production of large numbers of larvae[8], irradiated externally by means of sealed beta sources, would not be difficult. Investigations of the effects of chronic external beta and gamma radiation on mosquito populations are in progress.

J. E. GUTHRIE

Whiteshell Nuclear Research Establishment,
Atomic Energy of Canada Limited,
Pinawa,
Manitoba

R. A. BRUST

Department of Entomology,
University of Manitoba,
Winnipeg,
Manitoba

Received March 4, 1971.

[1] Whitten, M. J., and Taylor, W. C., *J. Econ. Entomol.*, **63**, 269 (1970).
[2] Willard, W. K., *Radiat. Res.*, **37**, 493 (1969).
[3] Willard, W. K., in *Radiation and Terrestrial Ecosystems* (edit. by Hungate, F. P.), *Health Phys.*, **11**, 1577 (1965).
[4] Riordan, D. F., *Canad. J. Zool.*, **43**, 497 (1965).
[5] Bugher, J. C., and Taylor, M., *Science*, **110**, 146 (1949).
[6] Leahy, M. G., and Craig, G. B., *Mosquito News*, **25**, 448 (1965).
[7] Smittle, B. J., *Florida Entomologist*, **51**, 59 (1968).
[8] Fay, R. W., McCray, M., and Kilpatrick, J. W., *Mosquito News*, **23**, 210 (1963).

Transmission of *Phytophthora* Pod Rot of Cocoa by Invertebrates

Phytophthora palmivora (Butl.) Butl., the causal agent of black pod of cocoa (*Theobroma cacao* L.), accounts for an estimated 19% crop loss in Ghana[1], and losses are considerably higher in wetter countries such as Nigeria and the Cameroun Republic. The epidemiology of the disease is still uncertain but rain-splash from soil is considered by some workers[2,3] to trigger-off disease cycles.

During a study of 200 mature cocoa trees, black pod lesions were consistently associated with pods colonized by tent building ants. These tents serve as shelters for coccids (scale insects) which usually feed on the pod peduncle. The coccids are tended by ants which remove excess sugars for their own use. An extensive survey of black pod–ant tent relationships has been undertaken and five stands of mature cocoa trees, comprising ant-free and ant-colonized trees, have been selected for comparative studies. Preliminary results indicate a positive correlation between certain tent building ant species and the levels of black pod. The type of material used in the construction of the tents is the most important factor involved. For this reason ants of the genus *Crematogaster*, and specifically *C. striatula* Emery, appear to be the most important vectors of black pod. These ants remove the outer layers of healthy and diseased pods and use the material to construct tents and other temporary shelters. *C. striatula* in well shaded cocoa can assume complete dominance over wide areas. Black pods left on the ground after harvesting or left hanging on the tree are used as building material and small discrete tents are constructed primarily around the pod peduncle. The ants are, therefore, effectively transferring infected pod material from the ground or from on the tree sources to healthy pods. Ants moving from the base of the tree upwards have been trapped and in several cases material containing spores of *P. palmivora* was being carried for use in tent construction. *C. africana*, *C. clariventris* and *C. depressa*, in addition to *C. striatula*, have all been observed scraping and carrying away material from black pods left hanging on the tree or lying on the ground.

Other ant species seem to be more incidental in the spread of black pod as they rarely use diseased pod material in tent construction. *Camponotus acvapimensis* builds very large tents composed almost entirely of soil. Soil infested with spores of *P. palmivora* may be carried to healthy pods in "soil balls" and ultimately cause infection. *Pheidole* spp., mainly *P. megacephala*, and *Acantholepis capensis* also build large soil tents, although almost any freely available material may be included. The importance of these ants in the dissemination of black pod will depend very much on the amount of viable soil inoculum present in a particular area.

Tent building ant species, therefore, may be important vectors of black pod causing local epiphytotics. In particular, it is thought that *C. striatula* because of its building habits and its complete dominance under certain conditions may be responsible for serious black pod losses. The soil-tent ant species, although less directly involved in the dissemination of diseased material, may initiate disease outbreaks by carrying inoculum in soil to healthy pods and creating primary infection foci; secondary spread by rain-splash may follow. The proportion of cocoa trees colonized by tent building ants may be very high[4] and their significance in the vertical spread of black pod should not be overlooked.

It is considered that ants are responsible primarily for vertical disease transmission, but horizontal spread may be attributable to other invertebrate vectors. Thorold[5] noted the absence of infection gradients in the field and concluded that horizontal dispersion of black pod follows a random pattern of the type expected for an airborne disease. Nevertheless, sporangia of *P. palmivora* have never been isolated from the air[6,7] and horizontal spread is still speculative and largely unexplained. Microscopic examination of faeces of herbivorous invertebrates commonly found on black pod revealed the presence of *P. palmivora* spores. Faecal material, wound-inoculated into healthy pods, produced typical black pod lesions. Viable spores have now been recorded from the faeces of eight beetle species, three millipede species, four caterpillar species and four snail (slug) species. Beetles are by far the most prevalent invertebrates on black pods and one species in particular (family *Nitidulidae*) occurs on 50–60% of black pods. The invertebrates feed on the outer tissues of black pods and in the process ingest mycelium and spores of *P. palmivora*. These remain intact during passage through the gut and are ejected together with cutin, pod hairs and stomatal guard cells. An extremely high percentage of the sporangia pass through the alimentary tracts of snails, millipedes and caterpillars without damage and pod inoculations from these faeces invariably cause infection. Most sporangia in beetle faeces are devoid of contents and infectivity is low. It is thought that at a certain stage in development the sporangial papilla becomes susceptible to enzyme attack and the sporangial contents are digested by the insect. Chlamydospores seem to remain unchanged after ingestion by invertebrates.

Flying insects, carrying inoculum of *P. palmivora* in the faeces, may explain the random occurrence and "over-dispersion" of black pod recorded by Thorold[5,8] which led him to suggest air dispersal as a means of dissemination. The importance of the other invertebrates in the transmission of black pod is difficult to assess. But the widespread occurrence of these animals in cocoa farms implies that they may be responsible for some horizontal spread. By feeding on black pods left on the ground after harvesting they may move around large amounts of inoculum, possibly introducing spores in previously disease free soil from where they may be dispersed by ants or rain-splash.

The answer to black pod control may lie partly in the efficient application of insecticides and Weststeijn[9] noted that the addition of a DDT-formulation to lime Bordeaux mixture distinctly improved the control of black pod. The effective removal of diseased pods from the cocoa farm after harvesting is an obvious control method, thereby limiting the amount of infective material available to tent-building ants and herbivorous invertebrates. This preliminary evidence of the transmission of black pod by invertebrates may partly explain the vertical and horizontal spread of this economically important pathogen.

I would like to thank Mr B. Bolton for initial identification of the ant species. I am a member of the British Research Team of the British Overseas Development Administration.

H. C. EVANS

Cocoa Research Institute (CSIR),
Tafo,
Ghana

Received March 22, 1971.

[1] Blencowe, J. W., and Wharton, A. L., *Rep. 6th Commw. Mycol. Conf. Kew*, 139 (1961).
[2] Grimaldi, J., *Rep. Cocoa Conf.*, London, 90 (1957).
[3] Okaisabor, E. K., *Nigerian Agric. J.*, 2, 67 (1965).
[4] Strickland, A. H., *Rep. W. Africa Cocoa Res. Inst., 1947–48*, 33 (1949).
[5] Thorold, C. A., *Nature*, 170, 718 (1952).
[6] Wharton, A. L., *Rep. W. Afr. Cocoa Res. Inst., 1954–1955*, 56 (1955).
[7] Okaisabor, E. K, *Third Intern. Cocoa Res. Conf., Accra, Ghana, 1969* (in the press).
[8] Thorold, C. A., *Trans. Brit. Mycol. Soc.*, 38, 435 (1955).
[9] Weststeijn, G., *Ann. Rep. Cocoa Res. Inst. Nigeria, 1966–1967*, 75 (1968).

Metabolic Implications of Polymorphism as an Adaptive Strategy

ENZYME polymorphisms are known to be widespread in natural populations[1–3]. No adequate theory has been advanced to account for their frequent occurrence; some workers support the proposition that they are selectively "neutral"[4–6], while others present evidence that they are maintained in natural populations through natural selection rather than by non-selective processes[1,7–9].

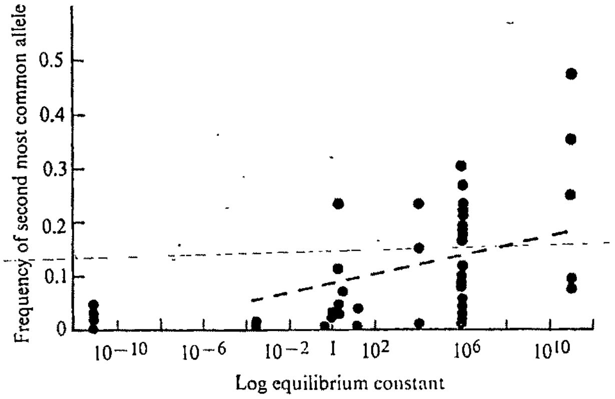


Fig. 1 Relationship of polymorph frequencies to equilibrium constant. For each of fourteen enzymes, the reported frequency of the second most common allele for a given population is plotted as a function of the corresponding equilibrium constant. The coefficient of correlation for a linear regression analysis of these data is 0.483, significant at the 0.1% level. Non-specific enzymes were not analysed. $Fn4 = 27.08$, $P \ll 0.005$.

The hypothesis that selection acts to maintain enzyme polymorphisms in natural populations has important metabolic implications not previously considered. Within any metabolic pathway a given catalyst will affect the rate of end-product formation according to its role in the kinetics of the overall sequence; those enzymes which exert acute control over flow through the pathway (that is, irreversible and rate-limiting steps) should be most individually sensitive as sites of action of selective forces (in contrast to selection on generalised metabolic systems[10]). Thus if natural selection affects the output of metabolic pathways by modulating the extent of molecular heterogeneity among the enzymes of the pathway, such selection should be most efficient if applied at those metabolic steps which most specifically control flow through the pathway.

The results of applying selection to enzymes with different kinetic roles in metabolism can be seen most clearly in irreversible reactions. In a freely reversible reaction, mass action must strongly dilute the effect of any change in the kinetics of the reaction on previous or subsequent reactions. An irreversible reaction may act by mass action to promote the operation of the pathway both proximal and distal to it, previous reactions being drawn along by its removal of substrate, and subsequent reactions being driven by its products; thus the activity of an enzyme catalysing an irreversible reaction has effects extending over the whole pathway. The action of selection in maintaining a natural, stable enzyme polymorphism would be enhanced if applied to such an "acute control" point, as it would critically affect the efficient operation of the entire metabolic sequence.

Table 1 Enzyme Polymorphism in *Drosophila*

Enzyme	K_{eq}	Sample size	Populations significantly polymorphic (%)	Index of polymorphism
Alcohol dehydrogenase (1.1.1.1)	1×10^{11} (19)	1,150	100	0.330
Aldehyde oxidase (1.2.3.1)	2×10^{6} (20)	850	66	0.120
Xanthine dehydrogenase (1.2.3.2)	Very large (21)	703	66	0.131
α-Glycerophosphate dehydrogenase (1.1.99.5)	1×10^{4} (12)	831	40	0.083
Glucose-6-phosphate dehydrogenase (1.1.1.49)	3×10^{2} (20)	527	28	0.054
Hexokinase (2.7.1.1)	155 (23)	141	50	0.007
Isocitrate dehydrogenase (1.1.1.41)	20 (20)	743	40	0.029
Malic enzyme (1.1.1.40)	20 (24)	687	20	0.012
Phosphoglucomutase (2.7.5.1)	17 (25)	464	75	0.137
Fumarase (4.2.1.2)	4 (26)	577	0	0.013
6-Phosphogluconate dehydrogenase (1.1.1.44)	1 (27)	187	0	0
Phosphohexose isomerase (5.3.1.9)	0.3 (28)	251	0	0.002
Aldolase (4.1.2.13)	2×10^{-4} (29)	515	0	0.006
Malate dehydrogenase (1.1.1.37)	7×10^{-13} (20)	1,012	10	0.015

Reported values of the equilibrium constants of the fourteen enzymes are listed; for enzymes with variation in reported values, the value closest to 1.0 was chosen. In all cases, the physiological direction of the reaction in insects was used in determining the equilibrium constant. Also listed for each enzyme is the sample size, *n* (the total number of individuals from all populations examined for a given enzyme), the percentage of the populations examined in which high frequency polymorphisms (two or more alleles with frequencies $\geq 4\%$) are reported, and the index of polymorphism $(1-P)$.

These metabolic considerations predict that if enzyme polymorphisms are maintained in natural populations by selective forces, a survey of the occurrence of polymorphisms in various enzymes of individuals of natural populations would reveal a greater degree of polymorphism in enzymes catalysing physiologically irreversible reactions; significantly less polymorphism would be expected for enzymes whose reactions are freely reversible, or possess equilibria strongly favouring the reverse of the physiologically significant direction.

Unfortunately, most surveys of enzyme polymorphism reported so far cannot adequately test this hypothesis, as they have been conducted on very small samples of natural populations[1,11–13]. Investigations of inbred laboratory strains[14] are unsatisfactory for this purpose as the selective advantage of polymorphisms in such strains is less clear. Within the past few months, however, extensive surveys of major metabolic enzymes in natural populations of numerous species of *Drosophila* have been reported[15–17], which allow a meaningful test of the hypothesis outlined here.

Table 1 is a compilation of these studies; the frequencies of polymorphism for as many as fourteen enzymes in individuals collected from a total of ten populations of *Drosophila* (six species) were reported. Although the frequency with which a given polymorphism occurs may vary for the different populations (as the selective factors maintaining it may vary), the examination of data obtained from many populations should minimize the effects of this problem and reveal a consistent pattern, if there are metabolic reasons for certain reactions being sensitive targets of polymorphism-promoting selection. Such a pattern exists, as is clearly demonstrated in Fig. 1, in which the polymorphic allele frequencies of the fourteen enzymes are plotted against their equilibrium constants: the frequencies observed are markedly higher for reactions, with equilibria which lie far to the right.

To obtain an estimate of the average degree of polymorphism associated with each enzyme, the frequency of an enzyme's most common allelic class in each population is multiplied by the sample size of that population, and the products for all populations used to calculate a frequency P

$$P = \Sigma f_i x_i / \Sigma x_i$$

where, for sample i, f is the frequency of the most common allele and x is the sample size. P is thus the average frequency, weighted for sample size, with which the class "most common allele" occurs. One may then consider $(1-P)$ as an index of polymorphism, values of which calculated from the reported surveys suggest that a close correspondence exists between the frequency of enzyme polymorphism and the equilibrium constants of the reactions (Fig. 2).

This same correspondence can be seen in the distribution of examined populations in which high frequency polymorphisms ($\geq 4\%$) are reported (Table 1): high frequency polymorphisms are reported in every case where K_{eq} is large, while no high frequency polymorphisms are reported for freely reversible reactions.

Recent data on the frequency of enzyme polymorphism in extensive studies of human populations are consistent with the above findings[18]: high frequency polymorphisms are reported for glucose-6-phosphate dehydrogenase and phosphoglucomutase, and are not found for 6-phosphogluconate dehydrogenase.

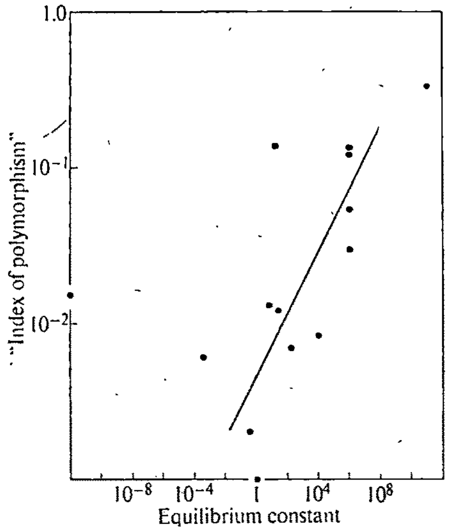


Fig. 2 Relationship of "index of polymorphism" to equilibrium constant. The "index of polymorphism" values are those of Table 1, and reflect the frequency in an average population with which the most common allele of an enzyme does not occur.

Thus, as theoretical considerations predict, a relationship is seen between the occurrence of enzyme polymorphisms and the equilibrium constants of the enzyme reactions involved. The existence of such a relationship argues strongly that enzyme polymorphisms are generally of adaptive significance in nature, and suggests that the magnitude of the observed enzyme polymorph frequencies in natural populations reflects not only the strength of the selection maintaining the polymorphism, but also the "responsiveness" of the metabolic step to that selection.

GEORGE B. JOHNSON

Department of Biological Sciences,
Stanford University,
Stanford, California 94305

Received December 21, 1970; revised March 12, 1971.

[1] Lewontin, R., and Hubby, J., *Genetics*, **54**, 595 (1966).
[2] Burns, J., and Johnson, F., *Science*, **156**, 93 (1967).
[3] Harris, H., *Proc. Roy. Soc.*, B, **174**, 1 (1969).
[4] King, J., and Jukes, T., *Science*, **164**, 788 (1969).
[5] Selander, R., and Yang, S., *Genetics*, **63**, 653 (1969).
[6] Kimura, M., *Nature*, **217**, 624 (1968).
[7] Koehn, R., *Science*, **163**, 943 (1969).
[8] Gibson, J., *Nature*, **227**, 959 (1970).
[9] Richmond, R., *Nature*, **225**, 1025 (1970).
[10] Simpson, G., *Science*, **146**, 1535 (1964).
[11] Selander, R., and Yang, S., *Genetics*, **58**, 599 (1967).
[12] Selander, R., Hunt, W., and Yang, S., *Evolution*, **23**, 379 (1970).
[13] O'Brien, S., and MacIntyre, R., *Amer. Nat.*, **103**, 97 (1969).
[14] Henderson, N., *Ann. N.Y. Acad. Sci.*, **151**, 429 (1968).
[15] Kojima, K., Gillespie, J., and Tobari, Y., *Biochem. Genet.*, **4**, 627 (1970).
[16] Prakash, S., *Proc. US Nat. Acad. Sci.*, **62**, 778 (1969).
[17] Prakash, S., Lewontin, R., and Hubby, J., *Genetics*, **61**, 841 (1969).
[18] Harris, H., *Brit. Med. Bull.*, **25**, 5 (1969).
[19] Racker, E., *J. Biol. Chem.*, **184**, 313 (1950).
[20] Johnson, M., in *The Enzymes* (edit. by Boyer P., Lardy H., and Myrback K.), **3**, 407 (Academic Press, New York, 1960).
[21] Morell, D., *Biochem. J.*, **51**, 657 (1952).
[22] Baranowski, T., *J. Biol. Chem.*, **180**, 535 (1949).
[23] Robbins, E., and Boyer, P., *J. Biol. Chem.*, **224**, 121 (1957).
[24] Harary, I., Korey, S., and Ochoa, S., *J. Biol. Chem.*, **203**, 595 (1953).
[25] Najjar, V., *The Enzymes*, **6**, 161 (1962).
[26] Hill, R., and Bradshaw, R., in *Methods in Enzymology* (edit. by Lowenstein J.), XIII (Academic Press, New York, 1969).
[27] Pontremoli, S., Grazi, E., de Flora, A., and Mangiarotti, G., *Arch. Sci. Biol.*, **46**, 83 (1962).
[28] Kahana, S., Lowry, O., Schulz, D., Passonneau, J., and Crawford, E., *J. Biol. Chem.*, **235**, 2178 (1960).
[29] Lehninger, A., Sice, J., and Jensen, E., *Biochim. Biophys. Acta*, **17**, 286 (1955).

Evidence for Selective Differences between Cells with an Active Horse *X* Chromosome and Cells with an Active Donkey *X* Chromosome in the Female Mule

THE glucose-6-phosphate dehydrogenase (G6PD) locus is known to be *X*-linked in the horse and donkey[1,2]. The female mule, an interspecific hybrid of these two species, is an obligatory heterozygote for two electrophoretically distinguishable G6PD alleles[1,2].

Studies of the DNA replication patterns of the chromosomes of two female mules by Mukherjee *et al.*[3,4] have shown that, in about half the cells investigated in each animal, the *X* chromosome derived from the female parent (the horse *X*) was late replicating and the *X* chromosome derived from the male parent (the donkey *X*), was early replicating; the reverse pattern obtained in the other half of the cells. Hamerton *et al.*[5] found in three female mules, however, that most lymphocytes and fibroblasts had a late replicating donkey *X* chromosome and early replicating horse *X* chromosome. The distribution was significantly different from the roughly equal representation of cell types noted by Mukherjee *et al.* In the only cell line studied electrophoretically by Hamerton *et al.*, horse G6PD was preferentially expressed, consistent with the hypothesis that the early replicating mammalian *X* chromosome in females is the active one[6]. The observations of the latter workers suggest that in some female mules, either (1) the donkey *X* chromosome is preferentially inactivated early in development, or (and) (2) cells with an active horse *X* chromosome have a selective advantage in at least some tissues during ontogeny.

Table 1 G6PD Phenotypes of Female Mules

Predominant phenotype	Cervical cord	Pancreas	Parotid	Liver	Lung	Thyroid
H>D	20 (18)	19 (12)	23 (15)	9 (6)	8 (8)	8 (6)
H≃D	1 (1)	4 (4)	4 (3)	12 (7)	11 (5)	12 (9)
D>H	2 (2)	4 (3)	3 (3)	13 (8)	6 (4)	9 (5)

The number of animals with the observed phenotypes are listed. Not all organs from every animal could be investigated, but in twenty-two of the fifty-four animals at least five of the six organs listed in the table were studied. The results for these twenty-two animals are listed in parentheses. The patterns were scored independently by E. B. H. and L. D. B. and the sample rerun if there was a discrepancy or uncertainty (see Fig. 1). Examination of blood samples revealed that the horse band was the usual predominant one here also. Mixing experiments with variable proportions of donkey and horse blood samples with known activity indicated that, when the bands of approximately equal intensity were observed, there was at least 35% activity of the major component in the mixture. In a mule in which expression of donkey and horse phenotype was close to equality, there was no observed difference in the proportion of parental phenotype between reticulocyte-rich and reticulocyte-poor preparations, although there was less activity in the latter. Similarly, there was no observed difference between fresh and stored cells in the proportion of parental phenotype, although activity was lower in stored cells. The specific activities of G6PD in peripheral blood of a young donkey and of a young horse were within 10% of each other. Earlier studies[8] suggested that the donkey phenotype was preferentially expressed in liver, but this no longer seems likely.

We have investigated this question by studying the G6PD phenotypes of samples from tissues and organs of fifty-four female mules. The results for representative organs are listed in Table 1. Examples of patterns and their interpretation are illustrated in Fig. 1.

There were two organ types: those in which the horse phenotype was usually predominant, and those in which expression was not significantly different from what would be expected on a random basis. In none of the organs studied was the donkey phenotype the usual predominant one. If *X* inactivation were non-random, and occurred at the early

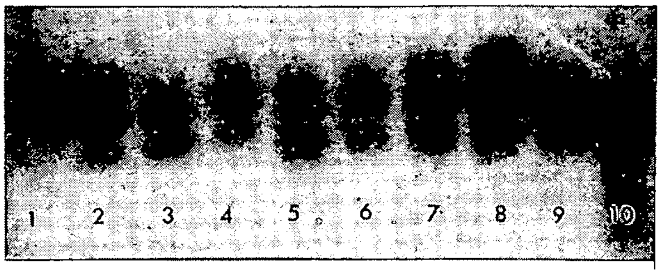


Fig. 1 The bands in the slots at extreme left (1) and right (10) illustrate donkey (D) and horse (H) patterns respectively. The other samples, from pancreas and parotid, were interpreted from left to right as follows: (2) D > H, (3) H > D, (4) ?, (repeat), (5) H > D, (6) H > D, (7) ? H≃D, (repeat), (8) D > H, (9) H > D. All organs were studied using starch gel electrophoresis in EBT buffer with standard methods[9] at *p*H 8.6. Almost all animals were relatively old. Organ samples were collected at the abattoir on dry ice about 60–120 min after death and exsanguination. Blood was obtained in citrate about 5–15 min after death and stored at 4° C.

stage usually hypothesized in the mammalian female embryo, representation of the favoured allele should be relatively uniform in all derived tissues, unless cell selection in particular organs had also occurred. Since X inactivation is thought to occur at about the blastocyst stage[7], before observable organ differentiation, it seems unlikely that preferential inactivation has occurred just in the cell precursors of particular organs. It seems more probable that X-linked alleles of the donkey and horse have had different effects during development and maturation, with a relative selective advantage for cells with an active horse X chromosome in organs such as the parotid gland. But preferential expression of the horse X chromosome in these latter organs did not occur in all animals studied, indicating that the observed phenomenon represents a trend rather than a uniform developmental event in the female mule. Thus discrepancies between studies such as those of Hamerton *et al.* and Mukherjee *et al.* seem likely to reflect sampling differences in animals studied.

An alternative explanation for the observed distribution should be mentioned. Unknown organ specific factors may have differentially suppressed (or enhanced) expression of a particular parental G6PD phenotype (for example, perhaps inhibiting expression of the donkey band in cervical cord, pancreas and so on). Although this seems unlikely, it cannot be completely excluded. But when samples of pancreas with horse phenotype were mixed with samples of other organs which expressed the donkey phenotype, there was no evidence for an inhibiting factor.

ERNEST B. HOOK
LORETTA D. BRUSTMAN

Birth Defects Institute,
New York State Department of Health,
and Department of Pediatrics,
Albany Medical College of Union University,
Albany,
New York 12208

Received February 18, 1971.

[1] Trujillo, J. M., Walden, B., O'Neil, P., and Anstall, H. B., *Science*, **148**, 1603 (1965).

[2] Mathai, C. K., Ohno, S., and Beutler, E., *Nature*, **210**, 115 (1966).

[3] Mukherjee, B. B., Mukherjee, A. B., and Mukherjee, A. B., *Nature*, **228**, 1321 (1970).

[4] Mukherjee, B. B., and Sinha, A. K., *Proc. US Nat. Acad. Sci.*, **51**, 252 (1964).

[5] Hamerton, J. L., Gianelli, F., Collins, F., Hallett, J., Fryer, A., McGuire, V. M., and Short, R. V., *Nature*, **222**, 1277 (1969).

[6] Lyon, M. F., in *Adv. Teratol.*, **1** (edit. by Woolam, D. H. M.) (Logos Press, London, 1966).

[7] Kinsey, J. D., *Genetics*, **55**, 337 (1967).

[8] Hook, E. B., and Brustman, L. D., *Genetics*, **64**, 530 (1970).

[9] Motulsky, A. G., and Yoshida, A., in *Biochemical Methods in Red Cell Genetics* (edit. by Yunis, J. Y.), 51 (Academic Press, New York, 1969).

New Determination of Australopithecine Height

AN estimation of the body height of two robust australopithecines (Sk 82 and Sk 97) is attempted here on the basis of a ratio between femoral head diameter and body height in modern *Homo sapiens*. Coon, using the fibula and tibia from the "*Zinjanthropus*" living floor at Olduvai, has estimated[1] three different heights for this creature or creatures: 144.5 cm (4 feet 8 inches); 136 cm (4 feet 6 inches); and 152.4 cm (5 feet). Lovejoy and Heiple, using specimens from Sterkfontein (STS 14, STS 34 and TM 1513), have reconstructed[2] a height for the gracile australopithecines at 106.7 cm–109.2 cm (3 feet 6 inches–3 feet 7 inches). An estimation has never been made, however, for the height of an undisputed robust australopithecine.

Because australopithecines were fully erect and bipedal[3] it is possible to apply postcranial information derived from modern *Homo sapiens* to australopithecines; the mechanical relations which hold true for modern bipedal *Homo sapiens* will also apply therefore to bipedal australopithecines. I selected fifty specimens (thirty males and twenty females) at random from the Hamann-Todd Collection of modern *Homo sapiens* in the Cleveland Museum of Natural History. I defined terms and measurements as follows. Femoral head maximum diameter: the diameter of the femoral head taken in the horizontal plane with the femur resting on the condyles and trochanter. Femoral head minimum diameter: the diameter of the femoral head taken at right angles to the plane of maximum diameter. I took care to avoid measuring non-weight bearing surfaces or protrusions. Circumference: the circumference of the femoral head taken at the greatest measurement of weight bearing area on the head. Calculated area: the head of the femur was considered as a half sphere. I used the formula, $\text{area} = 2\pi r^2$ (r, radius), half the value of the surface sphere.

The specimen files of the Hamann-Todd Collection provided body heights. All measurements are in centimetres or, in the case of areas, in centimetres^2, and were taken with a Vernier calliper accurate to a hundredth of a millimetre and a cloth tape accurate to one millimetre. Rechecking occasional specimens for all measurements showed that the values obtained originally were at least 98% accurate.

Determinations were made to indicate which measurement, area or diameter would relate best with body height. By correlation analysis, I found that the average diameter of the head (midpoint between maximum and minimum) correlated better than the surface area (a correlation of 0.648 as opposed to 0.627) and I used it in all further calculations and considerations.

Using a least mean squares two variable regression I then calculated the line of best fit for the data. The equation of this line is

$$\text{body height} = 13.29\times + 108.59$$

The average distance of actual body height from this line is 3.2%, or 5.4 cm. I calculated absolute range of error using the body heights furthest removed from the line of best fit even though these heights may be anomalous. This range of absolute error is +8.2%, −7.4%. I then used this formula to predict the body height of Sk 82 as 151 cm (4 feet 11.5 inches) and Sk 97 as 157 cm (5 feet 1.5 inches). Taking average range of error into account, the range of probable heights for these specimens is

Sk 82	146–156 cm
Sk 97	152–161 cm

I see no reason why this method and formula cannot be used to estimate the height of any bipedal, erect hominid within the average range of error.

I thank Miss Patricia Helwig, Assistant Curator of Collections, and the Cleveland Museum of Natural History for their assistance in making the Hamann-Todd Collection available.

PETER E. BURNS

Department of Anthropology,
Case Western Reserve University,
Cleveland, Ohio 44106

Received April 6, 1971.

[1] Coon, C. S., *The Origin of Races*, 285, 294 (Knopf, New York, 1969).

[2] Lovejoy, C. O., and Heiple, K. G., *Amer. J. Phys. Anthrop.*, **32**, 33 (1970).

[3] LeGros Clark, W. E., *The Fossil Evidence for Human Evolution*, 157 (University of Chicago Press, Chicago, 1964).

BOOK REVIEWS

Empiricist Physician

Fundamenta Medicinae. By Friedrich Hoffmann. Translated and introduced by Lester S. King. (History of Science Library: Primary Sources.) Pp. xxvi + 142. (Macdonald: London; Elsevier: New York, January 1971.) £3.

THE seventeenth century was fertile with new ideas in medicine and science. Long established Aristotelian doctrines began to crumble before the assault of Baconian empiricism and atomism modified by Descartes, Gassendi and Boyle: the emergence of the iatrochemical and iatromechanical "schools" of medicine completed the rout. It is tempting to classify seventeenth century physicians into various "schools" such as Galenist, iatrochemist or iatrophysicist, but these categories, favoured by latter-day interpreters, were not so clearly defined. In spite of the increased knowledge of scientific medicine, adherents of the chemical or mechanical schools did not really succeed in discarding Galenic remedies or dispensing with the basic tenets of humoral pathology. In this respect Friedrich Hoffmann (1660–1742) was typical of his times.

Born in Halle, Hoffmann studied medicine at Jena and Erfurt, qualifying in 1681. After visiting medical schools in France, Holland and England (where he met Boyle) he returned to practise in Minden and Halberstadt. In 1693 Frederick III invited him to become Professor of Medicine at Halle and, apart from a few years in Berlin as court physician, Hoffmann spent the rest of his life there as a university professor. His interest in medical education led him in 1695 to publish his *Fundamenta Medicinae* as an introduction to systematic medicine.

Hoffmann's faith in scientific medicine is clear from his introduction. "Mechanics alone, wedded to anatomy and chemistry," he wrote, "has so far led natural and medical science from a low position to an exalted one." Although he was able to interpret physiological processes in mechanical terms (his theory of disease too is a mixture of humoral pathology and atomic theory). Hoffmann found it difficult to explain mental processes in similar terms. After removing the theological concept of "soul" beyond the realm of his treatise, Hoffmann tried to reconcile the rational, volitional functions of the mind with the mechanical functions of the body. He postulated that sensory perception and muscular movement were due to the animal spirits controlled by the sensitive soul—a concept expounded by Willis in *De Anima Brutorum* (1672). His diagnostics, hygiene and therapeutics were concerned with the "art" of medicine and, like Sydenham, Hoffmann followed Hippocratic principles based on generalizations drawn from his own experience. He regarded body fluids as the site of disease and stressed the diagnostic importance of analysing blood, saliva, perspiration, lymph, urine and "spirits". An example of his diagnostic empiricism is the statement that "red urine without fever indicates scurvy". The maintenance of health he thought depended on the balanced interaction of the "six non-naturals—air, food and drink, sleep, useful activities and rest, evacuation and repletion and the emotions and passions". Hoffmann's therapeutics were based on his own experience of drug action within the theoretical framework of humoral pathology. He used Galenicals rather than chemical remedies, and although his theory of drug action was essentially mechanical, his actual prescribing was reassuringly empirical. Thus there was a conflict between the art of the physician using drugs based on past experience, and the scientific doctor anxious to propagate new discoveries in physics and chemistry.

This small volume precisely translated and usefully introduced by Dr Lester King is of general interest to the medical historian and essential reading for the seventeenth century specialist.

KENNETH DEWHURST

Small Mammals

Energy Flow Through Small Mammal Populations. Edited by K. Petrusewicz and L. Pyszkowski. (Proceedings of IBP Meeting on Secondary Productivity in Small Mammal Populations, held in Oxford, England, July 29th to August 2nd, 1968.) Pp. 298. (Polish Scientific: Warszawa, 1969/1970.)

THIS volume contains the proceedings of a meeting held under the auspices of the International Biological Programme and yet, from the contents, one might get the impression that work on small mammals is non-existent in Africa, Asia, Australasia and South America. Of the fifty-six participants from fourteen countries, twenty-two came from central or eastern Europe five from North America and the remainder from western Europe. Twenty-two of the thirty-one contributions were written by ecologists from eastern Europe and surely this emphasizes the activity and leadership of these scientists within the context of IBP small mammal ecology.

The contents refer primarily to European work but this does not detract from their importance to small mammal ecology throughout the world. The contributions are grouped into four major sections: (1) standing crop evaluations and dynamics of numbers; (2) food resources and bioenergetic parameters; (3) reproduction and survival and (4) energy flow and productivity estimations. The eleven papers in the first section critically examine methods for estimating population density. It is clear that none of the currently used methods are entirely satisfactory for making absolute density estimates; however, both the mark-release-recapture and the standard minimum removal methods can, when combined with a knowledge of home range and residency, give rise to acceptable estimates. Ingenious methods involving the use of coloured baits and/or radioisotopes are suggested for the determination, among other things, of residency and home range.

The second section comprises nine papers and these, although dealing with the common theme of food resources and bioenergetic parameters, are a miscellaneous collection. In one instance the effect of small mammals on vegetation is considered, in others the effect of different food types on the animals is discussed. Yet other papers are methodological in nature, or deal with seasonal changes in the energy content or respiratory rate of single species.

Two of the papers in the third section tackle the difficult problems of estimating the number of young born into a population and the average length of life of small mammals. Criticism is levelled at the practice of assuming exponential population growth in certain species and different methods of estimating longevity are compared.

The final section on energy flow and productivity estimations contains six papers. It is interesting to note that although a bank vole has an average life expectancy of up to 3.66 months it is clear that some 60 per cent of its production occurs within the first 0.75 months of its life. This underlines the necessity of finding ways to study what is normally regarded as a non-trappable part of the population. The same conclusion is reached in another paper which deals with the field vole. The remaining papers are primarily concerned with food consumption by small rodents and it would appear that these animals rarely eat more than 1 to 2 per cent of the primary production potentially available to them. This suggests that food might not be a limiting factor to the size of some populations, although one author is careful to point out that food might become a critical factor which limits those overwintering populations which follow a peak in vole numbers.

Clearly, this volume summarizes a wealth of information about diverse matters relating to energy flow through small mammal populations. Typographical errors occur frequently in the text but in no instance did I find them particularly misleading and therefore I have refrained from detailing them. In conclusion it must be said that any mammalogist or ecologist professing an interest in small mammal population density estimates, production or energy flow would be doing himself a disservice if he did not consult this publication.

JOHN PHILLIPSON

Scientific Psychiatry

Psychiatry as a Behavioral Science. Edited by David A. Hamburg. (The Behavioral and Social Sciences Survey: Psychiatry Panel.) Pp. xii+114. (Prentice-Hall: Englewood Cliffs, New Jersey, 1970.) $5.95.

SOMEONE once described a camel as a horse that had been designed by a committee. A book written by a committee starts off at a similar disadvantage. This work, one of a series of reports for the public initiated by the US National Academy of Science and the Social Science Research Council, was prepared by a distinguished panel of American scientists. It aims to provide a comprehensive review of psychiatry as a behavioural science, especially in its scientific and research aspects, and, hopefully, to "constitute a basis for an informed, effective national policy to strengthen and develop" these fields. With the public view of psychiatry dominated by the images of the couch and the asylum, the treatment of neurotic private patients by psychoanalysts, and the confinement of psychotic patients in large mental hospitals, there is certainly room for an entertaining and stimulating review of the areas of expansion in modern psychiatry.

Although this volume provides a fairly wide ranging survey of the field of recent and current research in psychiatry, the survey is patchy and by no means as up to date as one expects from a work published in 1970—much of it could have been written five years ago or more. Psychoanalysis is perhaps over-represented for a study of behavioural science; and the emphasis is almost entirely on American work. The presentation is terse and irregular, and manages to be too specific and technical for the lay reader in many passages, as well as too vague and lacking in detail for the professional reader. There is an intelligent and reasonably selective list of annotated references.

The style of writing is turgid and uniformly woolly, which is unpardonable when dealing with such a lively and potentially exciting subject. The layout and design are singularly uninspiring, and the long slabs of grey type are not always easy to read. Perhaps the major weakness of the book is the common one of aiming at a great many targets and missing most of them. It is neither sufficiently clear and attractive to interest and inform many members of the informed public, nor sufficiently critical and specific to be really helpful in guiding future research and developments in psychiatry.

MICHAEL A. SIMPSON

Poor Substitute

Optical Properties of Solids. Edited by E. D. Haidemenakis. (Lectures and Seminars presented at the Fifth Chania International Conference, held at Chania, Crete, Greece, June 30–July 10, 1969.) Pp. xv+520. (Gordon and Breach: New York and London, November 1970.) £12.25; $9.50.

THIS publication covers optical studies in the alkali halides, the inert gases, semiconductors and magnetic insulators but gives only slight coverage to optical studies of metals. Of especial interest is the review by Seraphin of the use of modulated reflectance in the study of band structures; he shows how the technique can be used to separate the massive background of non-critical optical transitions from the smaller contribution due to the critical points, which are nevertheless essential in the adjustment of theoretical band structures.

The contributions by Button and Kaplan on spectroscopy at sub-millimetre and far infrared wavelengths, respectively, bear on semiconductor problems. The use of high frequency photons to excite cyclotron resonance or some other transition between allowed electron states makes possible an order of magnitude increase of resolution compared with that available at microwave frequencies and allows the application of Fourier spectroscopy and the use of resonant photoconductivity as a detection system.

The optical properties of the alkali halides and the inert gases seem to be the simplest possible, and several papers in these conference proceedings concentrate on these materials. Optical properties are evidently better understood than for semiconductors. A paper by Zawadzki develops the analogy between free relativistic electrons and electrons in a narrow gap semiconductor, applying the ideas to the behaviour of electrons in crossed electric and magnetic fields and especially to the optical properties of semiconductors under such conditions. Moriya's review of optical excitation of magnons in spin systems describes a field of growing interest rather sketchily and with no experimental details, only references to the original literature.

Such papers draw attention to the conflict between the more conventional review literature and conference proceedings, in that the inadequacies of some of the reviews in this publication force the reader back to the original literature at an earlier stage than is usual in the study of a review. If the excessive cost of this book is set beside its value to the physicist, it is probably not a book to own but one to be borrowed from a library.

ALEXANDER D. C. GRASSIE

Analysing Proteins

Protein Sequence Determination: Methods and Techniques. By S. Blackburn. Pp. ix+292. (Marcel Dekker: New York, September 1970.) £8; $16.75.

THIS excellent account of protein sequence analysis will be useful to chemists, biochemists, molecular biologists and others who require a practical guide to the subject. Automated analysis is still very much in its infancy and, as the author points out, sequencing a protein is not yet a routine operation, each protein presenting a problem in its own right. In twenty chapters the sequencing strategy is revealed with the enthusiasm to be expected from an experienced worker in the field.

The book covers cleavage of disulphide linkages; specific and non-specific cleavage of proteins by enzymes, electrophilic reagents, acid and alkali; fractionation of mixtures of peptides by precipitation, gel filtration, ion exchange chromatography, paper chromatography and electrophoresis; end group determination, ordering the peptide fragments, radioactive techniques; and finally, mass spectrometric and X-ray crystallographic techniques. The latter techniques have for good reasons been discussed only briefly, but sufficient information is provided to make possible a useful comparison of data obtained by X-ray analysis with those obtained by conventional chemical techniques. At the beginning of each chapter the principles involved are stated concisely in a homely style and these are followed by practical details of experimental procedures. An excellent job has been done in selecting these practical examples, most of them allowing the worker to take his problem directly to the bench without reference to the original literature. A list of sources is, however, available at the end of each section.

The book is not overburdened with tables and figures, and abounds in sound advice and hints on how to avoid pitfalls. It forms a companion volume to the author's earlier *Amino Acid Determination: Methods and Techniques*, but can be read and used independently. The level of proof reading was quite good although a curious chapter heading was missed on page VIII. The publisher could have been a little more generous with the subject index. Overall the book fulfils its aims and should be well received by research workers who have an interest in peptide and protein sequence data. J. LEGGETT BAILEY

Chain Genetics

The Genetic Markers of Human Immunoglobulins. By R. Grubb. (Molecular Biology, Biochemistry and Biophysics, Vol. 8.) Pp. xii+152. (Chapman and Hall: London; Springer–Verlag: Berlin and New York, November 1970.) £4.90.

THE immunoglobulins (Igs) are unique among proteins in their diversity of structure and function. The discovery in 1956 of genetic markers on human and rabbit Igs by the author of this book and by Oudin, respectively, was therefore of particular significance. The subsequent intensive study of these markers has provided an essential framework for understanding the genetic control of antibody synthesis, and has delineated several features of great interest with regard to the assembly of antibody molecules and the differentiation and cooperation of cells which produce antibodies. Genetic markers have also been useful in studies of the phylogeny, ethnic features and ontogeny of immunoglobulins, and forensic pathologists have used them with practical effect in cases of disputed paternity and confusion of newborn infants in maternity hospitals. They can be of unique value in the latter case because the infant's genotype for Ig synthesis is not expressed at birth and its circulating IgG is for practical purposes exclusively of maternal origin.

Genetic markers are now known for three of the seven major classes of immunoglobulin heavy and light chains. Identification of their many polymorphic forms involves the use of human antisera which specifically agglutinate red blood cells coated with human immunoglobulin. The anti-human γ-globulins used in these tests originate principally in patients who have had multiple blood transfusions or in subjects who may be apparently healthy or afflicted with rheumatoid arthritis. The clinical significance of these antibodies in the latter context and their possible role in the pathogenesis of disease are matters of continuing controversy and interest.

All these intriguing ramifications of an important topic are lucidly outlined in the first eighty-five pages of this admirable, small book. The text is supported by a wealth of tabulated data and a concluding appendix of thirty pages provides a comprehensive source for the original references, mode of detection, ethnic distribution, molecular localization and inheritance of some thirty genetic factors which have been detected on human immunoglobulins. This book will be of great value as a succinct and authoritative source for immunologists, geneticists, clinicians and others interested in the many sided problems of antibody synthesis. It is regrettable that the author's economy and accuracy were not emulated by the publishers who are responsible for the inflated price and several printing errors—some diverting, such as that on page 82, which concerns "the mouse lamb dalight chains" of IgM. S. COHEN

Vibrations

Low-Frequency Vibrations of Inorganic and Coordination Compounds. By John R. Ferraro. Pp. xiv+309. (Plenum: New York, 1971.)

IMPORTANT developments in far infrared instrumentation during the past ten years have led to a considerable increase in the published data on low frequency vibrations of molecules and ions, which are not only of interest to spectroscopists but of potential value to other chemists. In this book the author, who has published a considerable number of research papers in this field, sets out to demonstrate the usefulness to inorganic chemists of infrared absorption measurements below 650 cm^{-1} on inorganic compounds.

A brief introduction sketches the history of far infrared measurements and the experimental and interpretative problems of this region of the spectrum, and is followed by a chapter describing commercial instruments including interferometers, some in considerable detail but others only in briefest outline. A comparison of the relative merits of spectrometers and interferometers, probably of greater general interest than the rest of the chapter, is unfortunately presented in brief tabular form, with references to other texts. The third chapter summarizes useful data on window materials and some common organic solvents. Calibration is also briefly discussed but one is referred to other texts for many of the calibration data. The fourth chapter sketches the use of inelastic neutron scattering and high pressure techniques in vibrational studies, again with references to other sources for details.

The next four chapters, comprising more than two-thirds of the book, present data (including those from Raman measurements) and discussion on metal–oxygen, metal–halogen, metal–nitrogen and miscellaneous metal–ligand vibrations. Many of the data are in tabular form and the presentation is similar to that in other books of this type. The final chapter very briefly summarizes some results on ion-pair and lattice vibrations, clathrate vibrations and low frequency electronic transitions. Two appendices give tables of crystal site symmetries and subgroup correlations, and describe a method for factor group analysis of crystal vibrations.

This book undertakes to cover a very wide field embracing almost the whole of metal–ligand vibrations, which is scarcely reduced by the rather artificial limitation to frequencies below 650 cm^{-1}. In order to save space much use is made of references to other texts for detailed discussion, and on many topics no detailed or critical account is given. References are included up to 1969, but, the author says, the book does not claim complete coverage of topics or references, and there are some obvious gaps. The book therefore seems to be chiefly of value to newcomers and non-specialists in this field, giving in one volume brief accounts of both experimental and interpretative matters. While also of use to specialists as a recent source of references, its value as a convenient source of data is somewhat reduced by its lack of comprehensiveness on details.

J. A. CREIGHTON

Spectra Galore

Spectroscopy in Inorganic Chemistry. Vol. 1. Edited by John R. Ferraro and C. N. R. Rao. Pp. xiii+410. (Academic: New York and London, October 1970.) $19.50; £9.10.

THE two volume treatise of which this volume is the first part is more specialized than its title suggests. It describes recent advances in inorganic spectroscopy but omits well known aspects of spectroscopy such as electronic or vibrational spectroscopy and their applications to coordination chemistry as well as lengthy discussions of instruments and methods. The articles are intended not as complete compilations but as reports of the present position and of future potentialities.

There has not been much time for some of the new results to be digested, and many of the chapters are oriented towards methods and methodology (even when these can be found elsewhere) rather than towards the interpretation of spectra in terms of structure and bonding. Many chapters are anecdotal rather than correlative, some are too elementary. Thus section D of the chapter on high-resolution NMR begins: "The electronic environment in molecules does one more subtle thing. It couples magnetic nuclei present in the same molecule. Such a coupling is experimentally observed as a splitting of NMR lines." (Surprise, surprise.) This chapter describes some useful inorganic studies, but fails to mention superconducting magnets, liquid crystals and Fourier transforms.

Some of the chapters are competently written, a few well written. The foreign language literature, however, and its English translations, is often poorly served. The first article refers to one German and to two (translated) Japanese papers in 65 references. The review of Mössbauer spectroscopy, which is a German invention, refers to two German papers (by Mössbauer), two Japanese and one (translated) Russian paper among 90 references. Editors (on both sides of language barriers) should encourage their authors in scientific internationalism, for this lack of effort, which is not necessarily chauvinism, is only too common. In a volume costing £9 (£1 per article of about 45 pp.) we expect coverage of the less accessible, and no less relevant, parts of the chosen fields.

There are other examples of parochialism. Thus the description of the early work in matrix isolation spectroscopy refers to Pimentel but not to Norman and Porter, Rice or Lewis and Lipkin[1].

While counting references, we could congratulate the authors of the chapter on soft X-ray spectroscopy on their activity in this field, for 23.5% of their references are to their own work. They tell us that their examples were chosen to show "typical results", but the references, if not the text, should aim at a reasonable coverage and should mention other useful accounts that are available[2]. Their suggestion (p. 210) that the future significance of this method lies in the interpretation of spectra rather than in the determination of concentration is misleading; they themselves report (p. 242) that the analysis of rocks by X-ray emission spectroscopy was shown (1965) to be better than with wet chemical techniques.

As the book goes on, the proof reading flags, and a strange chemistry, of nigrogen, cynides and ferrocynides, develops. We need these reviews; but the high cost of distribution justifies a plea for a high standard of preparation.

J. MASON

---

[1] Norman, I., and Porter, G., *Nature*, **174**, 508 (1954); Rice, F. O., and Freamo, M., *J. Amer. Chem. Soc.*, **73**, 5529 (1951); **75**, 548 (1953); Lewis, G. N., and Lipkin, D., *J. Amer. Chem. Soc.*, **64**, 2801 (1942).

[2] *Practical X-ray Spectrometry* by R. Jenkins and J. L. De Vries, Macmillan, London; Springer, New York (1971); and "X-ray Fluorescence Spectrography" by K. Norrish and B. Chappell, in *Physical Methods in Determinative Mineralogy*, Academic Press, London (1967).

Hot Polymers

Thermal Stability of Polymers. Vol. 1. By Robert T. Conley. (Monographs in Macromolecular Chemistry.) Pp. x+644. (Marcel Dekker: New York, October 1970.) $44.50.

THE wide uses to which polymeric materials, especially those of synthetic origin, are now applied make it imperative that information on properties and performance should be as complete as possible. Plastics, rubbers and fibres are often subjected to environmental conditions which may have a profound effect on their chemical, physical and mechanical properties. Behaviour at elevated temperatures is an important factor when selecting suitable materials, and this book provides a welcome collective survey on thermal and thermal/oxidative degradation. Edited by Professor Conley of Wright State University, Dayton, Ohio, it has fourteen chapters, by experts in US academic, industrial and research organizations and one contributor from the UK (Dr Wright of the Royal Aircraft Establishment, Farnborough).

In two initial chapters, Professor Conley discusses the general concepts of polymer properties, degradation processes, molecular structure, and stability criteria, drawing attention to structures which contribute to thermal resistance. Chapters by R. H. Boyd and A. V. Tobolsky and co-authors then review the more theoretical aspects of thermal depolymerization, scission processes and polymer oxidation, providing a background essential for appreciation of later chapters where important classes of polymers are considered. These deal with polyolefines (R. H. Hansen), rubbers (E. M. Bevilacqua) and fluorocarbon polymers (W. W. Wright). Professor Conley and co-authors provide chapters on vinyl and acrylic polymers; on polyamide, polyimide and related condensates, polyesters and polyethers; on thermosetting polymers (phenolic, nitrogen, furan and epoxy resins); and on cellulosic products. The remaining chapters are by G. P. Shulman who deals with heterocyclic polymers and to some extent covers the same field as Conley's chapter ten, and by J. Economy and J. H. Mason on the behaviour of some inorganic polymers.

Each chapter is accompanied by lists of references to the more important papers (usually up to 1967), so giving an extensive but sometimes noncritical review since the treatment varies with the individual contributors. While the editor has endeavoured to bring the contributions together in a uniform manner, some repetition or overlap is evident (particularly in the tenth and thirteenth chapters). To some extent this may be an advantage in that the chapters become self-contained. There are places where the reader will see omissions or be critical of the authors' interpretation of published information, evident in discussions on acrylic, vinyl and olefine polymers. Another feature is the apparent insufficient attention given to technological implications, particularly on the use of stabilizers, antioxidants and modified process conditions, all of major importance in polymer-using industries. Again, one notes that while there is much on the degradation of polyacrylonitrile, the bearing of this on carbon fibre formation is not stressed; nor are polyurethanes dealt with.

Author and subject indexes are provided, although the latter could have been more comprehensive, and I noticed some typographical errors throughout the text.

The book presents a useful addition to the literature and those who are concerned with the behaviour of polymers in conditions where thermal and oxidative influences are encountered will find it to be of particular value. Unfortunately, the high price may well limit its general availability and it is likely to be consulted in libraries rather than purchased for personal use. I look forward to the second volume, now in preparation, and although the possible content has not been indicated it is hoped that this will be complementary to the first volume and perhaps be concerned with other polymer types and with thermal degradation as it affects processing and properties rather than chemical structure.

R. J. W. REYNOLDS

CORRESPONDENCE

Smoking and Cancer

SIR,—In his letter (*Nature*, **231**, 543; 1971) on smoking and lung cancer, Dr Sterling states that he had set up a panel to provide authoritative guidance on the link between smoking and disease, and invited Dr Cuyler Hammond to meet this panel. He also requested Dr Hammond to provide the panel with the unpublished data from his own studies: Dr Hammond refused.

He then develops a highly personal attack on Dr Hammond in which he implies that this refusal casts serious doubt on Dr Hammond's reports on the association between smoking and lung cancer.

More seriously, however, Dr Sterling's letter also implies that this refusal by Dr Hammond casts similar doubt on other studies on the relationship between lung cancer and smoking. Thus, Dr Sterling impugnes the integrity and competence of the numerous scientists and committees in many countries who have reviewed exhaustively the extensive data published on this subject[1,2]. If Dr Sterling disagrees with their conclusions he should do so in a carefully documented critique of all the published data rather than by singling out Dr Hammond's study for personal criticism.

I suspect the reason for Dr Hammond's unwillingness to meet with Dr Sterling's panel is that he does not feel that his time would be best spent discussing and presenting data to yet another group of individuals apparently unfamiliar with the material in the widely explored area of cigarettes and lung cancer. There must be few experts in this field who have studied the literature and not yet reached a decision.

Thus, I believe that Dr Hammond's colleagues will consider that Dr Sterling's tasteless attack constitutes a form of blackmail, and that he was fully justified in his decision not to discuss his data with Dr Sterling's panel. Further, many will draw the conclusion that this attack implies that any scientist who fails to make his data available to any Tom, Dick or Harry is lacking in credibility. This attempt to discredit scientific data and conclusions by character assassination through inference should not be part of the modern scientific approach, and I am surprised that this letter was published in *Nature*.

I have disagreed from time to time with the American Cancer Society on its comments on individual scientific papers, but I have not doubted its good faith and the basic correctness of its wish to bring to public notice the danger of a popular habit, which at best is a thankless task.

Lastly, Dr Sterling should confirm that neither he, nor his department nor his research, are supported by the grant reported to have been made recently to his university by certain industrial interests.

Yours faithfully,
JOHN HIGGINSON

World Health Organization,
International Agency for Research on Cancer,
16 *Avenue Maréchal Foch,*
69 *Lyon* (*6ème*)

---

[1] *Smoking and Health Report of the Advisory Committee to the Surgeon General of the Public Health Service* (Public Health Service Publication No. 1103, 1964).

[2] *Smoking and Health Now* (A Report of the Royal College of Physicians, 1971).

Defoliation by Gypsy Moth

SIR,—In the article "St George versus a Gypsy Moth" (*Nature*, **231**, 142; 1971), *Nature* shows signs of adopting the style of its American contemporary, a consummation devoutly to be abhorred. Your Washington correspondent says that the battle of words over the gypsy moth "has been a walk-over for those who argue that gypsy moths may in all likelihood be less pernicious than the measures taken to exterminate them". Who decided this ? On June 24, 1970, the *New York Times*, which is scarcely a friend of pesticides, reported from Riverhead, Long Island (p. 59): " . . . The caterpillars arrived in large cans and plastic bags brought to the legislative session by more than 50 irate residents of the communities of Shirley, Mastic and Ridge. The residents demanded immediate emergency action to curb this year's invasion which is, according to some agricultural experts, the largest since the moth was introduced into this country in 1869. 'We are in a state of emergency', Mrs Helen Gaylord, of 32 Maple Avenue, in Shirley, told the legislature, 'Our children cannot go out. Our pools are finished for the summer. It's a question of survival—the caterpillars or us'."

Nature states that the USDA, "like many bureaucracies, is kept in motion by force not of reason but of habit", a generalization that also could be unkindly applied to Washington correspondents. Your correspondent also asks the rhetorical question: "Why does the Department of Agriculture persist in pursuing a programme of which the objectives are ill defined, the benefits clearly temporary, the biological consequences unknown because of inadequate research, and the intellectual foundations so shoddy as to destroy any pretensions to academic respectability the department might still hold ?"

The objective of the programme is clearly defined—kill gypsy moth caterpillars. All benefits can be described as temporary, and all biological consequences as unknown. The final phrase is merely name-calling. Stripped of its emotional rhetoric, the article in *Nature* is a one-sided plea for not interfering with "the natural evolution which must take place while gypsy moths find their natural niche" (column 3). This is the standard neo-Luddite plea against pest control. It is voiced most commonly by dwellers in air-conditioned urban buildings. Meanwhile, after a century to "find their natural niche", the gypsy moths are on the move, and are defoliating vast new areas of forest and woodland[1].

Yours faithfully,
THOMAS H. JUKES

Berkeley,
California

---

[1] Nichols, J. O., *Pennsylvania Forests*, Fall 1970 (Pennsylvania Forests Association, 5221 E. Simpson Street, Mechanicsburg, Pennsylvania).

Reports and Publications

not included in the monthly Books Supplement

Great Britain and Ireland

Forestry Commission. Booklet No. 28: Gwydyr Forest in Snowdonia—a History. By Donald L. Shaw. Pp. vii+107+26 plates. 40p net. Bulletin No. 42: Conifer Woolly Aphida (Adelgidae) in Britain. By C. I. Carter. Pp. iv+51. 75p net. Forest Record No. 74: Development of Glasshouse Techniques for Early Progeny Test Procedures in Forest Tree Breeding. By R. B. Herbert. Pp. 34. 20p net. Forest Record No. 75: Design, Construction and Maintenance of Earth Dams and Excavated Ponds. By E. F. Granfield. Pp. 75. 17½p net. Booklet No. 30: Metric Conversion Tables for Forestry. Compiled by J. E. Everard. Pp. v+71. 50p net. (London: HMSO, 1971.) [245

Queen Mary College, University of London. Annual Report 1969/1970. Pp. 149. (London: Queen Mary College, 1971.) [255

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 269, No. 1199 (13 May 1971): A Discussion on Architectural Aerodynamics. Organized by M. J. Lighthill and A. Silverleaf. Pp. 321–554+plates 8–12. £6.50; $16. Vol. 269, No. 1200 (13 May 1971): Splashing of Water Drops on Solid and Wetted Surfaces: Hydrodynamics and Charge Separation. By Z. Levin and P. V. Hobbs. Pp. 555–586+plates 13–16. £1.10; $2.85. (London: Royal Society of London, 1971.) [255

Other Countries

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Meteorological Physics, 1969/1970. Pp. 41. Division of Forest Products—Research Review 1969/1970. Pp. 55. Rangelands Research Unit—Report 1968/1969, and 1969/1970. Pp. 49. (Melbourne: CSIRO, 1971.) [304

Zentralblatt für Pharmazie, Pharmakotherapie und Laboratoriumsdiagnostik, 6, Band 109, 1970. (Berlin: Veb Verlag Volk und Gesundheit, 1970.) [304

Queen Victoria Museum and Art Gallery, Launceston, Tasmania. Annual Report 1968/1969. Pp. 8+4 plates. Records of the Queen Victoria Museum. No. 35: *Allanaspides helonomus* Gen. Et. Sp. Nov. (Crustacea: Syncarida) from Tasmania. By R. Swain, I. S. Wilson, J. L. Hickman and J. E. Ong. Pp. 7. No. 36: The Geology and Petrology of the Tertiary Volcanic Rocks of the Tamar Trough, Northern Tasmania. By F. L. Sutherland. Pp. 58. (Launceston: Queen Victoria Museum and Art Gallery, 1970 and 1971.) [304

Australia: CSIRO. Division of Fisheries and Oceanography Technical Paper No. 30: The Hydrological Environment of Moreton Bay, Queensland, 1967–68. By B. S. Newell. Pp. 35. (Melbourne: CSIRO, 1971.) [304

The Royal Society of London (1660–1970). By Sir David Martin. (Lecture delivered to the Israel Academy of Sciences and Humanities on May 7, 1970.) Pp. 15. (Jerusalem: The Israel Academy of Sciences and Humanities, 1970.) [304

Journal of Vocational Behavior, Vol. 1, No. 1, January 1971. Edited by Samuel H. Osipow. Pp. 1–204. Vol. 1, 1971 (4 issues). Institutional subscriptions: $25. Private subscriptions (for the subscriber's personal use only): $10. (New York: Academic Press, Inc., 111 Fifth Avenue, 1971.) [304

Commonwealth of Australia. Department of Supply. Australian Defence Scientific Service—Defence Standards Laboratories. Technical Memorandum No. 34: Comparison of Tentacle Extracts from *Chiropsalmus Quadrigatus* and *Chironex Fleckeri*. By T. E. B. Keen. Pp. 8. Report 377: Further Studies on the Biochemistry of the Toxins from the Sea Wasp *Chironex Fleckeri*. By H. D. Crone and T. E. B. Keen. Pp. 8. (Ascot Vale, Victoria: Chief Superintendent, Defence Standards Laboratories, 1970.) [304

US Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Resource Publication No. 87: Review of the Problem of Birds Contaminated by Oil and Their Rehabilitation. By John W. Aldrich. Pp. iv+23. $0.35. Resource Publication No. 65: Effects of Surface Mining on Fish and Wildlife in Appalachia. By Joseph A. Boccardy and Willard M. Spaulding, Jr. Pp. iv+20. $0.35. Resource Publication No. 68: Effects of Surface Mining on the Fish and Wildlife Resources of the United States. By Willard M. Spaulding, Jr., and Ronald D. Ogden. Pp. iv+51. $0.60. (Washington, DC: Government Printing Office, 1970 and 1971.) [35

Carnegie Institution. Annual Report of the Director of the Department of Terrestrial Magnetism, 1969/1970. (Reprinted from *Carnegie Institution Year Book 69* for the year July 1, 1969—June 30, 1970.) Pp. 367–550+7 plates. (Washington, DC: Carnegie Institution, Department of Terrestrial Magnetism, 1971.) [35

World Health Organization. Technical Report Series, No. 464: Joint FAO/WHO Expert Committee on Brucellosis—Fifth Report, Geneva, June 29–July 6, 1970. Pp. 76. (Geneva: WHO; London: HMSO, 1971.) 4 Sw. francs; 40p; $1.25. [35

Official Records of the World Health Organization, No. 188: The Work of WHO 1970—Annual Report of the Director-General to the World Health Assembly and to the United Nations. Pp. xvii+305. (Geneva: WHO; London: HMSO, 1971.) 9 Sw. francs; 90p; $3. [35

National Institute of Nutrition, Hyderabad. Annual Report for the period October 1, 1969, to September 30, 1970. Pp. viii+140. (Hyderabad: National Institute of Nutrition, Indian Council of Medical Research, 1970.) [35

Population Bulletin, Vol. 27, No. 1 (February 1971): The Future Population of the United States. Pp. 32. (Washington, DC: Population Reference Bureau Inc., 1971.) [45

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 141, No. 1 (December 17, 1970): West Indian Xenodontine Colubrid Snakes—Their Probable Origin, Phylogeny, and Zoogeography. By Vincent J. Maglio. Pp. 53. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1970.) [45

Fondation Universitaire. 50e Rapport Annuel, 1969/1970. Pp. 161. (Bruxelles: Fondation Universitaire, 1971.) [45

US Department of the Interior: Geological Survey. Bulletin 1315: Geochemical and Geological Reconnaissance in the Seventymile River Area, Alaska. By Sandra H. B. Clark and Helen Foster. Pp. iii+21. $0.25. Bulletin 1313–A: Variable Azimuth Schlumberger Resistivity Sounding and Profiling Near a Vertical Contact. By Adel A. R. Zohdy. Pp. iv+22+1 plate. $0.65. Bulletin 1333: Geology of the McCarthy B–4 Quadrangle, Alaska. By E. M. MacKevett, Jr. Pp. iii+31. $0.25. (Washington, DC: Government Printing Office, 1970 and 1971.) [45

Umweltforschung—Aufgaben und Aktivitäten der DFG 1950 bis 1970. Pp. 184. (Bonn–Bad Godesberg: Deutsche Forschungsgemeinschaft, 1971.) [45

Alfred P. Sloan Foundation—Report for 1970. Pp. viii+72. (New York: Alfred P. Sloan Foundation, 1971.) [65

Science Council of Canada. Report No. 11: A Canadian STOL Air Transport System—a Major Program. Pp. 33. $0.75. Report No. 12: Two Blades of Grass—The Challenge Facing Agriculture. Pp. 61. $0.75. Background Study for Science Council of Canada. Special Study No. 20: Prospects for Scientists and Engineers in Canada. By Frank Kelly. Pp. 61. $1. (Ottawa: Information Canada, 1971.) [65

Smithsonian Contributions to Zoology. No. 82: Pontoniinid Shrimps from the Ninth Cruise of RIV *Anton Bruun*, IIOE, 1964. I: *Palaeomnella* Dana and Periclimenes Costa. By A. J. Bruce. Pp. 13. $0.25. No. 83: The Distribution and Patterns of the Major Arteries of the Iguanids and Comments on the Intergeneric Relationships of Iguanids (Reptilia: Lacertilia). By George R. Zug. Pp. 23. $0.40. No. 72: Revision of the Fish Genus *Ecsenius* (Blenniidae, Blenniinae, Salariini). By Victor G. Springer. Pp. 74. $1.25. No. 73: Synopsis of the Tribe Salatiini, with Description of Five New Genera and Three New Species (Pisces, Blenniidae). By William F. Smith-Vaniz and Victor G. Springer. Pp. 72. $1.25. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [65

Geological Survey of Canada. Paper 69–56: A Collection of Papers prepared for a Study of the Earth Sciences in Canada, commissioned by the Science Council of Canada. Edited by C. H. Smith. Pp. iv+318. $3.50. Paper 70–40: Basins and Geosynclines of the Canadian Shield. (A Collection of Papers presented at a Symposium held in Ottawa, March 17/18, 1970.) Edited by A. J. Baer. Pp. vi+165. $3.50. Paper 70–64: The Adsorption and Coprecipitation of Silver on Hydrous Oxides of Iron and Manganese. By Willy Dyck. Pp. iii+23. $1.50. Bulletin 180: Stratigraphy and Structure of the "Keno Hill Quartzite" in Tombstone River—Upper Klondike River May-Areas, Yukon Territory. By D. J. Tempelman-Kluit. Pp. 102. $3. (Ottawa: Information Canada, 1970 and 1971.) [65

The 1971 Eruption of Cerro Negro, Nicaragua. By J. G. Viramonte, E. Ubeda and M. Martinez. Pp. 28. (Cambridge, Mass.: Smithsonian Institution. Center for Short-Lived Phenomena, 1971.) [75

Institutt for Atomenergi, Kjeller Research Establishment. Kjeller Report No. 142: Instrumental Neutron Activation Analysis of Zircalloy Using a Ge(Li) Detector. By E. Steinnes. Pp. 11. (Kjeller, Norway: Institutt for Atomenergi, Kjeller Research Establishment, 1971.) [115

Role Playing and Teacher Education: a Manual for Developing Innovative Teachers. By David L. Lehman. Pp. 59. (Washington, DC: The Commission on Undergraduate Education in the Biological Sciences, 1971.) *gratis*. [115

Pesticide Residues in Food: Report of the 1969 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Group on Pesticide Residues, Rome, 8–15 December 1969. (FAO Agricultural Studies No. 84; World Health Organization Technical Report Series, No. 458.) Pp. vi+43. (Rome: FAO and WHO; London: HMSO, 1970.) [115

Sveriges Geologiska Undersökning, Stockholm. Lovö Geomagnetic Observatory, Year Book 1968. Pp. 38. Lovö Geomagnetic Observatory, Year Book 1969. Pp. 38. (Stockholm: Sveriges Geologiska Undersökning, 1971.) [115

Carnegie Institution of Washington, Year Book 1969, 1969–1970. Pp. x+767. (Washington, DC: Carnegie Institution of Washington, 1971.) [115

Smithsonian Studies in History and Technology. No. 6: History of Letter Post Communication Between the United States and Europe, 1845–1875. By George E. Hargest. Pp. ix+234. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) $4.25. [115

Annals of the South African Museum. Vol. 57, Part 8: Amphipoda from Southern Angola. By Brian Kensly. Pp. 149–156. (Cape Town: South African Museum, 1971.) [125

Annual Report of the Mauritius Institute for 1968. Pp. 12. Annual Report of the Mauritius Institute for 1969. Pp. 24. (Port Louis: Mauritius Institute, 1969 and 1970.) [135

US Department of the Interior: Geological Survey. Professional Paper 599–F: Summary of Lunar Stratigraphy—Telescope Observations. By Don E. Wilhelms. Pp. iii+47. $0.60. Professional Paper 638: Geology and Magnetite Deposits of the Franklin Quadrangle and Part of the Hamburg Quadrangle, New Jersey. By Donald R. Baler and A. F. Buddington. Pp. v+73+2 plates. Professional Paper 639: Ordovician Brachiopods, Trilobites, and Stratigraphy in Eastern and Central Nevada. By Reuben James Ross, Jr. Pp. iv+103+19 plates. Professional Paper 640–C: Eocene Mollusks from Eua, Tonga. By Harry S. Ladd. Pp. vii+10+5 plates. $0.55. Professional Paper 644–B: Hydrocarbons in Thermal Areas, Northwestern Wyoming. By J. D. Love and John M. Good. Pp. 23. $0.40. Water-Supply Paper 1904. Surface Water Supply of the United States 1961–65. Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Vol. 1: Basins from James River to Savannah River. Pp. x+942. (Washington, DC: Government Printing Office, 1970.) [135

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin No. 200: Biostratigraphy of some Early Middle Silurian Ostracoda, Eastern Canada. Additional Silurian Arthropoda from Arctic and Eastern Canada. By M. J. Copeland. Pp. 13+4 plates. $1.50. Paper 70–12: Geology, Colville Lake Map-Area and Part of Coppermine Map-Area, Northwest Territories. By D. G. Cook and J. D. Aitken. Pp. vi+42. $1.50. (Ottawa: Information Canada, 1971.) [145

National Research Council of Canada. Annual Report on Support of University Research 1969/1970. Pp. viii+412. (Ottawa: National Research Council of Canada, 1971.) [185

Australia: Commonwealth Scientific and Industrial Research Organization. Mineral Chemistry–Research Report 1969/1970. Pp. 20. (Port Melbourne: CSIRO, 1971.) [185

US Department of the Interior: Geological Survey. Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A1: Methods for Collection and Analysis of Water Samples for Dissolved Minerals and Gases. By Eugene Brown, M. W. Skougstad and M. J. Fishman. (Book 5, Laboratory Analysis.) Pp. vii+160. (Washington, DC: Government Printing Office, 1970.) $2. [185

Bulletin of the American Museum of Natural History. Vol. 144, Article 1: Stratigraphy of the Santa Fe Group, New Mexico. By Ted Galusha and John C. Blick. Pp. 1–128. $6. Vol. 144, Article 2: Gastropoda and Monoplacophora of the Solsville Member (Middle Devonian, Marcellus Formation) in the Chenango Valley, New York State. By Harold B. Rollins, Niles Eldredge, and Judith Spiller. Pp. 129–170. $2.30. (New York: American Museum of Natural History, 1971.) [185

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature+ Nature Physical Science	**£24**	**$83**	**$90**
Nature+ Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431

American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004

All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, July 30 1971

NATURE Volume 232 AUGUST 6 1971

Black Arrow Aborts

THE only complaint to make about the abandonment of the development of the Black Arrow rocket (see page 362) is that the decision was not taken several years ago. Since 1964, when the programme was begun, only a few enthusiasts have professed conviction that they knew what Black Arrow might accomplish. To most people, it has been clear all along that the fully developed rocket would be too small to accomplish the tasks which the then government specified for it. The result is that more than £11 million has been for practical purposes wasted on the development programme and on the launching of three rockets at a cost of £2 million each. The question whether the programme should continue has no doubt been brought to a head by the failure last year of an attempt to attain an Earth orbit. In the circumstances, there will clearly be a great many pairs of crossed fingers as the time approaches, later this year, for the attempt to launch the X3 satellite. Whatever happens, however, that will be the last monument to Black Arrow. Thereafter, the British Government will turn, as it might have done in the first place, to the National Aeronautics and Space Administration in the United States. It is earnestly to be hoped that the talk now to be heard of rekindled British interest in a European launcher rocket is, for the time being at least, all smoke and no fire. In the meantime, three questions should be answered—what can be done to prevent similar mistakes in the years ahead, what strategy should be adopted for the development of space research in Britain and what opportunities are there, in this hard-headed world, for making money out of space?

Black Arrow was born of compromise. Quite sensibly, after the unhappy experiences of the early sixties, with the concept of a military rocket able to carry nuclear warheads from one place to another, the British Government was persuaded that it could not set up in business as an independent satellite launcher. That, in any case, was what the European Launcher Development Organization (ELDO) was meant to accomplish but there was a seductive line of argument that there would be serious dangers in contracting out of what was called space technology —the design and operation of solar cells, ion rockets and the like. The result was the decision to develop a launch vehicle capable of putting experimental packages into orbit but not big enough to launch satellites of any practical value in applications. (The scheme for using electrical propulsion driven by solar cells as a means of progressively driving out the orbit of such a satellite to geostationary distances is ingenious enough but unlikely to compete successfully with techniques of satellite launching that achieve a geostationary orbit at one fell swoop.) Worse still, however, the Black Arrow development programme was run on a shoestring. For the first year and a half, until the beginning of 1966, contracts were awarded for periods of three months at a time, with the result that those working on the programme were frustrated and, predictably, by the time Black Arrow was in service, most of what it might have achieved had already been done elsewhere. It is also now a familiar experience, in several other countries as well as the United Kingdom, that it is not safe to base a rocket development project on a single launching once a year. The waste of time and money if one rocket should fail (as one and a half have done with Black Arrow) cancels out the economies that parsimony brings. This is the point which the Estimates Committee of the House of Commons singled out for special complaint in 1967, but nothing much has been done since then to put the programme on a better footing. The moral, of course is that there is no point in trying to enter a quickly growing branch of advanced technology with only just enough resources to wet the feet. Black Arrow has been an unnecessary but expensive product of wishful thinking.

What should happen next? Plainly the British Government cannot hope to mount programmes comparable in scale even with the malnourished Black Arrow programme without spending much larger sums of money than it is accustomed to devote to work like this. In the past five years, the Black Arrow programme hasaccounted for more than one-sixth of expenditure on space research and technology carried out by British organizations. At the moment, Black Arrow is running at about twenty per cent of this same expenditure. Evidently it would be quite out of the question to mount a more effective programme to succeed Black Arrow without increasing the space budget by several million pounds a year and by recognizing that close on a half of all national expenditure on space research and technology would go on this new venture. That, evidently, is out of the question. In any case, the defence research programme, which has already led to the valuable Skynet communications satellite, is probably a sufficient guarantee that British laboratories will know what the Joneses are doing even if they cannot keep up with them in every respect. In the circumstances, British policy should aim at making sure that at least some British organizations will be able to make effective bids for the Intelsat contract that should then become available for building communications satellites. Nobody should be ashamed of asking NASA to keep on launching scientific satellites labelled with a Union Jack. What matters there, as every scientist will say, is whether the satellites are launched successfully and whether they function well when once in orbit.

But is there money to be made? It is perhaps salutary to recall that communications satellites are potentially valuable because they are, above all, cheap. The first five copies of the Intelsat IV satellite, each with up to 6,000 telephone circuits or their equivalent, cost Intelsat $72 million and it seems to be acknowledged that the price would have been lower if Comsat had been a better manager. The second five copies cost less than half as much, and are likely to satisfy the needs of Intelsat for the rest of this decade. The questions that governments like the British should ask themselves is how much it may be worth spending to win a chance of competing successfully for a business that seems to amount to something of the order of $100 million a decade, or $10 to $20 million a year. On the face of things, it would be safer to put the money into a programme for developing a technology in some more reliable market, typewriters for example. To be sure, the enthusiasts such as the British Aircraft Corporation say that in due course there will be a rich market for supplying communications satellites to individual countries or groups of countries wishing to have one to themselves, or even in broadcasting educational television. The trouble here is that the market may not exist at all—much depends on the outcome of experiments still to be mounted as well as on the regulations adopted by governments to control the importation of television programmes from outside. And applications of communications satellites other than in telecommunications, socially valuable though they may be, are necessarily less prolific markets for the satellite builders. From a strictly national point of view, in other words, as well as from all others, it is essential that the British Government, now embarked on yet another review of "the national space technology programme", should try to head off the enthusiasm of the country's aerospace industry as it calls itself, and that it should persuade them to put their backs into more mundane activities such as telephone switching equipment.

Sheep and Goats

GOVERNMENTS on both sides of the Atlantic have in the past week been perplexed by the need to make decisions about the future of important enterprises. In the United States, the Senate on Monday this week did finally agree, over Mr William Proxmire's protesting but much alive body, to provide a guarantee for a bank loan to the Lockheed Company so as to provide it with the finance necessary to keep the company alive. In Britain, the government has taken a similar view about its attitude towards the computer company International Computers Limited, which in the past few months has from time to time been alarmed by harsh words from government ministers anxious to impress on all kinds of industries that nothing stands between a company and bankruptcy except profit. Although no money will change hands in an obvious sense, ICL will be helped along by government research contracts and will also benefit from a procurement policy likely in the future to be more effective than in the past (see page 360). Other computer companies in Britain are understandably upset by this development, especially because many of them must have been calculating that the impending entry of Britain into the EEC would have been a splendid way of extending their influence on the mainland.

In case the British Government should be considered to be backsliding from the spartan views which Mr John Davies at the Department of Trade and Industry has been advocating in the past year, however, no less a person than Mr Davies himself stood up in the House of Commons last Friday to pronounce the end of government support for Upper Clyde Shipbuilders Limited, an amalgamation of four Scottish shipbuilding yards which has been supported since 1967 with a total of £27 million and which is now in bankruptcy. No doubt this decision would have been an even more dramatic incident if the workers affected had meekly joined the dole queue; instead they have quixotically kept on working. But for them and taxpayers everywhere, it may be important to know by what criteria conservative governments in the United States and Britain have decided to go against their principles and prop up lame ducks. Why is Upper Clyde Shipbuilders less deserving than International Computers Limited?

The most obvious difference is that shipbuilding is, so far as Britain is concerned, an ageing industry, while International Computers Limited is at least an enterprise that may bring profit in the years ahead. In other words, it may well seem that what the British Government is doing is prudently to invest in the future and sensibly to wash its hands of the past. The difficulty, of course, is that industrial issues are never as simple as this. Ever since the publication of the Geddes report on shipbuilding five years ago, it has been clear that there is nothing wrong with British shipbuilding except the way in which the industry is organized. There have been too many yards, too many builders of marine engines, too little equipment which is modern and efficient and too casual a sense of the difference between profitability and failure.

Upper Clyde Shipbuilders came into being partly because of the then government's wish to make progress along the lines suggested by the Geddes Commission and partly because of its wish to encourage some novel, almost syndicalist, experiments in labour relations at the Fairfield's yard, one of those now amalgamated into Upper Clyde Shipbuilders. Perhaps, in retrospect, nobody should be surprised that the prospect of closure has stimulated schemes for enabling workers to assume and retain control. Yet the case of British shipbuilding is entirely similar to that of British textiles two decades earlier—too many companies, too little productivity insufficiently compensated for by low wages, too little new machinery and no semblance of financial understanding, let alone control. The Geddes Commission in 1967 would have done well to have spelt this out more clearly. The last government would have done the shipyards as well as its reputation a service by looking for radical solutions to shipbuilders' problems, not by attempting to paper over the cracks with what is now acknowledged to be a theoretical merger.

As things have turned out, the steps that might have been painless four years ago have now been forced on the shipyards, on the government and on the people of Clydeside. In his first statement, last week, Mr John Davies spoke as if the issue was simply a question of cash, and there is of course no doubt that in strictly commercial terms, Upper Clyde Shipbuilders are bankrupt. Even if they were able to finish off their ships on time, they would be almost certain to lose money on them. No doubt the independent (but some say biased) committee which provided the government with advice is right to have said that work would have to be concentrated on two yards, not four, and it is logical for the government to take the line that even such a solution will be acceptable only if private industry seems inclined to take a risk in building ships on the Upper Clyde. Yet is it proper for a government to wash its hands as easily as Mr Davies has done of responsibilities implicitly incurred by its predecessor? Rightly or wrongly, most probably wrongly, Mr Anthony Wedgwood Benn, when Minister of Technology, allowed public funds to be engaged in keeping the Upper Clyde Shipbuilders going from one crisis to the next. By doing so, he helped to create an illusion of security among the 9,000 people on the payroll. Is it permissible now for his successor to pretend that such an implicit commitment can be dismissed simply because the government has changed? It is creditable that Mr Davies's contribution to the debate in the House of Commons last Monday did more to acknowledge that it is unseemly to throw 4,000 people out of work in an area in which unemployment is already high. It is a question whether, in circumstances like these, the government does not owe the community a much more active campaign to help ensure that the consequences of shutdown are ameliorated. This, after all, is one of the continuing roles of governments in advanced societies in which old industries must continually be overtaken by new.

A second complaint against what may be a perfectly respectable decision to recognize the bankruptcy of Upper Clyde Shipbuilders Limited is that the government has in its present way of thinking no means of encouraging the kind of rationalization of the shipbuilding industry which needs to be attempted. It is all very well to say that governments should let industries and companies demonstrate by performance whether they can sink or swim but this is too passive a point of view. There are, after all, carrots as well as sticks, and it should not be beyond the wit of governments such as even the present to devise ways of moulding the shipbuilding industry in Britain into a pattern which is commonly held to be viable. To be sure, there are some theoretical difficulties in the way for those who believe that only the natural tug of war to which industrial companies are naturally exposed can be relied upon to chart the course for the years ahead, but this is like saying (as some heuristically inclined educationists insist) that if you put a child in an empty room and leave him for long enough, he will rediscover Newton's laws. The truth is that there are plenty of ways of combining carrots with sticks so as to catalyse the process of self-discovery. What the shipbuilding industry in Britain needs is more encouragement to invest in new equipment and more vigorous criticism of its performance. The government would do a public service if it recognized that it must play a part in bringing out these conflicting pressures in the industry. After all, there are several candidates in line for similar treatment. After shipbuilding will come the motor car industry. Can machine tools be far behind?

By these standards, the plan to give ICL a sense of security which it has not entirely earned by its own efforts leaves much to be desired. The chief difficulty is that there is no means of knowing whether it is possible to organize a computer company on a strictly national basis. With shipbuilding, it is true, comparatively small enterprises can succeed—the Scandinavians and the Japanese have shown precisely that. But with computer manufacturers, the high cost of meaningful research and development puts computer manufacturing in a class with aircraft manufacture and petroleum production. The chances are that the most straightforward way of launching such an enterprise is to have access to several markets and to several sources of technical skill. It may be the case that the British Government is building up ICL as a kind of dowry with which to join the EEC. It is also of course true that ICL itself has been enterprising in forging links with companies abroad, notably the Control Data Corporation (but its association with RCA has come to an end in the past few weeks). In all the circumstances, there is a danger that the British Government may be seeking to prop up an organization in the wrong pattern. This is the error which its predecessors made, but it may be a little more demeaning that the mistake on this occasion resembles that by means of which tiny governments give themselves an air of going places by sponsoring with taxpayers' money airlines which cannot be commercially viable.

The case of Lockheed is very similar. Ironically, the Senate's narrow endorsement by one vote of the earlier decision by the House of Representatives to guarantee a loan of $250 million to a corporation already in the red to the tune of $400 million came just a week after the first flight of the Douglas DC-10, an aircraft that would be entirely competitive with the Lockheed Tri-Star airbus if the two manufacturers had not insisted in advance that potential customers should place firm orders and make appropriate non-returnable deposits as marks of their good faith. As it happens, the putative customers now regret having placed their orders, for air traffic has lagged behind the forecasts of a few years ago. The ideal outcome of the trouble about Lockheed would have been a merger with Douglas, even though that might well have left the luckless Rolls-Royce with nothing to keep the RB211 engine alive.

What Congress has now done is to help support an entirely laudable organization which is nevertheless cast in the wrong mould—in spite of the buoyancy and the commercial resilience of the American based airlines, the United States is already too small to support three large suppliers of civil aircraft. As with computers, so with aircraft, the development costs are necessarily so high that only multinational companies can hope to be successful. But Lockheed is, like its competitors, also closely tied to national programmes for defence research and development. Congress would have done better for Lockheed, for airlines and for American taxpayers if it had found some way of pushing the company towards a different strategy for organization with less dependence on the domestic market and the local government. Like the British shipbuilders and the British computer manufacturers, American aircraft manufacturers are in danger of strangling themselves with too little imagination. There are no sheep, they are all goats.

100 Years Ago

THE charge against Mr. Hampden of libelling Mr. Wallace was tried on Thursday week in the Secondary's Court. Our readers will remember that a wager of 500*l*. having been made between Mr. Hampden, who affirmed that the world was flat and not round, and Mr. Wallace, it was decided against the former, who thereupon abused Mr. Wallace as a liar and a swindler. The action now tried was for damages for these and other similar libels, and Mr. Hampden was condemned to pay 600*l*. damages.

From Nature, **4**, 287, *August* 10, 1871.

OLD WORLD

GAS CENTRIFUGE

All Set for Bensberg

EUROPEAN uranium enrichers in Britain, Germany and Holland are now able to point to at least one tangible sign of progress—the formal establishment of the German-based company called CENTEC at Bensberg, near Cologne, on July 29. The new company, formally known as CENTEC Gesellschaft für Centrifugentechnik mbH, will be responsible for the research and development programme which, the sponsors hope, will yield a couple of functioning pilot plants by about 1975, one in Britain and one in Holland. When the technical and economic feasibility of gas centrifuge machines for enriching uranium has been assessed, CENTEC will be empowered to design and build enrichment plants to the orders of its customers. As at present foreseen, the principal customer will be a company called URENCO, similarly sponsored by the three governments of Britain, Germany and Holland, which will be able to process and to sell enriched uranium to potential users. Theoretically, CENTEC will also be able to sell enrichment plants to countries not involved in the development programme, but obviously the sponsoring governments will be more anxious to set up as successful suppliers of enriched uranium, with the continuing profit that that will bring, than to supply outside parties with opportunities to set up in competition. URENCO has not yet been formally established, but its shareholders will be, as with CENTEC, the Dutch company Ultra-Centrifuge Nederland NV, the German company Gesellschaft für Nukleare Verfahrenstechnik mbH and British Nuclear Fuels Limited.

Those involved with the new development seem to be as optimistic now as were the three sponsoring governments when news of the possible use of gas centrifuges in uranium enrichment was first made public two years ago. By all accounts, individual centrifuge machines have been tried out separately and in cascade, with the result that the designers are confident that the machines will function. The questions to be decided chiefly concern the cost of production. Mr T. Tuohy, managing director of British Nuclear Fuels Limited and a director of CENTEC, said earlier this week that the obvious target at which to aim is the United States Atomic Energy Commission price of $32 per ton of separated work, the equivalent of $35 per ton delivered in Europe. He said that he and his colleagues were optimistic about the prospects—that this goal could be attained.

The chairman of the board of the new German-based company is Dr Ir Han Hoog, recently retired as a director of Shell Petroleum Limited and its Dutch equivalent. The general manager of the new company is Mr David Peirson, secretary of the UK Atomic Energy Authority for the past sixteen years. The new company's budget is not publicly available, although it is expected that total development costs will be several million pounds. Under the arrangements which have been worked out so far, research contracts will be farmed out by CENTEC to the three shareholding companies. The result is that only the technical staff of CENTEC will be in a position to make an overall assessment of the programme of research and development.

SCIENTISTS' PAY

Calm Before the Storm

BOTH sides involved in the scientists' pay dispute are, this week, taking stock of the situation before the official arbitration tribunal sits next Thursday, August 12. The Institution of Professional Civil Servants (IPCS) has organized a public meeting scheduled for 7.00 p.m. on August 10 at the Central Hall, Westminster, to bring to a climax its campaign that started at Abingdon three weeks ago.

These meetings have attracted large numbers of people but they have produced no visible change in the attitude of the Civil Service Department. A spokesman for the department said earlier this week that he could not comment on the strength of feeling shown at the IPCS meetings, but that the official side's case—based on the findings of the Pay Research Unit—had already been submitted to the tribunal. The unit's report has been interpreted by the government to show that a scientist within the Scientific Civil Service is as well off financially as his counterpart in industry.

The institution counters this by saying that the comparison is unfair in the sense that several of the companies included in the pay research exercise base their salaries on what the government pays scientists. It also says that the government's offer makes no allowance for the fact that the administrative grades within the Civil Service received a pay award earlier this year and so this present offer destroys the concept of internal relativity within the Civil Service.

Mr William McCall, the general secretary of the IPCS, said earlier this week that any independent body would be certain to recognize the merits of the institution's case and that he was confident that the outcome of the tribunal would be favourable to the scientists. Mr McCall also said that if the tribunal does not produce what he expects, he will press for a review of the basis for determining scientists' salaries within the Civil Service.

Whatever the findings of the tribunal, there will be pressure to change the procedure used to determine government scientists' salaries. If there is no award, the IPCS will evidently seek to change the system. If, on the other hand, the tribunal recommends all or even part of the claim, then the whole of the pay research investigation will be invalidated. For outsiders, however, it remains a paradox that the report of this Pay Research Unit is still confidential between the Civil Service Department and the IPCS.

The question of whether the report of the Pay Research Unit should be made public was raised in the House of Commons on July 29 when the Prime Minister, Mr Edward Heath, told Mr Tam Dalyell that the decision to abide by the recommendations of the Pay Research Unit was originally made by the previous government, but that "it does not seem justifiable, when the pay is above those already working outside, that those concerned should still have increases of salary". The Prime Minister added that he would enquire into the possibility of making public the report of the Pay Research Unit.

COMPUTERS

No Breadline for ICL

THE announcement last week by Mr Frederick Corfield, Minister for Aerospace, that the British Government would continue to support International Computers Limited is certainly a shot in the arm for the company, but the decision has aroused some strong feelings among the other computer companies in Britain. These, mostly offshoots of United States corporations, naturally feel hard done by and their strongest complaint is that the minister has apparently reversed previous government policy in granting further research and development money to ICL.

ICL has always been in a privileged position in the supply of computers to government departments, and the new statement will ensure that it remains so. The company's position is determined by single tender orders which involve no competition from other manufacturers. Such preferential treatment should ensure a thriving company, but the signs are that all is not well at ICL, with 1,800 redundancies announced last week to add to the 1,600 announced earlier this year. Sir John Wall, the chairman of ICL, has said, however, that the company is in good shape and that the pessimism about the future is not well founded. Sir John also stated that the cut back in staff was evidence of ICL's determination to live within its resources.

International Business Machines Limited has been maintaining a dignified silence about the events of the past few days, but the Honeywell company has spoken out against the government's action. Mr Donald Brosnan, chairman and managing director of Honeywell, said this week that "the policy works against the British computer industry as a whole and in particular against the development of computing in the public sector. Not only does it stifle competition but it also limits access to international technology".

The question that remains unanswered is whether the confidence placed in ICL is well founded. Are the performance and cost of ICL computers comparable to or better than their competitors? Such a comparison is not possible without a detailed analysis of the needs of a particular user; in particular it depends on the scientific/commercial balance that the computer user needs to achieve.

The computer companies stress that they will only quote performance figures for a particular job assignment. Despite this, however, it is apparent that the Honeywell 6000 series, the IBM 370 series and the ICL 1900 series are comparable and competitive.

With ICL receiving such support from the government and also in view of the pressure that is being exerted on industry and public sector bodies in Britain to buy ICL computers, it is not difficult to understand why the other companies are annoyed. Mr Brosnan expressed the feelings of these companies when he stated that Mr Corfield's statement "ignores the interest of foreign owned companies which manufacture and employ significant numbers of people in the United Kingdom (Honeywell employs 8,000). In so doing it conflicts with other stated government policies of attracting overseas investment to the nation's development areas".

EARTHQUAKE RESEARCH

What Future for Tokyo?

from a Correspondent

THERE is great anxiety among seismologists about the future of the International Institute of Seismology and Earthquake Engineering, set up in Tokyo in 1962 and supported jointly by the Japanese Government and the United Nations Development Programme. The immediate problem is to know how the institute can continue after August 1972, when the present agreement runs out. At what seems to have been a sombre meeting of the senior consultants of the institute in Tokyo in March this year, it was accepted that continuing support for the institute would have to come from the government of Japan and from UNESCO.

The institute has a permanent staff of about twenty provided by the government of Japan. UNESCO also provides two or three "experts" each year, usually seismologists and earthquake engineers from outside Japan. The institute conducts regular lecture and laboratory courses, as do the visiting lecturers.

An average of some twenty students a year have been trained at the institute. Over the years, they have come from more than forty countries, including developing countries in Asia, Africa, South America and the Pacific region. Stipends have been provided by the government of Japan and through the UNDP Special Fund.

During 1963–68, the institute concentrated on routine training. The programme has now been extended to include an advanced course in which the emphasis is on research training. The students, some five a year, have previously been PhD graduates or hold equivalent qualifications. The advanced students assist in the lecturing programme.

The institute has a special role in fostering seismology and earthquake engineering in developing countries, where there is an urgent need both to mitigate the effects of earthquake disasters and to fill conspicuous gaps in the global recording of earthquakes.

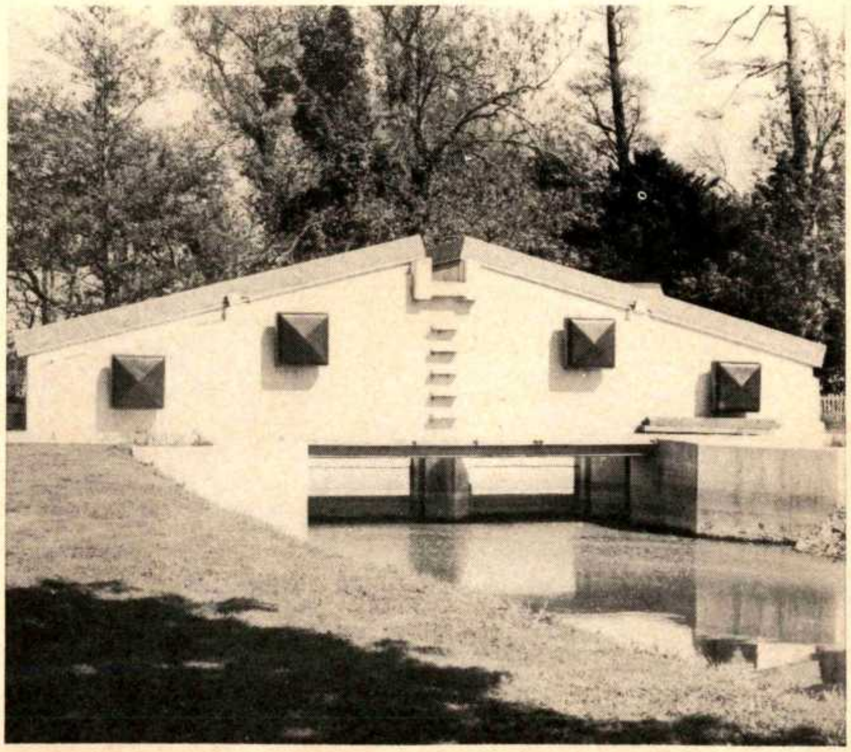
ASTRONOMY

South African Debate

EMOTIONS are still running high at the closing of the only strictly South African observatory, the Republic Observatory at Johannesburg. There have been bitter exchanges in the correspondence columns of *Monthly Notes of the Astronomical Society of South Africa*. The British Science Research Council is involved to the extent that the rationalization of South African astronomy was stimulated by the review of astronomy in the southern hemisphere completed late in 1968 by

Freshwater Biology

THIS is a fluvarium which was opened on July 23, together with other extensions, at the site of the River Laboratory of the Freshwater Biological Association in East Stoke, Dorset. The fluvarium, which was opened by Mr Peter Walker, Secretary of State for the Environment, has been so called because it is an aquarium in which the tanks contain flowing water. Built where once a corn mill stood, the building is a water-tight concrete box, 30 feet by 12 feet, sunk 9 feet into the ground. The roof is made of glass, and the flow of water through the fluvarium is maintained by gravity, without recourse to pumps.



a committee of four set up by the council.

Like the more recent review of astronomy in the northern hemisphere (see *Nature*, **232**, 289; 1971) the report of the southern hemisphere review committee was never published. But it is understood that the committee was particularly concerned with the reduction of British commitments in South Africa with the building of the Anglo-Australia 150-inch telescope.

As a part of this plan, the Royal Observatory at the Cape of Good Hope, which has been in the hands of the Science Research Council since 1965, is to be run as a joint venture with the South African Council for Scientific and Industrial Research. At the end of this year, the Royal Observatory at the Cape will become the headquarters of what is to be known as the South African Astronomical Observatory, having as its first director Sir Richard Woolley who retires as Astronomer Royal at the end of the year. The chief facilities of the observatory, however, will not be at Cape Town, where of course city lights have long been a problem, but at a new site which is being established 235 miles to the north-east at Sutherland.

It is understood that the British contribution will be of the order of R1 million over ten years, compared with R2 million from the CSIR over the same period, and that the CSIR will have the controlling interest.

What some astronomers in South Africa and elsewhere are objecting to is that the rationalization of South African astronomy also involves the closing by the CSIR of the observatory at Johannesburg. The most vociferous support for keeping the Republic Observatory comes from Dr W. S. Finsen, who was director from 1956 (when the observatory was known as the Union Observatory) to 1965, and Dr W. H. van den Bos, director from 1941 to 1956. But Drs Finsen and van den Bos are being encouraged in their campaign by astronomers outside South Africa. Among others, Drs A. F. Cook and B. G. Marsden of the Smithsonian Astrophysical Observatory have expressed their concern about the future of long-term programmes on the positions of asteroids and on double stars at present being carried out by the Republic Observatory.

The reaction of the CSIR is that omelettes cannot be made without breaking eggs. Unfortunately, however, the continuing debate between the opposing factions is being conducted at an acrimonious and personal level, in *Monthly Notes* as well as in the Johannesburg newspapers.

Among the concerns of Drs van den Bos and Finsen seem to be that the new arrangements will put the centre of gravity of South African astronomy in the Cape Town area. They are suspicious that the role of the Royal Observatory at the Cape as the headquarters of the new observatory was decided before the location of the new observing site was chosen, rather than the sequence of events presented by the CSIR in which the site at Sutherland was chosen first, after which the selection of the Royal Observatory as headquarters became obvious because of its proximity. But Drs van den Bos and Finsen also dispute the choice of Sutherland because its "bleak and isolated locality" (on the western edge of the Karroo) will not be attractive to observers, because of their argument that the programmes on asteroids and double planets would not benefit from being transferred to Sutherland from Johannesburg and because of their claim that South Africa contains better sites than Sutherland.

The CSIR case has been put by Dr F. J. Hewitt, chairman of the South African National Committee on Astronomy and vice-president of the CSIR. He says that the Sutherland site was selected on its merits, that the choice of the Royal Observatory as headquarters then became logical, that the Republic Observatory is in any case run down, and that "a particularly unfortunate aspect has been the attempt to involve astronomers overseas in the campaign against these new proposals".

Particularly galling to Drs van den Bos and Finsen is that the CSIR did not appoint a new director to the Republic Observatory after Dr Finsen's retirement. Since then the observatory has been making do with an acting director, Mr J. Hers.

It is regrettable that the debate is taking place only after the decision on the South African Astronomical Observatory was announced by the CSIR on September 23 last year. As things stand, the efforts of Drs van den Bos and Finsen are in danger of being regarded as a lost cause being conducted by the old guard of South African astronomers reluctant to be moved from Johannesburg. Nevertheless, since the CSIR took over the running of the Republic Observatory from the South African Government in 1964, the CSIR has had the responsibility of seeing that rearrangements are made only after proper consultation, an obligation which Drs van den Bos and Finsen would say has not been carried out.

Meanwhile, it appears that the 26½-inch refractor at the Republic Observatory—the largest instrument and about fifty years old—is likely to continue in operation at Johannesburg for two or three years more. The observatory Franklin-Adams used for work on asteroids and comets at a site 50 miles outside Johannesburg is expected to remain where it is for a year or two. Discussions are going on about what to do with the other aspects of the work of the observatory—chiefly the maintenance of the national time service—and the observatory's library is being moved to Cape Town.

The rationalization now planned takes no account of the Radcliffe Observatory at Pretoria, which has in the past been financed by the British Science Research Council. For some time, the council has been saying that its support will end in 1974, and the Radcliffe Trustees, the formal owners of the observatory, would be hard pressed to know where to look for continued support. But the Radcliffe Observatory has a splendid 74-inch reflector, and the plans for the Sutherland site so far include no large telescope. Is it too much of a coincidence that 1974 would be a good time to move the Radcliffe instrument from Pretoria? And is there now a danger that that would be a further thorn in the flesh of the Afrikaaners on the Rand, ever suspicious of the Cape? After all, a site three-quarters of the way to the Cape does not seem like a no man's land even if it is mostly desert.

The consensus among astronomers is probably favourable towards the efforts by the CSIR to provide a strong centre for South African astronomy. In spite of several successes, astronomy in South Africa has not had the reputation that should have come to one of the few countries favourably placed to observe the relatively unexplored southern skies, which include the galactic centre and the Magellanic Clouds. The South African observatories have already been eclipsed by the observatories in Chile, where the United States and others are investing in the remarkably clear skies of the Andes.

Nevertheless, most astronomers will also wish that the CSIR had gone about its plan with more tact. And it is obviously important that some way should be found of continuing the long term programmes on the asteroids and double stars that are being carried out by the Republic Observatory.

SPACE POLICY

Black Arrow Scrapped

THE British Government has decided to end the Black Arrow satellite launcher programme. This is no surprise and indeed the decision has been foreshadowed on several occasions during the past few months especially in the evidence of the Ministry of Aviation Supply and the Department of Trade and Industry before Subcommittee B of the Select Committee on Science and Technology. It is nevertheless surprising that the government should have chosen to make its announcement only three months before the select committee is expected to publish its report and recommendations on the future of space research in Britain.

The Black Arrow rocket has been an integral part of the National Space Technology Programme for several years. Its end is a part of the review of the programme being carried out by Mr Frederick Corfield, Minister of Aerospace at the Department of Trade and Industry. The department has always argued that the programme should be oriented to achieve the best results for the smallest expenditure and with an eye on the need that British industry should gain technologically. At present, about £4.6 million a year is spent on the programme, about 20 per cent of the total for all British space activities (see Table). Of this total, about 55 per cent is spent on scientific research. The government evidently now feels that Black Arrow does not represent sufficiently good value for money. Some scientists had hoped that a British rocket would eventually be used to launch a British scientific satellite but that idea too dies with Black Arrow.

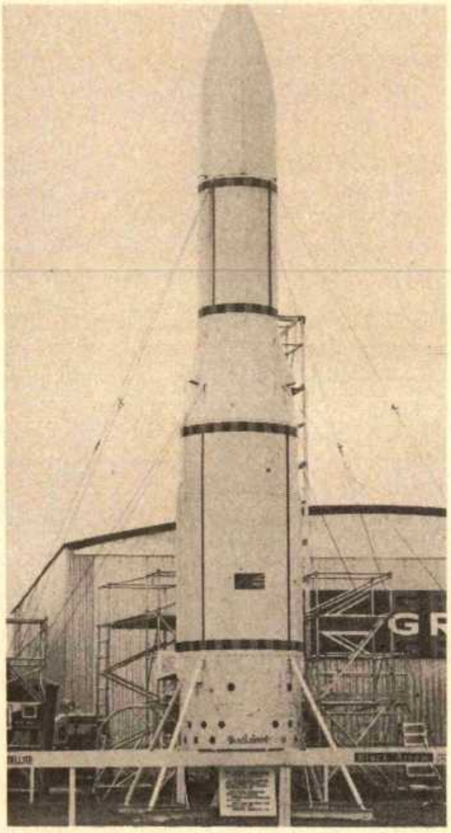
The X3 satellite due to be launched by Black Arrow in September.

The DTI seems to have convinced itself that there is very little expertise to be won from small satellites in the low level polar orbits which could be reached with Black Arrow. Nowadays, interest centres on satellites in geostationary orbits. The next launch of Black Arrow, with the X3 satellite, from Woomera during September will be the last. The planned X4 satellite will be launched from the United States on a Scout rocket.

British Space Expenditure, 1970-71 (£000 estimated)

International	
ELDO	£1,840
ESRO	£5,450
Intelsat	£1,130
Post-Apollo studies	£230
National	
Defence	£6,730
Commercial satellite communications (Post Office earth terminals)	£1,600
Scientific space research	£3,930
National Space Technology Programme	£4,640
Total	£25,550

Apart from being limited in capacity, Black Arrow is neither dependable nor cheap even allowing for the long intervals between launches, which have meant that it has never been fully tested. During the six years of development from the Black Knight rocket, £11.5 million has been spent with each launching costing £2 million. One launch has failed, one has been a success and one a partial success. The total cost of a Scout launching will be about £1.25 million. The whole rather unsatisfactory situation was summed up by the companies involved in the manufacture of the satellite who advocated earlier in evidence to Subcommittee B that Black Arrow should be fired oftener or scrapped altogether.



The Black Arrow Rocket.

The government's plans for testing much larger satellites and complete applications systems in geostationary orbit are unlikely to be spelled out before the end of the year—almost certainly after the Select Committee has presented its report. But the X4 satellite will contain a prototype system for maintaining the triaxial stability which is vital for most applications satellites, particularly for those in geostationary orbits. The Thor Delta is the obvious launcher but would cost about £3.3 million a time.

The British Government seems to be talking as if there will be no difficulty in being able to use whichever American rockets appear best to suit its plans for launching satellites, but no formal agreement yet exists and this is a field in which private understandings are notoriously capable of being undermined by the ins and outs of governments. In the circumstances, it would not be surprising if the British Government might yet choose to collaborate in the construction of some rocket launcher within the framework of ELDO, although it is clear that many hearts would have to change before that became a painless operation.

The preliminary decisions taken last month by the council of ESRO to go ahead with a space applications programme as distinct from the scientific programme which has been ESRO's chief concern since its inception in 1962 must also have influenced the decision to scrap Black Arrow. Britain at present contributes about £5.5 million a year to ESRO (see Table) and this sum is likely to increase quite rapidly if the applications programme goes ahead smoothly. France, Germany, Italy and Britain have agreed to provide £33 million by the end of 1973 and then a minimum of £30 million a year until 1980. It must have become clear to the DTI that if British industry is to contribute in a significant way to a European applications programme and thus provide some economic feedback for the money invested in ESRO, it must gain the expertise with geostationary systems that could not be properly attained with satellites launched by Black Arrow.

The increasing involvement of the DTI with ESRO also means that the department will take on an increasing share of the representation of the UK at ESRO. At present the Department of Education and Science provides most of the representatives although it is advised by members of the Department of Trade and Industry.

One of the unresolved questions is that of British or European participation in the post-Apollo programme which still suffers from lack of definition and whose very existence seems doubtful. One of the suggestions which the United States Government made last year was that Europe should contribute "at least 10 per cent" towards the development of the post-Apollo transportation system whose total development cost would be "about £4,000 million" during the next ten years. It has been suggested, for example, that agreement to join the post-Apollo programme could pave the way for NASA to launch European applications satellites and presumably UK payloads of the same type as well.

NEW WORLD

Davis Committee Exposes the Problems

by our Washington Correspondent

THOSE who believe that the United States has a workable and explicit policy for the support of research and development had only to attend three meetings last week of the Science, Research and Development Subcommittee of the House Committee on Science and Astronautics to discover that their faith may be groundless. The subcommittee, under the chairmanship of Mr John Davis, took a speculative bite at a problem that will keep it occupied throughout the autumn and winter, but which could well require many more years of probing before worthwhile conclusions can be formed. The subject that the committee intends to study is "science, technology and the economy"; whether the committee has bitten off more than it can chew remains to be seen. Nevertheless, to judge by what the hearings uncovered last week, the subcommittee could do a valuable job in bringing even some of the more obvious problems to public attention.

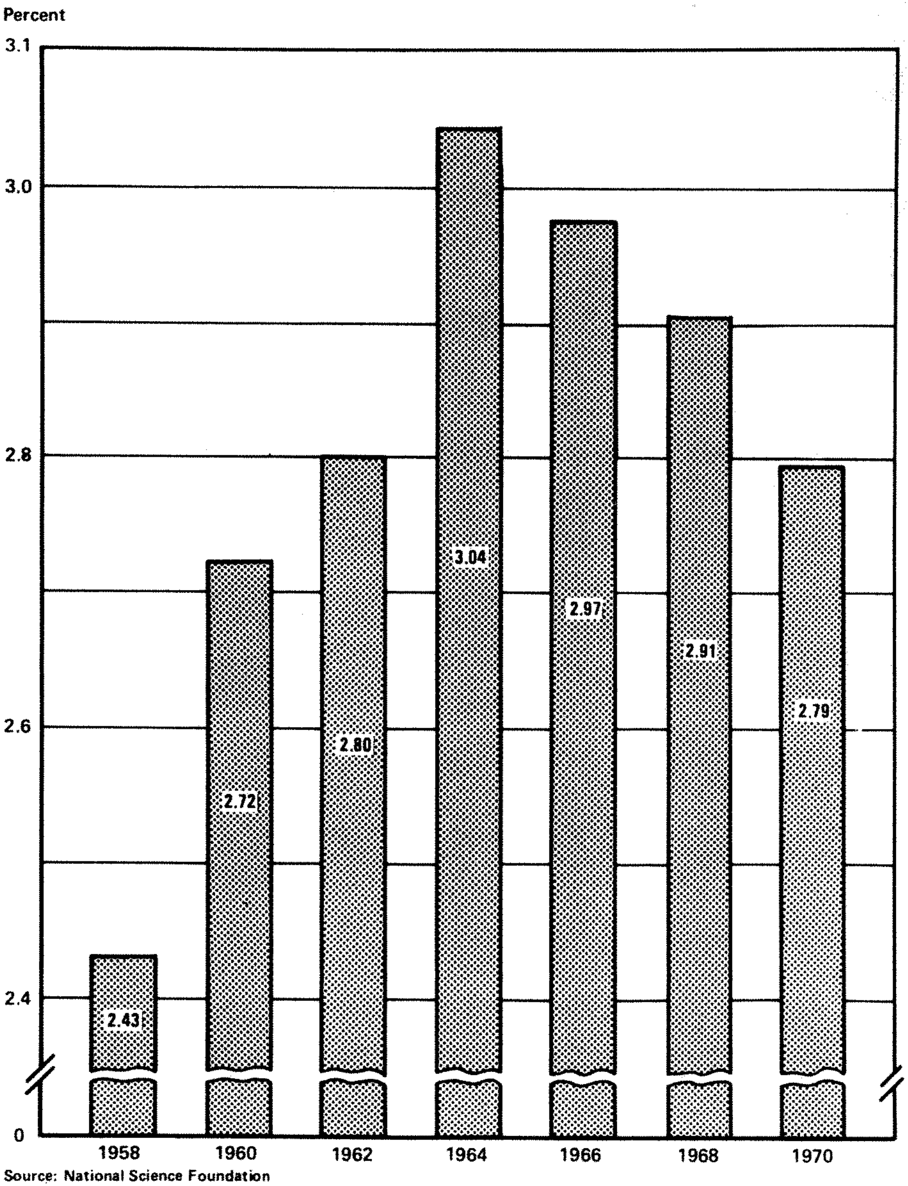
Chief among the factors that will occupy the committee's attention is that the growth in expenditure on research and development in the United States has been decelerating during the past decade. During the past five years, for example, expenditure on research and development has been growing at only 0.3 per cent a year taking inflation into account, compared with a real growth rate of about 11 per cent in the late 1950s. It seems that what is most at fault is the mechanism for promoting research and development in the private sector.

What policies are needed to ensure that more funding for research and development will stimulate the flagging economy of the United States, and reverse the slide in the nation's trade balance? According to the American Federation of Labor and Congress of Industrial Organizations (AFL-CIO), the answer may be found in simply imposing federal restrictions on the outflow of capital and technology from the United States.

The theme of the AFL-CIO's argument, presented to the subcommittee by Mr Andrew J. Biemiller, a former congressman, is that uncontrolled foreign investment has enabled other nations to match the technology of the United States in some crucial areas. The upshot is that United States capital has stimulated other nations' economies and has provided job opportunities abroad, while unemployment in the United States has steadily been getting worse. In short, the AFL-CIO accused the United States of exporting technology at the expense of home investment. Citing the textile industry as an example, Mr Biemiller pointed out that Asian textiles were no threat to the American market at one time because they could be relied on to shrink, but that situation was changed when the sanforization process was licensed outside America.

Economic isolationism is, however, unlikely to find many friends in Congress, and the AFL-CIO was given a fairly rough ride by the committee and by Murray L. Weidenbaum, assistant secretary to the Treasury for economic policy, at a later hearing. Referring to the ideas as "crude protectionism", Mr Weidenbaum said, "one thing you don't do is to prevent the international movement of technology or inhibit licensing in that way". He pointed out in particular that the international flow of technology is a two-way process, and that the United States is also at the receiving end of some foreign technologies.

Mr Weidenbaum's own suggestion is that "what is needed is a relatively steady trend of funding for research—steady in 'real' terms to protect research budgets against the inroads of inflation". But, while it is relatively easy to plan the rate of funding for research and development in the public sector, the private sector is a much tougher nut to crack. And the impor-



Source: National Science Foundation

The ratio of expenditure on research and development to the gross national product, 1958–1970.

tance of providing sufficient incentives for research and development in the private sector is emphasized by the fact that about 85 per cent of the development part of R & D is performed by private industry, chiefly with financial support from the companies themselves.

Maurice H. Stans, Secretary of Commerce, left the subcommittee with a few suggestions to chew over, however. "We should examine," he said, "the feasibility of establishing a single federal focus for several activities directly related to enhancement, assessment, and forecasting of industrial technology." This suggestion is an echo of an old cause of the subcommittee's, which held a number of hearings last year under the former chairman, Emilio Q. Daddario, to explore the feasibility of setting up an Office of Technology Assessment, and which has recently been putting that into effect.

The second option suggested by Mr Stans is that the committee should carefully consider the feasibility of creating direct or indirect financial incentives to stimulate new technology. Federal assistance could be provided to private companies—for example by loan guarantees, cost sharing, grants or procurement incentives, and the risk of setting off to develop a new technology could also be offset to some extent by tampering with the tax structure, giving depreciation allowances, and a host of other indirect subsidies. And, taking a sideways glance at his own National Bureau of Standards, Mr Stans suggested that there could be considerable tightening up on the setting of industrial standards. "Potential manufacturers," he said, "hesitate to invest heavily in the introduction of innovative equipment when they are uncertain about the requirements or specifications which will define the market for such equipment, especially in export trade."

But voluntary standards made by private industry are likely to fall foul of the anti-trust laws. Although the overt reason for the anti-trust laws is to prevent the formation of cartels and similar price-fixing rings, they can also have the effect of slowing research and development, for with the exception of a few special cases it is illegal for companies to collaborate on research projects. Mr Stans suggested that "it may well be time to modernize the anti-trust laws which evolved to deal with the different problems of an earlier era in order to permit the pooling of funds and risks that must accompany major technological advances". He was also quick to point out when questioned by the committee that it is premature to draw the conclusion that changes in the laws are on the way. Nevertheless, it seems clear that in areas such as research and development on pollution control, some relaxing of the anti-trust laws is being considered at least in the Department of Commerce.

Mr Stans told the subcommittee that in some areas American companies are being hamstrung by the anti-trust laws in such a way that they find it difficult to compete on equal terms with joint research projects conducted by their foreign competitors. But at home the anti-trust laws are increasingly becoming a source of concern for the smaller motor manufacturers who must meet stringent exhaust emission standards by 1975.

Mr Leonard Woodcock, president of the International Union, United Automobile, Aerospace and Agricultural Implement Workers of America (the union which represents most of the motor workers in the United States), wrote to Mr John N. Mitchell, the Attorney General, on May 24 suggesting that unless a crash programme combining the resources of all manufacturers in the automobile industry is conducted, the problem of exhaust pollution may not be solved before the 1975 deadline, and moreover, many of the smaller automobile companies are likely to go to the wall simply because they have insufficient funds to invest in pollution research. "This cooperative effort," he said, "can be maintained within both the spirit and the letter of our anti-trust laws." But Mr Richard McLaren, Assistant Attorney General in the Anti-trust Division, did not agree, for in reply to Woodcock's letter he said "we cannot agree . . . with your statement that there should not be competition in such vital areas as human safety and pollution control. Competition has served us well as a spur to the enormous technological gains achieved in this and previous decades. It should not be abandoned as we strive to meet the critical challenges to the safety and health of American citizens".

Perhaps the best explanation of the policy of the Department of Justice towards collaboration on pollution research was given by Mr McLaren in a letter to Mr John A. Volpe at the Department of Transportation, when he said "the Department of Justice feels that government agencies which are charged with the responsibility of meeting specific public goals, such as promoting automobile safety, should proceed in a manner which promotes competition to a maximum extent consistent with the achievement of their specific statutory responsibilities". Obsolete or not, the anti-trust laws are plainly a fundamental part of the American industrial ethos.

Pesticides in the Open

A MUCH criticized obstacle to the free flow of scientific information about pesticides and herbicides was removed last week by Mr William D. Ruckelshaus, Administrator of the Environmental Protection Agency. The EPA has now decided to release scientific advisory reports on controversial pesticides as soon as it receives them. The reports, which often constitute an important part of the mechanism by which an allegedly dangerous agent is banned or allowed for use, have in the past been kept secret until a final decision is taken. This has made it impossible for the scientific information contained in the reports to be criticized or discussed by professional scientists until it is too late to affect the final decision.

The scientific advisory reports are filed under the provisions of the Federal Insecticide, Fungicide and Rodenticide Act, which allows a manufacturer of a product whose use has been cancelled by the EPA to request that the issue be referred to an advisory committee or to ask for a public hearing. This procedure can delay cancellation by six months or more, for the manufacturer has 30 days after the serving of a cancellation notice to request referral to the advisory committee and the committee, whose members are nominated by the National Academy of Sciences, has 60 days in which to make its report. Within 90 days, Mr Ruckelshaus must then decide either to confirm or to set aside his earlier notice of intention to cancel or suspend the product. During the whole of these proceedings, the manufacturer is allowed to market the product.

The issue of secrecy has been brought into sharp focus recently with the controversy surrounding a report on the herbicide 2,4,5-T, which was submitted to Ruckelshaus in May. A few copies of this have found their way into circulation, and it has come in for strong criticism from some sectors of the scientific community. One of the charges levelled at the report is that the scientific information which it contains would not be acceptable for publication in a scientific journal, because some of it is based on superficial experiments conducted without proper controls. That information will, however, now be open to criticism, for the 2,4,5-T report is available from the EPA upon request.

ATOMIC ENERGY

Seaborg to Leave AEC

by our Washington Correspondent

DR GLENN T. SEABORG, chairman of the Atomic Energy Commission for the past decade, will leave the AEC next year, probably in November, to return to the University of California at Berkeley. His resignation, which was announced recently, was obviously expected in the White House, for President Nixon wasted little time in making public his choice for a successor, and sent the name of James R. Schlesinger, an assistant director of the Office of Management and Budget and a lifelong Republican, to the Senate for approval. Seaborg pointed out in his letter of resignation that last year he expressed the desire to return to California to resume his research on transuranium elements—research for which he won the Nobel Prize in 1951.

Although Nixon accepted Seaborg's resignation "with regret", and was unstinting in his praise for Seaborg's record of achievements at the AEC, it has been suggested that he was looking for a change of leadership for the commission. During the past year, the AEC's nuclear energy programme has faced mounting opposition from environmentalists, and strong doubts have been cast on its nuclear safety programme. Nevertheless, Seaborg has skilfully ensured that the AEC's programme has gone forward largely unmodified, and there is little doubt that he has served the commission well during his ten years in office.

Seaborg is the president-elect of the American Association for the Advancement of Science, a position to which he was elected last December in the face of considerable opposition from some members of the association. The chief complaint raised against his candidacy was that as head of a much criticized government agency, he would find a conflict of interest in many of the issues with which the AAAS is involved—the issue of reactor safety which the AAAS was asked by Senator Edmund Muskie to investigate, for example. Few, however, opposed his election on personal grounds, and his resignation from the AEC may help to assuage those fears, although for the major part of his term of office at the AAAS, which starts on January 1, 1972, he will be wearing both hats.

Schlesinger, Seaborg's proposed successor, is widely regarded as a whiz kid in the Nixon Administration. A graduate in economics from Harvard, where he later obtained a PhD, he has been a member of the faculty of the University of Virginia, and immediately before joining the Nixon Administration in 1969 he was engaged on a study of nuclear proliferation at the Rand Corporation. His first and only job in the Administration was in the old Bureau of the Budget and later in the Office of Management and Budget, where his chief area of concern has been defence spending, but he has also been involved with funding for environmental protection. He was also said to have been considered by Nixon for the post of Under Secretary for the Interior, but that suggestion was opposed by Republicans from the Western States, and his name was never put forward for nomination.

Environmentalists will be able to take some heart from the fact that his hobby is bird-watching. But as head of the AEC he will face a difficult task in resolving the conflict between environmentalists who oppose the nuclear power programme and the strong advocates of nuclear energy in the Congress and the Administration. Nixon will be looking to Schlesinger to put into effect the commitment to have a liquid metal fast breeder reactor in operation by 1980—an important part of the message to Congress in June in which Nixon requested $3,000 million for a crash programme for developing clean energy. The decision to concentrate on nuclear power production at the expense of other possible fuels has met with considerable opposition from environmentalist groups, who are urging a closer scrutiny of the environmental and public health hazards from reactors. Schlesinger's job at the AEC will evidently be to make sure that the nuclear power programme is not seriously disturbed by such fears, although he must obviously satisfy his critics that he is not deaf to their warnings.

James R. Schlesinger, Nixon's nominee as AEC chairman.

Uranium Enrichment

A FUNDAMENTAL change of policy for the sharing of information on uranium enrichment was announced last week by the US Atomic Energy Commission. The AEC is prepared to enter into discussions with the governments of ten other nations to explore possibilities for cooperating in multinational uranium enrichment facilities. The AEC is quick to point out, however, that these talks "would not involve a commitment at this time on the part of the US to make such technology available for use abroad". The talks are therefore simply concerned with exploring the financial and security arrangements under which the United States may be prepared to grant licences for the operation of gaseous diffusion plants in foreign countries. The nations to be represented at the talks are Britain, France, West Germany, Holland, Belgium, Luxembourg, Italy, Australia, Canada and Japan.

The Joint Committee on Atomic Energy has long opposed any move towards the sharing of American gaseous diffusion technology with other nations, chiefly because it fears that the technological and economic lead that the United States now holds in this process may be lost, and some members clearly view the announcement with misgivings, for the committee has asked in no uncertain terms to be kept informed of developments. The commitment to enter exploratory talks is the fruit of a review of US policy on uranium enrichment conducted by the administration and announced by President Nixon in his foreign policy report to Congress on February 25.

The Atomic Energy Commission also points out that gas centrifuge technology will not form a part of the exploratory talks, so that the United States is not seeking to join Britain, West Germany and Holland in their multinational research effort on the gas centrifuge process (see page 360). An official at the AEC pointed out that the talks will be limited to gaseous diffusion because that process has already been proven, and it offers the best approach to meeting the demand for enriched uranium in the late 1970s.

This change in policy is bound to be welcomed by the nations involved, as it will expedite the use of uranium for peaceful purposes.

NEWS AND VIEWS

New Baskets for Old

UNICELLULAR organisms reflect the extremes to which cellular morphogenesis can be stretched, as any protozoologist will testify. However esoteric their appearance they also retain the ability for clonal growth—to generate repeatedly two "finished" cells from one. They, and indeed most cells, contain organelles made up of microtubules—arrays of globular protein subunits which form hollow tubes of diameters around 250 Å. Protozoa should therefore spell out the range of possible structures which can be formed from microtubules and yet be reduplicated in a spatially precise manner at each cell division. The control of such a system presents intriguing problems. On page 387 of this issue of *Nature* J. B. Tucker of the University of St Andrews, Scotland, raises questions about the spatial discrimination that must underlie the formation of microtubular components in the ciliated protozoan *Nassula.*

The largest organelle in *Nassula* is the feeding apparatus the pharyngeal basket), a palisade of rods made up from longitudinal microtubules and attached to the cell surface. At division the old basket detaches from the surface and two new ones appear. Each new rod begins its assembly close to a basal body, another and more ubiquitous microtubular structure. The rods first appear in short rows; they align and detach from the basal bodies and then roll up to constitute the circular palisade. The completed baskets make microtubular connexions to other surface basal bodies. The two new baskets do not always include all the new rods, and these "errant" rods, after moving away, break down as do the old rods, while the integrated new rods elongate by addition of microtubular protein to their tips. How can rods in one area of the cell add subunits while apparently identical rods elsewhere in the same cytoplasm are being dismantled?

Like flagella elongation in *Chlamydomonas* the synthesis and assembly of rod microtubules are separable events. Cycloheximide inhibits synthesis and colchicine blocks assembly. Errant rods do not move away from the new baskets in the presence of vinblastine, nor are they resorbed, although the old rods continue to break down. Tucker erects two alternative hypotheses to explain this effect. Further research relevant to this study will be the resolution of the number of different types of microtubule subunit involved, and whether the microtubular assemblies appearing at different times and in different places are composed of identical subunits, and if so whether these share a common subunit pool, and are recycled when the structures are broken down.

The questions that Tucker raises—how the subunits are localized at the points of assembly and whether random diffusion of subunits synthesized throughout the cell accounts sufficiently for the observations—are two among several problems. They include the nature of the information system specifying the location of "nucleation" centres for organelles, the number of such centres and the regulation of the dimensions of the realized structures, and the self-assembly properties of the subunits. Tucker has previously observed in living cells that rod length fluctuates with feeding and starvation and that when the number of developing tubules is reduced they reach greater than normal length; both these facts are consistent with the available subunits determining the length. Dingle can upset the control of flagella number in *Naegleria* by heat shock at the moment of amoeboid-flagellate transformation (*J. Cell Sci.*, **7**, 463 ; 1970). The attraction of this system is that high developmental synchrony can be obtained in populations of these amoebae. Tilney (*Devel.* Biol., **2** (Suppl.), 63 ; 1968) and Roth (*J. Ultrastr. Res.*, **30**, 7 ; 1970) have suggested that the pattern of microtubules in the axopod of the heliozoan *Echinosphaerium* is a function of cross-linkages of fixed length and orientation between tubules rather than of an "orientation centre". Heat-shock disruption of rod tubules in *Nassula* and the subsequent abnormal arrangements of tubules argue in favour of the initiating sites as the determinants of the final pattern.

The solution of these problems promises to be of interest to all cell biologists and it may in addition shed light on the phenomenon of "cortical inheritance" in ciliates—alternative patterns of the same microtubular structures existing stably over hundreds of cell divisions in the absence of gene differences. Finally, it would be a glaring omission not to point out the close affinity in interest and attack between this field and that of phage morphogenesis (Levine, *Ann. Rev. Gen.*, **3**, 323 ; 1969).

Filling the Dating Gap

THE dating of the earlier part of the Pleistocene period still presents considerable problems but further exploitation of the various "radioactive clocks" should eventually overcome these difficulties. It is particularly encouraging that thermoluminescence dating may soon be extended to date bones and teeth from archaelogical deposits (Christodoulides and Fremlin, *Nature*, **232**, 257 ; 1971). The use of this method is well established for the dating of baked clay obects, and its potential for solving questions of authenticity has been illustrated in the past week by Martin Aitken and his colleagues at the University of Oxford who have demonstrated clearly that some "Etruscan" wall paintings on terracotta in European and American museums are at the most 12 years old (Aitken *et al.*, *Archaeometry*, **13**, 89 ; 971 ; Fleming *et al.*, *ibid.*, 143).

The promise of the thermoluminescence dating method of Christodoulides and Fremlin is that it will span the gap which still separates the time range of radiocarbon dating and the clocks of longer half-life. At the early end of the human time scale, potassium-argon dating has been useful in establishing a chronology for artefacts or hominid remains. The youngest dates obtainable from the uranium-lead and rubidium-strontium methods are about 10 million years, so that these isotopes, which are useful for longer geological time spans are not helpful here. The half-life of ^{40}K is 1.03×10^9 yr, however and mass spectrometry allows the determination of one of its decay products, the gas ^{40}Ar, in low concentrations. It is more difficult to date more recent ages than older ones because of the small quantities of argon involved, and the lower limit of the method is at present half a million years or a little less.

At the other end of the scale is radiocarbon dating. Radiocarbon dates now provide the essential basis for prehistoric chronology from Upper Palaeolithic times on and, until recently, radiocarbon seemed the most reliable of radioactive clocks. For a radioactivity-based age determination, it is usually necessary, of course, both to measure the quantity of the decay product present and the quantity left of the parent isotope. Radiocarbon dating was able to dispense with the former, because the original concentration of the parent isotope, ^{14}C, was assumed to be the same as the modern atmospheric concentration. The discovery that the atmospheric concentration of ^{14}C has fluctuated in recent millennia by up to 8 per cent of the present level has come therefore as something of a shock. The radiocarbon determination of dendrochronologically dated samples is now allowing the investigation of this effect back to 6000 BC, but its magnitude before this time remains uncertain.

The half-life of radiocarbon, approximately 5.7×10^3 yr, sets a more fundamental limitation on the age of samples which can be dated. The short half-life means that after a few tens of millennia there is very little radioactivity left in the specimen, and the counting of disintegrations in the sample is always complicated by a background activity caused by cosmic radiation which cannot be eliminated. Even the isotopic enrichment of gas samples only increases the effective time range of the method to a maximum of 70,000 yr.

Between the potassium-argon and radiocarbon dating methods lies a gap from 50,000 to 500,000 yr where other methods are required. Fission track dating is a possibility, especially when applied to volcanic glass. But like the potassium-argon method it gives the date of formation of the rocks under examination so that for more recent times, where the archaeological remains are not likely to be interstratified with eruptive deposits, this is a serious limitation. It is over this time range that thermoluminescence dating would be most useful.

Thermoluminescence dating depends on the principle that many minerals give off light when heated—the light emitted being proportional to the radiation dose experienced since the formation of the material. Archaeologically it has so far been principally applied to pottery and baked clay and the dating involves determining the radiogenic minerals in the clay as well as the thermoluminescence itself. The necessity of determining very small quantities of decay product (as in the potassium-argon method) or of the parent isotope (as in the radiocarbon method) is thus avoided. An early problem was how to eliminate light during the readout on heating, which derived from the energy used in pulverizing the sample. Fortunately, heating in an atmosphere of nitrogen considerably reduces this problem. Further improvement of this method came from the separation of different grain sizes in the pottery and an accuracy of less than 10 per cent is claimed for fine grain dating (Zimmerman, *Archaeometry*, **13**, 29; 1971).

Until recently the chief application of thermoluminescence dating has been in testing the authenticity of museum objects. The magnitude of the variations in atmospheric concentration of ^{14}C, suggested by tree-ring work, indicated a further useful line of approach, and the first TL results of Zimmerman and Huxtable on early neolithic pottery from the Balkans (*Antiquity*, **44**, 304; 1970) lend some support to the long chronology indicated by tree-ring work. Pottery was unfortunately not manufactured before about 7000 BC, but the same authors have dated baked clay figurines from the Gravettian site of Dolni Vestonice to 33,000 BP (*Archaeometry*, **13**, 57; 1971). The thermoluminescence age is 15 per cent higher than the radiocarbon age, with a probable error of 10 per cent. The method thus offers a useful check on radiocarbon dates.

The materials most readily available from Palaeolithic deposits are flint and bone, and it is these which make the work reported by Christodoulides and Fremlin, and the work which is currently in progress on thermoluminescence dating of flint, so promising. Once again the chief problem is luminescence arising independently of the radiogenic thermoluminescence which is to be measured. Christodoulides and Fremlin have reduced this effect to the extent that useful measurements can now be made on samples older than 100,000 yr.

If further work succeeds in reducing this background thermoluminescence by another order of magnitude, the method may yet yield useful dates in, and beyond, the 8,000 to 50,000 yr range at present covered by radiocarbon dating, but not yet checked by dendrochronology.

Mechanism of Polarity in Dispute

THE mechanism of genetic polarity has of late become the centre of one of those controversies which occasionally enliven the literature and the coffee room. One camp holds that nonsense codons, by stopping the movement of ribosomes translating nascent messenger RNA molecules, prevent further transcription because transcription is coupled to translation. Members of the other camp argue, however, that nascent messenger RNA chains continue to be elongated after a nonsense codon at some early position has prevented ribosomes translating the messenger. They claim that because the newly made stretches of messenger are devoid of ribosomes they are rendered susceptible to attack by nucleases and are degraded.

In next Wednesday's *Nature New Biology* there are two articles which present conflicting data to succour members of both camps. Imamoto and Kano, who favour the first hypothesis, have studied the synthesis of *tryp* operon mRNA in a strain of *Escherichia coli* with a temperature sensitive mutation in the structural gene for one of the 30S ribosome proteins, P10, which must be present for ribosomes to initiate translation of natural messengers. They present evidence which suggests that the overall rate of transcription of the *tryp* operon in this strain parallels the rate of protein synthesis. At the nonpermissive temperature protein synthesis is impaired and correlated with this synthesis of *tryp* messenger RNA is arrested.

By contrast, a few weeks ago Morse and his colleagues reported in *Nature New Biology* (**231**, 214; 1971) experiments which suggest that messengers devoid of ribosomes are degraded by a previously undetected endonuclease. Morse and Guertin now report new experiments on the effect of starving *E. coli* of amino-acids on the polarity of polar nonsense mutations in the *tryp* operon. Polarity is relieved when stringent cells carrying such mutations are starved; in other words, the *tryp* mRNA distal to the polar nonsense mutation, which is rapidly degraded in the presence of an adequate supply of amino-acids is stabilized when amino-acids are in short supply. By contrast, when relaxed strains are similarly starved there is no relief of polarity; indeed polarity is enhanced.

Morse and Guertin suggest that because starvation of stringent cells results in the cessation of ribosomal RNA synthesis as well as protein synthesis nucleotides must accumulate, and this might well result, by some feedback control mechanism, in the inactivation of nucleases. Messengers although exposed might therefore survive. By contrast, when relaxed cells are starved ribosomal RNA synthesis continues even though protein synthesis is halted.

HISTORY OF BIOLOGY

A "Non-event"

from a Correspondent

IT is a truth of history and an aspect of human behaviour that momentous occasions recalled in later life by men who took part often gain in grandeur and importance with the passing of time. A study of contemporary documents by J. W. T. Moody (*J. Soc. Bibliog. Nat Hist.*, **5**, 474 ; 1971) shows that the presentation of the Darwin–Wallace papers on natural selection to the scientific world on July 1, 1858, was no exception to this general rule. As Moody points out the occasion has been said to represent the beginning of a new era in scientific thinking—the beginning of modern biology or the Darwinian Revolution—but at the time of its presentation it was something of a non-event.

In the first place, the meeting at the Linnean Society at the critical day had been specially called by the president for the election of a new council member, but the opportunity was taken to deal with other business. A list of donations to the society's library and museum was read and there followed the election of the new councillor by ballot and a lengthy eulogy of the recently deceased vice-president, Robert Brown. Then the secretary read the text of the Darwin and Wallace papers on the laws which affect the production of varieties, races and species with an introductory letter from Sir Charles Lyell and Dr J. D. Hooker, and he followed by reading five other papers on miscellaneous and unrelated subjects.

According to the account of the event recollected by Hooker at the jubilee celebrations in 1908, only Darwin, Lyell, and Hooker himself, had any knowledge of the papers to be presented and Darwin for domestic reasons did not attend the meeting. Gage has noted (*A History of the Linnean Society of London*, 54; 1938) that at that date agenda were not sent to members, so the fewer than thirty members who attended the meeting can hardly have expected a momentous meeting. Hooker seems to have been the source of the impression that the audience was awed by the revelations read to them; in a letter thirty years after the event he wrote "the interest excited was intense . . . it was talked over after the meeting 'with bated breath'. Lyell's approval, and perhaps in a small way mine . . . rather overawed those Fellows who would otherwise have flown out against the doctrine . . .". Moody, however, suggests that the audience were not so much stunned by new ideas as they were overwhelmed by the sheer volume of information loaded upon them. No formal discussion took place at the meeting, the audience was expected to switch its attention instantly from the Darwin–Wallace papers to "Notes on the organization of *Phoronis hippocrepis*", and it is suggested that boredom and bewilderment on a July afternoon, as much as Lyell and Hooker's advocacy, contributed to the overawed silence which greeted the unveiling of the concept of natural selection.

GALAXIES

Circular Polarization

from a Correspondent

DURING the past two years astronomers have discovered circular polarization in the radiation from a variety of objects. At Jodrell Bank, for example, Conway and Gilbert measured significant circular polarization at 49 cm wavelength from several compact sources (*Nature*, **227**, 585 ; 1970), and a series of reports from Kemp and his colleagues has described the circular polarization in the light from white dwarf stars and planets (*Nature*, **232**, 151; 1971). The range of possible observations is now expanded still further with a terse announcement in *IAU Circular 2343* that circular polarization has been successfully detected in the optical radiation from two extragalactic sources.

For many years observers have determined the magnetic fields in the Sun by measuring the circularly polarized components in spectral lines split by the Zeeman effect. At the Crimean Astrophysical Observatory, Severny, Nikulin and Kuvshinov hooked up a modified solar magnetograph to their 100-inch reflector telescope, and monitored the light from the radio galaxy M87, quasar 3C 273 and a Seyfert galaxy NGC 4151. The condensed, superluminous nuclei in the last two objects gave a positive result: the degrees of circular polarization are 1.2±0.4 per cent for 3C 273 and 1.1±0.3 per cent for NGC 4151. One X-ray source in the galaxy Sco X–1 was also investigated, but like M87 it gave a negative result.

There are several ways of obtaining circularly polarized radiation, but theorists will understandably want to see a more detailed report before any firm conclusions can be drawn from this new discovery. It is important in that it adds another method for investigating physical processes inside remote compact objects. It is already well established that the synchrotron mechanism may be responsible for the continuum radiation at optical wavelengths, and with certain geometries for the magnetic field a circular component is also introduced into the radiation. An encouraging step in this direction is given by the 49 cm results, where the synchrotron theory seems to satisfy the observations.

SEAFLOOR SPREADING

Chemical Rates?

from our Geomagnetism Correspondent

SEAFLOOR spreading rates vary from ridge to ridge at any given time and probably even vary with time for any given ridge. The first of these propositions is easy to prove because the patterns of magnetic anomalies close to oceanic ridges may be compared directly with the well-dated geomagnetic polarity-time scale derived from continental rocks of the past 4 to 5 million years. When this is done it clearly emerges that average spreading rates for the past few million years are different for different ridges; and thus it is safe to say that current spreading rates are also different. Direct evidence for the

---

Dybbuks and Tachyons

A FEW years ago a suggestion was made that there might exist particles that travelled with a velocity greater than light. These particles, named tachyons, satisfy the fundamental equation of special relativity:

$$E^2-P^2=M^2$$

where E. P and M are the energy, momentum and mass of the particle. They differ, however, from ordinary particles in that their mass is imaginary but they have a real energy and momentum. In next Monday's *Nature Physical Science*, Raymond Fox of Haifa, Israel, points out that particles with imaginary mass, imaginary momentum and imaginary energy are also solutions of the fundamental equation, and Fox names such particles "dybbuks". The one important difference between tachyons and dybbuks is that whereas it has been argued that the concept of tachyons violates causality there is no likelihood of such a problem with dybbuks.

Fox gives a description of the possible interactions of these particles and applies the results to an evaluation of the gravitational interaction of a very dense star. An interesting prediction is that the gravitational radiation from a pulsar is very much less than presently estimated. Such a prediction is bound to arouse interest in astronomy circles and the basis of Fox's theory will no doubt be subject to intensive study.

second proposition is more tenuous, however, because it is difficult to assign ages beyond about 5 million years to either the continental reversal pattern or the series of oceanic magnetic anomalies. Nevertheless, although the uniform spreading rate is sometimes used as a working hypothesis in geophysics, there is now quite convincing evidence that spreading rates do vary with time.

The need to determine spreading rates as a function of time has led Bass (*Earth Planet. Sci. Lett.*, **11**, 18 ; 1971) to take the first steps in a novel approach to the problem. The point of his argument is that if it can be shown that the chemical composition of the basalt extruded at ridge crests varies systematically with spreading rate at the present time, then it might be possible to determine the relative spreading rates for older oceanic crust by chemical analysis. He has thus tried to demonstrate that such variations exist.

The first thing to be said about this approach is that there are known to be practical difficulties both in demonstrating composition–spreading rate correlations and, supposing such correlations exist, in extrapolating them to older rocks. It is an experimental fact, for example, that any dredge haul of basalt from the ocean floor is likely to contain more than one type of basalt. It thus becomes difficult to assign a particular chemical characteristic to any area, or even site, of the ocean floor. But even if this problem can be overcome by the selection and use of suitable criteria, the application of those criteria to older crustal basalts is likely to be invalidated by the possibility of secondary alteration. It is too soon to say whether or not alteration will ultimately prove to be an insurmountable objection to Bass's method; but Bass has, at least, been able to show that there are systematic relations between chemical composition and spreading rate although he admits that the data are "too sparse to establish a statistically compelling argument" for strong correlations.

The ridges Bass has chosen to examine are the rapidly spreading East Pacific Rise and Juan de Fuca Ridge, the moderately slowly spreading Gorda Ridge and the slowly spreading Mid-Atlantic Ridge. Not all samples from these ridges were accepted in defining the chemical characteristic for each ridge—several classes of rock which would have led to ambiguities in interpretation were excluded. But when the compositions of the rocks selected by Bass are plotted on the normative AN-OL-HY ternary diagram, systematic variations in the positions of the fields for the various ridges became apparent. Thus the fields for the ridges of lower spreading rate move progressively towards higher AN and OL values and the ends of the fields poorer in HY move progressively towards the AN corner. In short, the lower the spreading rate the richer in Al_2O_3 and the more undersaturated are the basalts.

According to Bass, the important conclusion to be drawn from these properties is that the lower the spreading rate for a ridge the greater is the depth from which magma can be derived. Thus if the maximum depth from which magma may be derived is, in effect, the maximum depth at which brittle fracture may occur, slowly spreading ridges must be underlain by thicker lithosphere. In other words, magma type or the chemical characteristics of the basalts may in principle be used as an alternative to seismic methods to determine the relative lithospheric thickness.

The correlation of lithospheric thickness with spreading rate then leads to conclusions about the processes which determine the maximum depth of brittle fracture. Whether the ridge is produced by stresses involved in the upwelling mantle material or by stresses imposed by the lithosphere sinking into trenches, the depth of brittle fracture (which determines the apparent thickness of the lithosphere) will increase with an increase in the rate of application of stress and thus with an increase in spreading rate. But working in the opposite sense is the effect of the geothermal gradient. The greater the spreading rate the greater will be the quantity of mantle material entering the ridge region from below and so the greater will be the heat flow. The higher geothermal gradient will effectively reduce the depth of brittle fracture. Although it is not possible to predict which of these processes will take precedence, Bass's correlation of the thickness-spreading rate in the lithosphere enables the relative effects of the two processes to be determined. Thus because increased spreading rates lead to thinner lithosphere it means that the geothermal gradient effect dominates.

HAEMOGLOBIN

Structural Variants

from our Molecular Biology Correspondent

SINCE Pauling's discovery rather more than twenty years ago of sickle-cell haemoglobin—the manifestation of what with unerring flair he dubbed a molecular disease—well over a hundred human haemoglobin variants have been documented. Some, but by no means all of these, are associated with pathological conditions, and a comparison of the stereochemical features of such species in the crystal with those of the normal protein should, one might hope, lead to a precise explanation of how a single-base mutation can encompass such dire physiological consequences. With the biochemical world still digesting the implications of his results on the relation between the structures of oxy- and deoxyhaemoglobin, Perutz, together with Greer, has recently embarked on

---

Uniaxially Reinforced Nylon Composites

IMPROVING the mechanical properties of polymers by inclusion of comparatively high strength and modulus fibres, such as glass or carbon, is well known. Unfortunately not all polymers are amenable to this method of reinforcement for, to utilize to the maximum the good mechanical properties of the fibres, the composite needs to contain long lengths of fibre. Any method of moulding such as by injection or extrusion, where there is drastic deformation and movement, will thus cause damage to induced fibres and so reduce their reinforcing effect.

The reinforced polymers which have had the greatest impact in engineering have therefore been constructed with thermosetting materials, such as epoxy and polyester resins, which require only simple "static" moulding conditions. The reinforcing fibres are placed in the mould with the desired orientation. Liquid resin is then added in the form of monomer or oligomer containing catalyst and the mould is heated. Polymerization then occurs and a solid moulding is formed. It is essential that no gaseous byproducts are produced during polymerization, for this causes disruption of the composite. Nylon is a well established engineering material which would benefit from fibre reinforcement. Some success has already been achieved by the incorporation of fibres before injection moulding, but fibre lengths in the composite are usually less than 1 mm and are almost randomly oriented.

Attempts to produce composites by this method of reinforcement fail because charging the mould with molten polymer is impracticable, and if normal monomers are used, water is evolved as steam during polymerization. But T. Bessell, D. Hull and J. B. Shortall report in next Monday's *Nature Physical Science* that this problem has been overcome by using caprolactam as the monomer and by inducing the ring opening type polymerization with anionic catalysts, so that the problem of gaseous byproducts is almost eliminated.

such studies of pathological variants (*Nature New Biology*, **230**, 261 ; 1971), and now Greer has contributed two new investigations of different types of structural aberration. To anybody except the young, the excessively blasé and the punch-drunk, they compel awe and fascination.

In the first article (*J. Mol. Biol.*, **59**, 99 ; 1971), Greer uses the difference Fourier method to study the structural effects of two different substitutions at a single position in the β-chain. Asn-102 is conserved in all known animal β-chain sequences, and may therefore be supposed to admit of little genetic tinkering. In haemoglobin Kansas, however, it is replaced by threonine, which causes the loss of cooperative function, and a decrease in oxygen affinity at physiological *p*H ; in haemoglobin Richmond it is replaced by lysine, without any clinical penalty.

The difference electron density map of deoxygenated Kansas shows a disturbance near the β-haem group, for the threonine causes crowding at a vinyl and methyl side chain. The haem is displaced, and with it the helix to which it is linked by way of the histidine iron ligand. This has other consequences, including a distortion at the interface of the α_1 and β_1 chains which affects even the α-chain structure at various points, including the haem surrounds. The threonine cannot fulfil one of the functions of the asparagine, of hydrogen bonding to an α-chain aspartate, and appears instead to form an intra-β-chain bond. In haemoglobin Richmond, the distortion of the structure under the influence of the substitution is much less, and the lysine is even able to bond to the α-chain aspartate. A consequence of one of the new contacts is to displace another arginine, which normally shows a prominent interaction with an α-chain tyrosine. Possibly for this reason the Richmond molecule dissociates much more readily than normal haemoglobin into dimers. In haemoglobin Kansas, in which an important saltbridge is absent, and the structure is generally distorted, this effect is still more pronounced.

Greer's second article (*ibid.*, 107) concerns itself with two haemoglobin M forms, Hyde Park and Iwate. The M variants are characterized by substitutions for haem-linked histidines, the corresponding haem groups being then inert with respect to oxygen, and normally in the ferric form. Haemoglobin Iwate has an α-chain substitution, and Hyde Park a β-chain, histidine being in both cases replaced by tyrosine. The chemistry of these forms shows many peculiarities—in one of them, for example, the isomorphous replacement by a mercurial occurs at the wrong thiol. The Hyde Park variant moreover has a tendency to shed its α-haem groups and in a proportion of the molecules in the crystal of the fully reduced pigment (and no doubt in solution) the haem is missing and its cavity is collapsed. The disturbance is localized, however, and is not transmitted to the rest of the molecule. Neither the aberrant tyrosine nor the distal histidine in this molecule approaches the haem.

In the α-chain Iwate variant the α-haem iron is evidently linked to the tyrosine and the normal distal histidine. The most striking feature of the structure in both the fully ferric and the β-deoxy-forms is that it is fixed in the deoxy-configuration, in spite of well-defined changes in the β-chain when its haem is liganded. This rationalizes the striking difference between the two M variants, Hyde Park having both normal oxygen affinity in its functional haems and a Bohr effect, and Iwate a low oxygen affinity and no Bohr effect. Evidently the tyrosine-iron bond prevents the normal steric transition to a state favouring the transition to the high-affinity quaternary structure, which, one may infer, originates in the α-chains. Molecular pathology aside, the particular value of these studies, especially when details can be seen at higher resolution, will be to reveal the nature of the intermediate states between the fully reduced and fully oxygenated forms, which could not otherwise be observed by crystallography.

STRATIGRAPHY

Fixing a Boundary

from a Correspondent

IT cannot be often that the Red Army is responsible for a geological article. In August 1968 the International Commission on Stratigraphy met in Prague and was about to consider a proposal that the boundary between the Silurian and the Devonian should be fixed at the base of the *Monograptus uniformis* Zone, which is appreciably below the traditional base of the Devonian in Russia and Czechoslovakia, but unfortunately well above its base in Britain. The practical consequence of this in the classic area of the Welsh Borderland would be to transfer the lowest Devonian strata as originally defined by Murchison to the Silurian system. The support for the proposal suggests that it would have been accepted had the work of the commission not been interrupted. Because of the invasion, however, the matter is still open to debate. Westoll, Shirley, Dinely and Ball have recently taken advantage of this respite to argue (*J. Geol. Soc.*, **127**, 286 ; 1971) that the traditional position of the boundary between the two systems should be retained.

This may seem a rather arid discussion of interest solely to specialists. In

Marine Bands and Tonsteins revealed in Derbyshire

THE Westphalian Coal Measures are remarkable for the continuity and persistence of certain rock types, especially the marine bands which have characteristic fossil fauna resulting from relatively short lived but widespread marine invasions of the coal delta. These bands have long been accepted as reliable stratigraphic markers on which to subdivide and to correlate the coal measures of the British Isles and Europe. The thin, curious, kaolinitic bands called tonsteins discovered more recently in the coalfields of Western Europe have provided welcome additional markers for correlation purposes.

Tonsteins were first found in North Staffordshire in 1961 and discoveries in other British coalfields have followed, particularly at one horizon below the equivalent of the *Edmondia* Marine Band. They are usually only a few centimetres thick, yet some of them are traceable over hundreds of square miles and, as in the case of some marine bands, a few can be traced throughout the coalfields of Western Europe. Their origin is problematical and probably varied; the volcanic ash idea is attractive in terms of widespread persistence but it is also held that they are the result of special soil forming processes.

In the forthcoming issue of *Nature Physical Science*, M. A. Calver, who has been associated with many discoveries of new marine bands in British coalfields, is joined by B. C. Worssam and G. Jago to demonstrate again that continuous core drilling coupled with examination can still reveal hitherto unproved marine bands and tonsteins. A series of boreholes drilled in the Pottery Clays of the South Derbyshire Coalfield during 1970 on behalf of the Institute of Geological Sciences and the National Coal Board have added considerably to the knowledge of the stratigraphy of these coal measures. Above the Overseal Marine Band Calver *et al.* have recorded the presence of the marine bands equivalent to the Top, Shafton and *Edmondia* Bands of the East Pennine Coalfields and they have also recorded a tonstein which occurs a little below the last of these bands at about the position of the Sub-High Main tonstein; another new tonstein is located between the Top and Shafton marine horizons. The elusive marine bands between the Overseal and underlying Two Foot have also been found and correlated.

fact, it arises from a more important philosophical question—how geological time should be measured. When stratigraphy was in its infancy, it was natural to fix on spectacular events the effects of which were readily apparent and to use these for dividing the geological record.

More recent ideas on the philosophy of stratigraphy, however, suggest that time is a continuum, and that stratigraphic boundaries are merely arbitrary divisions, much as the hours of the day are arbitrary. Once a boundary is agreed, and defined by a type section, stratigraphers can get on with their real business—correlation. From this it follows that type sections should be chosen in areas where the rocks have all the features useful in correlation—good varied faunas (preferably marine) and as few obvious breaks as possible. This means that the traditional type sections of the Silurian/Devonian boundary in the Welsh Borderland just will not do—not only are half the rocks continental, but the famous Ludlow Bone Bed represents a marked time break. A very suitable type area seems to be the Barrandium Syncline near Prague, which was protected from the major movements of the Earth which disturbed north-west Europe in early Devonian times and where the graptolites, all-important in correlation, were not forced to extinction.

Geologists are agreed that the new proposal presents British stratigraphers with some difficulties, as Westoll and his colleagues point out. They suggest that the boundary should be fixed at another graptolite zone rather lower in the succession, marked by *Monograptus ultimus*, which would give a boundary very close to the position of the Ludlow Bone Bed.

Not all their British colleagues take this view; there is considerable British support for the new proposal. It will be interesting to see whether Westoll, Shirley, Dinely and Ball are fighting a rearguard action or starting a successful counter attack.

OLFACTION

Smells Left and Right

from our Membrane Correspondent

IT has been reported that compounds, which differ only in their stereochemical configuration about one carbon atom, have distinguishably different odours. It is possible, however, that the difference in smell between these enantiomers can be attributed to that bugbear of olfactory research, the presence of unidentified impurities.

Earlier this year Leitereg *et al.* (*Nature*, **230**, 455; 1971) presented good evidence that the optical isomers (+)- and (−)-carvones, prepared from both natural and synthetic sources, have different odours—caraway and spearmint respectively—but these authors still paid lip service to the possibility that the odour difference was caused by impurities. This possibility has now been convincingly eliminated in some recent work reported by Russell and Hills (*Science*, **172**, 1043; 1971) and by Friedman and Miller (*ibid.*, 1044).

Russell and Hills investigated five different enantiomeric pairs of compounds—(+)- and (−)-carvones and four derivatives of each of these. The actual samples used for odour evaluation were purified fractions which were collected after successive passages through two different gas-liquid chromatography (GLC) columns. In each case there was a highly significant difference in odour quality. Using GLC and a variety of synthetic pathways, Friedman and Miller investigated eight different enantiomeric pairs which had distinct odour differences. All but one (amphetamine) of the pairs were terpenoid compounds and these included the carvones and also (+)- and (−)-limonenes; the last two had the odour of oranges and lemons respectively—as in the case of the carvones this is a far from subtle difference in odour. Friedman and Miller paid particular attention to the carvones; they resolved a racemic mixture, synthesized them by two different routes and also subjected them to a cycle of reactions which interconverted the + form to the − form and back again. The final products of all these manipulations were subjected to odour evaluation. The odours of the + forms were indistinguishable from each other as were the odours of the − forms, but the + and − pairs always had a significant difference in odour.

The postulated impurities now come to have unbelievable properties. There would have to be a different pair of impurities for many of the enantiomeric pairs studied, and in each case these impurities must survive both the GLC purification and the wide variety of chemical manipulations. Furthermore, just the same impurities would have to be present in both natural and synthetic compounds.

The vibrational and rotational modes of enantiomers are identical so that the now clearly established fact that enantiomers can have different odours spells death to any theory of olfaction which looks for correlations between odour quality and infrared spectra. Furthermore, other theories are now faced with the task of explaining why some enantiomeric pairs have different odours and other pairs have identical odours.

Some Pulsars May Emit Thermal X-rays

THE view that pulsars may consist of a superfluid core of neutrons surrounded by a rigid crust, as suggested by Baym, Pethick, Pines and Ruderman, is examined by George Greenstein of Princeton University Observatory in next Monday's *Nature Physical Science*. So far the attraction of the model of Baym and his colleagues has been that it accounts in a neat way for some of the irregularities in the behaviour of pulsars, interpreted in terms of starquakes occurring in the rigid crust. In this model the slowdown of the spin of the pulsar is accompanied by frictional heat as a result of slippage between the crust and the core.

Greenstein has evaluated the magnitude of this heating, which is on top of the temperature expected for pulsars on the basis of cooling theory, so that the temperatures which he obtains from an examination of the slowdown of pulsars can be considered as lower limits to pulsar temperatures.

It will not come as a surprise that the two pulsars for which the frictional heating process seems significant are the two fast pulsars, in the Crab Nebula and in Vela. For both these objects Greenstein finds temperatures caused by frictional heating arising between the rigid crust and neutron superfluid of 10^6 K. He suggests therefore that thermal X-ray emission ought to be detectable from these two objects—he expects them both to have X-ray fluxes of the order of 3×10^{-27} erg cm^{-2} s^{-1} Hz^{-1}.

It may also be possible to detect the thermal X-ray flux of 1×10^{-28} erg cm^{-2} s^{-1} Hz^{-1} that is expected from what is thought to be the closest pulsar, CP 0950. Another possibility is that pulsars the orientation of which is such that their pulsations are not detectable at the Earth may be discovered by their thermal X-radiation.

But for other pulsars the expected thermal X-ray emission is appreciably weaker—1×10^{-30} erg cm^{-2} s^{-1} Hz^{-1} for HP 1508 and 3×10^{-29} erg cm^{-2} s^{-1} Hz^{-1} for CP 1133. For these two objects the expected associated visual magnitudes are 32 and 27 respectively, far beyond the capabilities of the best optical telescopes.

Greenstein also reports that the heating at the inner boundary of the core takes place in a thin shell that can be a kilometre or so, or less, in thickness. He also points out that his equations indicate that fluctuations in the temperatures of pulsars are mirrored by corresponding fluctuations in the periodicity of the pulses, so that the regularity of pulsars implies that their temperatures are also kept amazingly constant.

NUTRITION

New Protein Foods

from a Correspondent

THE conference at the University of Reading, from July 13 to 15, on the topic of new protein foods differed from many others on the same subject in that it was confined to agricultural conditions in the United Kingdom. In his introduction, Professor F. Aylward (University of Reading) classified the different kinds of new protein foods and stated that a crop for the production of protein concentrates depends in practice on a combination of factors including (1) yield of protein, (2) technical problems of harvesting, extraction and processing, (3) nutritional value of protein and freedom from anti-nutrient or toxicological factors, (4) acceptability of product for direct use as a food or as a food component, and (5) economic and commercial considerations. The yield of protein depends on the percentage of protein in the crop, the total yield of crop, the actual yield of protein after harvesting, and the yield after processing.

Dr R. R. Robinson (University of Reading) described the use of bacteria, yeasts, fungi and chlorophyll-containing plants such as unicellular algae. There are many difficulties to be overcome regarding the extraction of protein from these organisms and oilseeds, legumes and cereals are likely to be the chief source of protein during the next two or three decades. Dr B. J. F. Hudson (University of Reading) ventured to predict the possible increase in consumption of these foods in the United Kingdom up to AD 2000. On the basis of careful calculations by forty different experts it seems that textured proteins and similar plant protein concentrates might replace as much as a quarter of the meat by AD 2000, or even earlier. Professor H. M. Sinclair (University of Oxford) dealt with the nutritional potential of these new protein foods. He emphasized the importance of ensuring a sufficient content of vitamin B_{12}, with fortification if necessary, and pointed out the valuable clinical work which is being carried out on vegans living only on plant foods.

Dr M. A. Crawford (Nuffield Institute of Comparative Medicine, London) emphasized the importance of other factors besides protein in the new foods and drew attention to the role of structural phospholipids in the brain and other parts of the central nervous system. Both Professor Sinclair and Dr Crawford said that the correlation between too high a level of total phospholipids and nervous diseases seems to be connected with the phospholipids breaking down, but they did not state the cause.

Meat production and demands in the United Kingdom were described by Mr H. F. Marks (Meat and Livestock Commission) and Dr A. M. Ashby (Unilever Ltd). Although Britain's entry into the Common Market would be expected to cause rises in meat prices probably greater than the corresponding income rises, they thought that the total consumption of meat would not fall much, although they agreed that much more consumption of these new protein foods will gradually develop. Dr W. F. Raymond (Grassland Research Institute, Hurley) suggested that the high cost of meat production might be curbed to some extent by using alternative feeding stuffs including perhaps those made from microorganisms.

Dr M. A. B. Boddington (Wye College, London) applied land use projections for the next 30 years to forecast possible developments in yields from land which might improve the present low conversion efficiency of only 6 per cent for beef and 12 per cent for pigs.

REVERSE TRANSCRIPTASE

Templates for Caution

from our Cell Biology Correspondent

As Speigelman in particular has of late taken great pains to emphasize, enzymatic activities detected by their ability to use synthetic RNAs and RNA/DNA hybrids as templates for the synthesis of DNA cannot be immediately equated with the reverse transcriptases found in animal tumour viruses. The enzyme or enzymes in such viruses—and there is still some dispute as to whether or not a single enzyme molecule can complete the synthesis of a double stranded DNA from the single stranded RNA viral genome (Robinson and Robinson, *Virology*, **44**, 457; 1971)—use endogenous viral RNAs, synthetic RNAs and hybrids as templates for the synthesis of DNA. But it does not inevitably follow that all enzymes which *in vitro* can be programmed by synthetic RNAs or hybrids to polymerize deoxynucleoside triphosphates function *in vivo* as authentic reverse transcriptases and have an endogenous and natural single stranded RNA template. Synthetic nucleic acids are useful templates for the detailed investigation of reverse transcriptases once the enzymes and their natural templates have been identified, but the identification of a reverse transcriptase in a new situation depends on the identification of its natural template.

Indeed if the ability of an enzyme to use synthetic RNA as a template for DNA synthesis *in vitro* is taken to be diagnostic of its function *in vivo* as a reverse transcriptase, the Kornberg DNA polymerase I of *Escherichia coli* must be classified as a reverse transcriptase. For as long ago as 1963–4 Cavalieri and his colleagues proved that DNA polymerase I can utilize double stranded synthetic RNAs as

Oncogenicity of Cleaved Adenovirus DNA

ALTHOUGH the adenoviruses apparently cause nothing more devastating than respiratory infections in their natural hosts, at least some of them induce tumours when they are injected into newborn rodents and will transform some of the cells which they infect *in vitro*. Attempts to analyse genetically the oncogenes in adenovirus genomes, by the isolation of temperature sensitive mutants, seem most promising, but meanwhile Mayne, Burnett and Butler have set about the physical isolation of fragments of the simian adenovirus 7 (SA7) genome which contain these genes. Next Wednesday's *Nature New Biology* contains their progress report.

Having first shown that intact DNA isolated from SA7 is not only infectious but capable of inducing tumours when injected into newborn hamsters, they have now fragmented this DNA, either by sonication or by shearing it through a syringe, fractionated the fragments and tested their oncogenic potential. Molecules roughly half the size of the complete genome, which can be separated by virtue of their density into heavy and light halves, induce tumours like intact molecules. By contrast smaller fragments obtained by sonication are not oncogenic.

The induction of tumours by both heavy and light "half" molecules, but not by smaller pieces, is of course disappointing to anybody bent on physically isolating the SA7 oncogenes free of any other genetic material. For although these experiments prove that the whole genome is not essential for oncogenicity the fact that both halves of the genome have oncogenic potential may well mean that several regions of the genetic material, rather than a unique segment, contain oncogenes. Alternatively and more optimistically, it might be argued that the oncogenes are centrally located in a small segment of the genome, which, depending on the precise point of cleavage, might be included in either the heavy or the light "halves". The hybridization studies between "half" molecules and DNA extracted from the cells of tumours induced by heavy and light "halves" that Mayne *et al.* promise may perhaps resolve this issue.

well as single stranded natural RNAs as templates for DNA synthesis. And, rubbing home the message, Cavalieri and Carroll reported two weeks ago in *Nature* (**232,** 254; 1971) that the addition of traces of calf thymus DNA to *E. coli* ribosomal RNA resulted in a three to ten-fold increase in the incorporation of the four deoxynucleoside triphosphates catalysed by *E. coli* DNA polymerase. This result is, of course, entirely reminiscent of the stimulatory effect of Goulian pieces on DNA synthesis catalysed by DNA polymerase I with natural DNA templates.

Mindful of these findings, it is perhaps rash of Werner *et al.* (*Biochem. Biophys. Res. Comm.*, **44,** 433; 1971), who have found that skin cells from people with xeroderma pigmentosum contain an enzymatic activity, which uses poly rG.dC as a template for DNA synthesis, that is absent from normal human skin cells, to conclude that xeroderma pigmentosum skin cells are infected with a tumour virus and contain a reverse transcriptase. In the light of Cavalieri's experiments and Spiegelman's cautionings one can offer an equally plausible alternative explanation of the data Werner *et al.* present.

There can be no questioning the fact that xeroderma pigmentosum cells lack some if not all DNA repair mechanisms and fail to excise pyrimidine dimers. Moreover this defect may well be responsible for the greatly enhanced incidence of skin carcinomas among people with xeroderma pigmentosum. Perhaps the different responses to poly rG.dC and native DNA of extracts of normal and xeroderma pigmentosum cells, which Werner *et al.* have detected, are the result of a mutation which has changed the template specificity of the cell's DNA repair enzyme(s). If human DNA repair enzymes resemble *E. coli* DNA polymerase I they will no doubt be induced to use, in the highly artificial environments set up by biochemists, synthetic RNAs and DNA/RNA hybrids as template. It does not take much stretching of the imagination to picture a mutation which would cause such an enzyme to use RNAs and hybrids in such experimental conditions in preference to native DNA, and at the same time impair DNA repair *in vivo*.

CONSERVATION

Rhinos Survive

from a South African Correspondent

DURING the early history of South Africa at least two big game species, the true quagga, *Equus quagga,* and the bluebuck, *Hippotragus leucophaeus,* were exterminated and many other species were severely curtailed in numbers and distribution. The approach to these matters has now improved considerably, however, as is shown by two recent reports.

One report by Dr U. de V. Pienaar, Nature Conservator of the Kruger National Park, points out (*Koedoe,* **13,** 157; 1970) that the southern white (or squarelipped) rhinoceros, *Ceratotherium simum,* is now safely re-established in the park after having been extinct in the eastern Transvaal for 65 years. The last white rhino in this area was apparently shot around 1896, when it seemed to be heading for extinction throughout its range. But it survived in the Umfolozi-Hluhluwe corridor complex in Zululand and gradually recovered so well that a danger arose of over-utilization and destruction of the available habitat. As long ago as 1928 the possibility of translocating rhino from Zululand to other parts of their former range was suggested by the naturalist Herbert Lang, but it was not until about 1960 that it became feasible for the Natal Parks Board to undertake large scale translocation. Even so, more than 1,200 animals remain in the Umfolozi-Hluhluwe corridor complex, so that the threat of over-utilization of the habitat has not been averted.

The second report, by Dr W. von Richter of the Mammal Research Unit, University of Pretoria (*Ann. Transv. Mus.*, **27,** 35; 1971), discusses the present status of the black wildebeest, *Connochaetes gnou.* This animal originally occurred extensively in the central open plains of South Africa, particularly the highveld grasslands of the southern Transvaal, Orange Free State and Karoo and the central and southern Cape grassveld. In addition it was probably a seasonal migrant into Natal. Within its range it was initially extremely abundant, but indiscriminate hunting, together with periodic disease outbreaks and gradual curtailment of the habitat to make way for human settlement, caused it to dwindle to about 600 animals, chiefly in the Orange Free State and southern Transvaal, by the turn of the century. Since then, however, numbers have increased steadily to more than 1,000 in 1945, about 1,800 in 1965 and 3,100 in 1970, as well as more than 155 animals living in zoological gardens in various parts of the world. The most marked increase has been in the Transvaal.

Von Richter concludes that, whereas it was thanks to the initiative of a few farmers that the black wildebeest was saved from extinction, an important factor in its survival has been the establishment of breeding herds in the national parks and provincial reserves.

Structure and Base Composition in DNA

IN the next issue of *Nature New Biology,* Bram contradicts the crystallographic dictum that DNA conformation is essentially independent of base composition. From X-ray scattering envelopes on gels or concentrated solutions, Bram previously found that the conformation of a DNA of typical base composition was little different from the B-structure found in fibres at high humidity. The same was true for DNA within calf thymus nucleohistone. A comparison of observed with calculated scattering curves indicated a small difference in the helix twist from that of B-DNA. Bram has now examined two bacterial DNA species containing 67 and 69 per cent of (A+T), as well as the alternating polymer, poly d(AT), and he reports that these do not fit the same model. The axial radius of gyration is similar, but the cross-section of the AT-rich species has much greater anisotropy. The curves fit neither a B nor an A nor a C-type of model, but all that can be said about the geometry of the new structure at the present stage is that the helix pitch is some 10 per cent larger than that of DNA of lower AT content. Bram suggests that the difference may be in the pucker of the sugar ring.

Bram notes that the transition of the DNA structure between the two forms is highly cooperative, in the sense that a large structural difference is observed between molecules containing 56 per cent and 67 per cent (A+T), but as between 56 per cent and 28 per cent (A+T) there is no further detectable change. Whether this apparent disagreement with earlier X-ray diffraction studies on fibres is a result of inadequate diffraction data on high-AT species, or on differences between the fibre and solution tates, remains to be established.

The biological implications of the findings that Bram reports may well be profound. If, as he suggests, AT-rich regions in a single chain cause alternation between the two types of structure, this offers a basis for a whole series of protein recognition processes. Some enzymes, repressors, and such molecules as polylysine, all show a strong binding preference for DNA species rich in (A+T). In bacterial DNAs tracts extremely rich in (A+T) have in fact been found. The observations would also make necessary a re-examination of the dependence of spectroscopic and other properties of DNA on the base-composition. The possibility exists that a similar effect may occur in RNA.

Value of Historical Records of Earthquakes

N. N. AMBRASEYS

Engineering Seismology Section, Imperial College, London SW7

Modern earthquake catalogues have been found unreliable in reporting earthquakes which occurred before 1900, because of a series of errors perpetuated at second and third hand. A new study of the original records provides significant data relating earthquake activity in the Eastern Mediterranean basin with plate tectonic activity over the past 2,000 years.

It is now recognized that any hope for earthquake prediction rests on increasing knowledge of earthquakes and their causes as well as of their effects. Almost all estimation of seismicity is probabilistic and the probabilities may be computed from observed occurrences of earthquakes. Yet the length of time covered by seismological data is negligibly short when compared with the geological time scale involved in the seismicity of a region. In a given sample period, small events are more numerous than large earthquakes, which are important but rare events, and are not easily counted unless the period of observation is large. Thus, in spite of the considerable volume of data for earthquakes in the present century, a much longer period of observation is needed to permit a meaningful statistical treatment. This can only be achieved by resorting to data for earthquakes before 1900 and by choosing for study seismic regions which have a long and well recorded history.

The region selected for study is shown in Fig. 1; it comprises the Eastern Mediterranean basin which has a very long and well documented history. Initially, the work was based on a number of earthquake catalogues and other modern works dealing with earthquakes before 1900. But after checking a number of suspect entries against the original authority quoted, I found that in general these catalogues were unreliable. Additional checking proved that entries were based on comparatively modern works and on early sources of information at third hand[1]. Apparently, seismologists who have often had to use literary sources at second hand have not been able to study early accounts critically. Thus, on many occasions, the same event, being described differently by various sources, was entered in their catalogues as two or even more events. Most of these catalogues have been copied by others without checking the mistakes, which are now being repeated in increasing numbers from publication to publication.

Thus, after experience of the difficulties caused by accepting the wrong data of modern writers, I decided to make a fresh collection of data based on contemporary evidence alone, with earthquake events set forth in an account of the local history and geography, starting with the first century AD, and avoiding the use of modern works.

Basic data were first drawn from the Teubner Series (3.5%)* as well as from the Patrologia Graeco-Latina (10.5%), and these were supplemented by information extracted from the Corpus Scriptorum Historiae Byzantinae (6.0%). Information from these sources provided a broad basis for the study of events before the sixteenth century. This information was augmented and cross-checked with data in the special publications of a considerable number of early writers (16.0%), bringing the period of observation up to AD 1700. At this stage, a number of problems arose in connexion with the dating of certain events, and also with the location of a considerable number of the sites mentioned in early documents. For the period between the sixth and fourteenth centuries, Syriac sources (2.5%) not only added new material but also resolved some of the chronological ambiguities. The use of contemporary Arabic sources (22.0%) also added a very substantial amount of fresh information, particularly for the period between the ninth and the seventeenth centuries, and helped disentangle some of the dating and siting problems; they did create, however, new difficulties in chronology, some of which were resolved using both Armenian and, to a lesser extent, Georgian sources (8.5%). The most invaluable sources of information were found in manuscripts (14.0%), particularly in the form of marginal notes and colophons. For the most part these are eye witness or contemporary accounts usually providing accurate dates or reliable information from which the actual dates could be derived; they describe events in remote areas, usually in monasteries far from large centres of culture, thus supplementing information from better known chronicles. For the whole period studied so far, inscriptions (1.0%) and a very small number of coins were found useful not only for dating events but also for assessing the degree and extent of damage caused by a particular earthquake; public assistance and private generosity are usually recorded. The issue of new coinage and the establishment of new mints were often measures taken by early authorities to relieve regions after destructive earthquakes. Archaeological evidence from recent excavations was also helpful, particularly in regions for which written evidence is either inconclusive or scarce.

Of the earthquake events identified in the course of the past ten years of the present work, most have been found in my own perusal of the original documents, which is still far from complete. The material available at present, however, is coherent enough to permit some conclusions to be drawn about the historical and scientific value of earthquake records for the first sixteen centuries AD.

The total number of all earthquakes, large and small, identified so far for the period AD 10 to 1699, is just over 3,000, or about twenty times the number of genuine earthquakes listed for the same period in modern catalogues.

---

* Percentage of the total number of original sources.

Because of serious gaps in recorded history of the Greek mainland, Macedonia and Albania, the homogeneity of the data for this area is relatively low. For the area east of the fortieth meridian, including the Red Sea, and for the period before the seventh century, the homogeneity of the data is even lower. For the rest of the region the sources of information are uniformly distributed in space and time and for the larger shocks, about 2,200 in all, the homogeneity of the material can be proved to be satisfactory for the whole period.

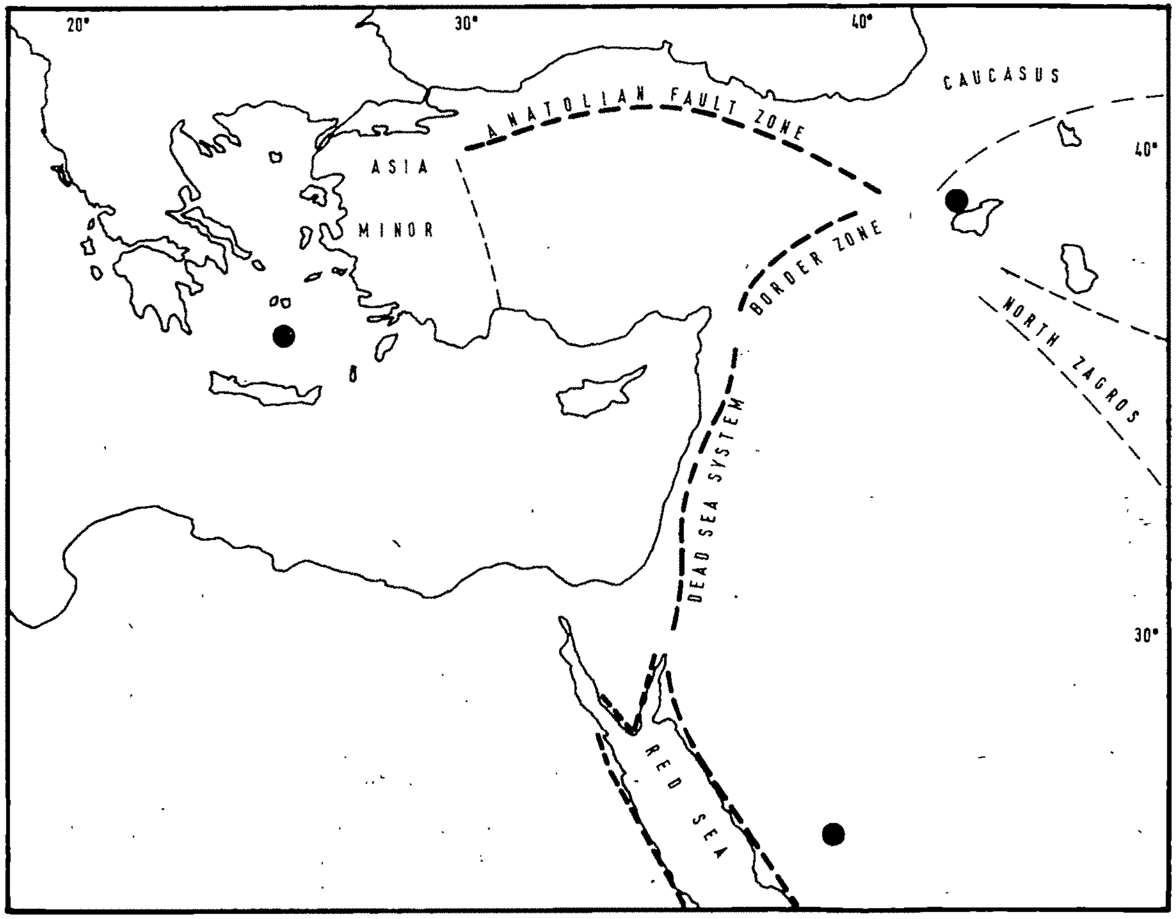


Fig. 1 The area of study.

It is often argued that it is the largest and most populous centres of culture that furnish most of the data about early earthquakes and that historical seismicity usually seems to be concentrated around large cities. This apparent concentration of seismicity is the result of the scanty and often inaccurate information given in modern earthquake catalogues. Modern writers seem to have been unable to study original sources critically or to identify localities affected by early earthquakes other than obvious ones. Thus, an unmodified use of the data in modern catalogues would lead to the absurd conclusion that the most seismic areas in the Middle and Near East are Istanbul, Antakya, Aleppo, and Jerusalem. But earthquakes felt in and reported from large cities originated in most cases from epicentral regions 50 to 400 km away where they caused heavy damage, accounts of which have been found either in less well known chronicles or in marginal notes, colophons and inscriptions written in remote areas which for centuries remained unaffected by significant population movements or by changes in the density of population.

The method used for the study of early events can best be illustrated by summarizing a typical example, that of the earthquake in AD 17 which affected the region recently damaged by a destructive earthquake on April 2, 1968. The earthquake of AD 17 destroyed twelve cities along the river Gediz, from the Aegean coast to Alashehir (Fig. 2). The earthquake happened at night and the destruction it caused was aggravated by conflagration. As a result of the earthquake certain parts of the Gediz valley were uplifted and others sank, the ground opened up in places and large landslides added to the damage. This earthquake is mentioned by Strabo (xii, 8; xiii, 3/4) who names only Magnesia and Sardis. Tacitus (ii, 47) gives more information about the twelve cities destroyed, which he names as Myrina, Aegae, Kyme, Temnus, Apollonis, Hyrkanis, Hierocaesarea, Mostene, Temnos, and Philadelphia. Pliny (n.h. ii, 200) and Seneca (n.q. vi, 1) call this earthquake a major disaster and give twelve as the number of the cities destroyed. Dio (1 vii, 17) attests the dispatch to Asia Minor of a senatorial commissioner with five lictors charged with the relief of the area as well as the remittance of large sums of money for reconstruction. Eusebius (2032) places this event in a.A. 2032, commencing October AD 16 and also in the third year of Tiberius, which are consistent with AD 17, but adds to the destroyed cities Dia and Ephesus. Hieronymus (566) adds Ephesus and Dia, but dates the event in the fifth year of Tiberius which is at least one year too high. *Chronicon Pseudo-Dionys* 93 gives the same date but adds only Ephesus. Later writers follow either Eusebius or Hieronymus, and some of them omit Kyme. Most of the earlier sources, Prosper (372), Dion. Telmar (46), Syncelus (319/603), record this earthquake in a.A. 2032, ending in October AD 17. Eusebius as well as Hieronymus add to the list a fourteenth city, Dia, which they do not count, however, and which is not mentioned in the Puteoli inscription. This inscription was found at Puteoli and bears the names of the twelve cities destroyed by the earthquake as well as that of Ephesus (CIL x, 1624). As this monument was set up in AD 30, twelve years after the earthquake, it is almost certain that Ephesus may have suffered and been relieved at a later date than the rest. In AD 22 a monument was erected in Rome by the restored cities in gratitude to Tiberius (BMC. Emp. i, Tib, 70); this monument is also described by Phlegon (42) and it refers to the twelve cities excluding Ephesus. It seems very likely that Dia has been derived from the dative case of Apollonis through an oversight of a later copyist of Eusebius's work (dat. Apollonidia = Apollonia-Dia). As Dia is found in texts always following the word Apollonis, or as in the Puteoli inscription the two names are joined together giving Apollonidea, we may assume that Dia did not suffer from the AD 17 earthquake. It must be pointed out, however, that Dia is given as a separate city damaged by this earthquake in Stephanus of Byzantium, who places it in Caria; perhaps Stephanus confused Dios Hieron, which is situated a few kilometres north of Ephesus in Lydia, with the alleged city of Dia. Sardis and Magnesia were almost totally destroyed

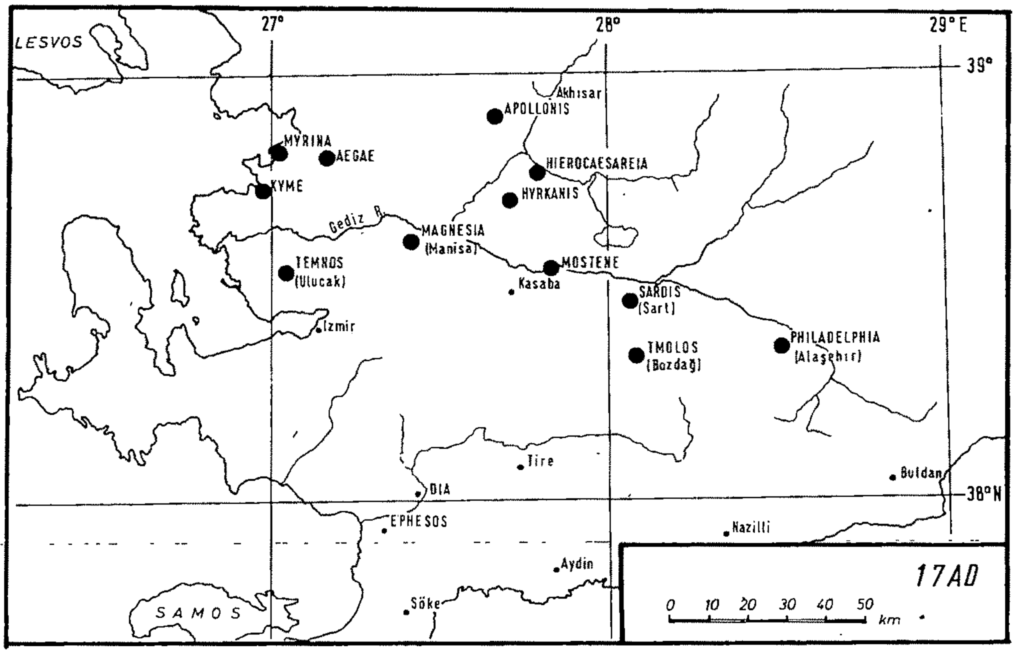


Fig. 2 Area affected by the earthquake of AD 17.

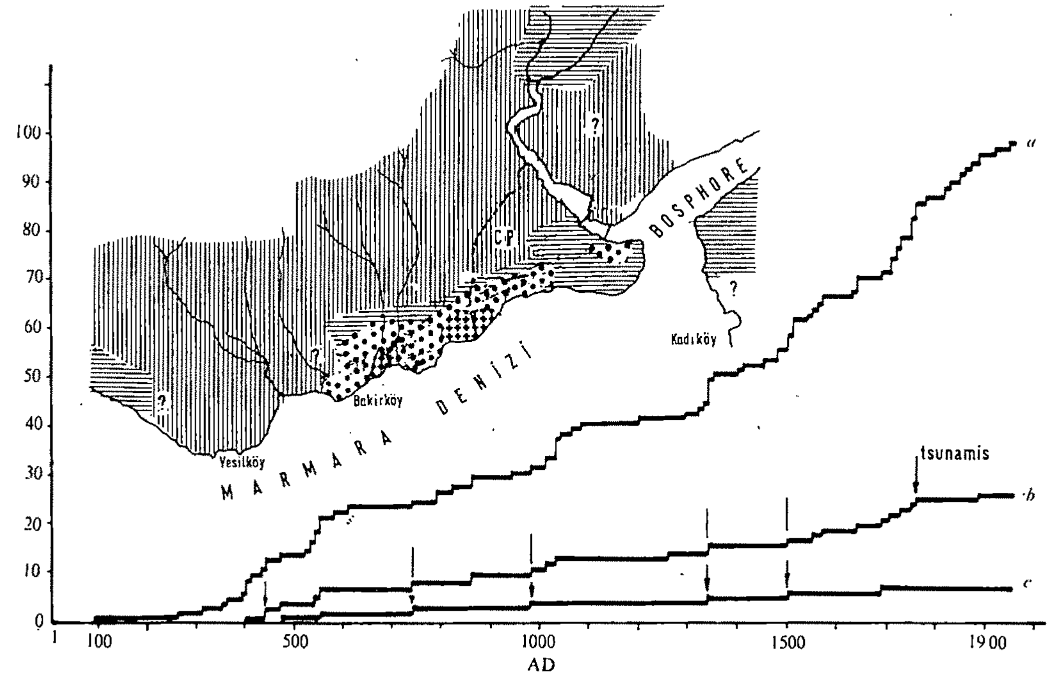


Fig. 3 Seismic activity in the Istanbul region.

and received considerable financial assistance from Rome as well as remission of all contributions to the Imperial Exchequer for a period of five years. Remission of tribute to the public exchequer for the same period was given to the other ten cities, *Res Gestae divi Augusti*. Sardis was rebuilt on an extensive plan. Recent excavations show an unusual type of foundation construction used in the reconstruction of the part of the city on the east bank of the Paktolos river—a grid of wooden beams under the foundation on which the structure was built. All evidence shows that this was a major earthquake.

From the study of individual events, in a manner similar to that given above, the seismicity of a particular area may be investigated over a long period of time. A typical though not the best example of early seismicity is that for the region of Istanbul. Fig. 3 (curve *a*) shows the time distribution of all strong earthquakes that caused alarm and also some damage in the city. There are two conspicuous periods of relative quiescence, and about 200 years long, centring on the seventh and twelfth centuries respectively. These are periods of genuine inactivity and are not due to lack of information. Indeed, the second of these periods coincides with the period of the Crusades for which we have the largest amount of information from occidental sources. From the same figure we notice that the frequency of occurrence of strong earthquakes in the fifth and sixth centuries is the same as that for the period after the fifteenth century. Fig. 3 (curve *b*) shows a similar plot for shocks that caused widespread concern over a large area and also considerable damage in Istanbul. Long periods of quiescence are again noticeable, but also groups of strong earthquakes, marking distinct increases in seismicity, with an average rate of about one damaging earthquake every 55 years. Finally, Fig. 3 (curve *c*) shows the distribution of destructive earthquakes that ruined large parts of the country around Istanbul, causing heavy damage with large numbers of casualties in the city itself. Some of these shocks were followed by comparatively short periods of high seismicity which often occurred near the end of long periods of relative quiescence; most of these large shocks were associated with seismic sea-waves, some of which were of catastrophic magnitude.

Frequency curves, similar to those shown in Fig. 3, have been prepared and studied for a number of regions. They show that earthquake frequency is subject to marked fluctuations rather than to any general monotonic variation. They show also that the data available for the seventeen centuries are sufficient for the detection of secular changes in local seismicity but inadequate to establish whether these changes are subject to a law. It is my impression that historical data are useful for the study of continental tectonics, of the migration of seismic activity in contiguous tectonic units as well as of the interaction between such units. For prediction purposes, however, a much longer period of observations is needed, for which no foreseeable means of data gathering seems likely.

The distribution of historical seismicity is shown by the shaded areas in Fig. 4. This map was constructed by superimposing all epicentral areas of individual earthquakes such as the one shown in Fig. 2. The map shows quite clearly that

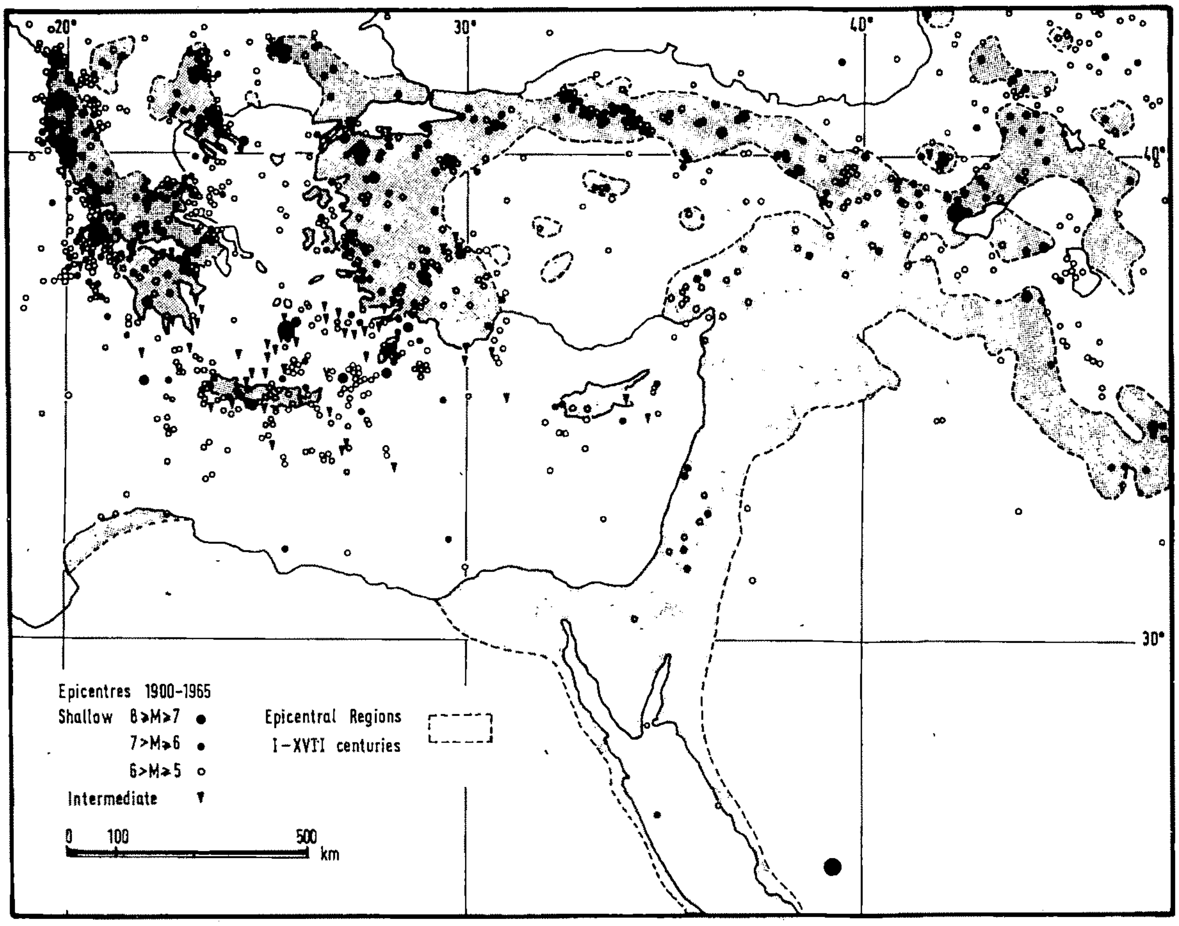


Fig. 4 Distribution of historical seismicity.

historical seismicity follows not only a well defined pattern but also that this pattern fits surprisingly well to boundaries of major tectonic units (Fig. 1). Thus the Red and the Dead Sea Systems, the broad Border Zone and its contact with the Anatolian Fault Zone and North Zagros, are very well defined. Complex zones in Greece and in western Asia Minor are less clearly depicted.

Instrumental data for the past 65 years, shown on the same figure, fit the pattern of early seismicity equally well. There is, however, a noticeable lack of instrumental data in the Border Zone, in the Zagros and in the Red and Dead Sea systems, which is obviously due to the relative quiescence of these regions during the present century. Consequently, it would seem that in certain cases of continental tectonics the formulation of theories on the basis of instrumental data alone, without due consideration of the time-scale involved in certain seismic processes, is bound to lead to erroneous or incomplete results.

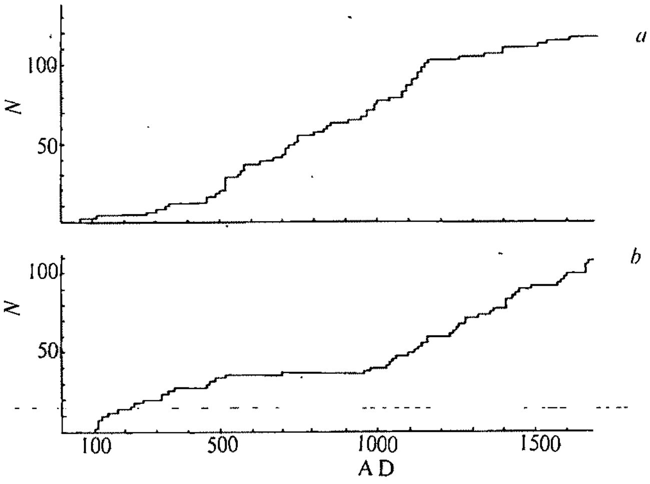


Fig. 5 Time distribution of damaging earthquakes. *a*, Border Zone; *b*, Anatolian Zone. N, Number of damaging earthquakes.

The study of interaction and migration of seismic activity in contiguous tectonic units is interesting. Fig. 5 shows the time distribution of damaging earthquakes (*a*) in the border zone and (*b*) in the Anatolian Fault Zone, the two units being contiguous. From this figure it is clear that during the first five centuries the Border Zone was comparatively quiescent while the Anatolian Zone was active. During the following six centuries the pattern was reversed, only to be reversed again during the eleventh century with a few centuries of overlapping activity. A similar cyclic pattern, but with longer periods of overlapping activity, was noticed for the Border Zone and the Dead Sea System. At this stage, however, a more detailed study of the interaction and correlation of activity of contiguous units is not warranted.

Faced with a high degree of uncertainty, but pressed by the requirements of a technical society for judgment and decisions, seismologists, geologists and engineers make daily estimates of seismicity with varying degrees of success. The study of historical earthquakes brings out two important points that should be taken into consideration when early events are used in seismicity assessments.

The first point concerns the interpretation of early earthquakes. To each of the curves shown in Fig. 3, or to any other frequency curve, corresponds a particular intensity, the precise value of which cannot be assessed. The reason for this is that intensity, being a measure of the effect of a particular earthquake on man-made structures, cannot properly be used to relate early and modern events. Modern buildings differ in many respects from old ones and to judge future damage from century old case histories can be seriously misleading. For instance, the exact repetition of an old earthquake at the present day will differ not only in the types of structures damaged but also in the degree of damage. In some cases modern structures will be more severely damaged than old buildings. A distant large earthquake ten centuries ago would have caused practically no damage in Istanbul, Ankara or Jerusalem; the same earthquake today would cause damage to a large number of long-period structures in these cities.

The second point concerns the assessment of seismicity using extant structures of old age. It is usually considered rather improbable that early structures, liable to damage by earthquakes, could stand for many centuries in seismic regions. The mere fact that quite a few early monuments are still to be found in a state of tolerable preservation in various parts of the world has led to the belief that these parts should be free from destructive earthquakes[2]. But historical and archaeological evidence shows that this is not the case. The early buildings that are still standing have in fact, during their lifetime, been subjected to a number of destructive earthquakes, and they have survived through a process of natural selection. They are a very small fraction of the total number of structures that existed in early times and they represent a sample of structures of the best final design and construction, achieved through the ages by a trial-and-error technique or by chance. Furthermore, frequent references occur in early literature to extensive repairs of walls, churches and other buildings, because of earthquake damage, where the occasion was taken to introduce changes in the design and construction which produced a more resistant structure. Thus, the mere fact that a few early monuments are still standing in various sites in Greece, Turkey, Iran or even in Skopje and in Istanbul, should not be taken to mean that these sites have been free from damaging earthquakes.

Perhaps it is one of our most interesting findings that the lasting effects of major earthquakes during the past twenty-five centuries would not seem to have been very significant. Soon after a large earthquake, vested interests invariably led people to act once again with disregard for the prospect of further such calamities. Yet modern writers[3,4] have attempted to use earthquakes to account for gaps in the sequence of civilizations and for movements of peoples, hypotheses for which I can find absolutely no historical justification. Earthquakes in the past twenty-five centuries have had little, if any, serious influence on historical developments in the Middle and Near East. They did often account for the premature decline of a local economy or for a crisis in local human affairs. But they have never caused the ruin of a culturally advanced state, far less the end of a civilization. In contrast with wars, epidemics and other long lasting calamities which have serious and prolonged effects, earthquakes, no matter how large, seem to have had little long-term impact on Man. It may be that men react to the inevitable hazard in a special way, distinct from the preventable hazards[5]. Personal, political and economic interests seem to overshadow, and in some cases suppress, the lessons to be learnt from destructive earthquakes. For example, villages ruined by early earthquakes are often rebuilt on different sites, whereas cities, where significant economic and political interests were involved, have been rebuilt, enlarged and embellished; invariably on the same site, even when it was known that the site was unsuitable and that it had contributed to the magnitude of the disaster.

This work, which began early in 1960 and which is still in progress, has been supported by UNESCO (1969–71).

[1] Sieberg, A., in Gutenberg, B., *Handbuch der Geophysik*, **4**, *Denkschr. Mediz.-Naturw. Gesellsch.*, Jena, **2** (1932).
[2] Frech, F., *Z. Gesellsch. Erdkund. Berlin*, 576 (1915).
[3] Marinatos, S., *Antiquity*, 3, 425 (1939).
[4] Schaeffer, C. F., *Stratigraphie compare et chronologie de l'Asie Occidental pour les IIIe et IIe millenaires* (Oxford, 1948).
[5] Burton, I., and Kates, R., *National Resources J.*, No. 3 (1964).

Discovery of Postcranial Remains of *Homo erectus* and Associated Artefacts in Bed IV at Olduvai Gorge, Tanzania

M. D. LEAKEY

Centre for Prehistory and Palaeontology, PO Box 30239, Nairobi

The discovery of a left femur shaft and hip bone of *Homo erectus* in association with an Acheulean industry in Bed IV represents the first direct association at Olduvai of a well defined artefact assemblage with *H. erectus*.

THE shaft of a left femur and a pelvic fragment attributed to *Homo erectus* (Olduvai Hominid 28) that are described by Dr M. H. Day in the following article[1] were found *in situ* during excavations in 1970 at site WK (Wayland's Korongo), Olduvai Gorge. This site lies on the south side of the gorge, 1.25 miles below the junction of the main and side gorges. Hand axes and cleavers had first been observed there on the scree below Bed IV during 1932, but no excavations were undertaken before 1970, when a systematic exploration of Bed IV was carried out.

The two hominid bones were found on a well defined surface and lay 1.5 m apart, with the anterior aspect of the femur shaft and the interior or pelvic aspect of the innominate bone upwards. Several artefacts and broken remains of fauna lay nearby (Fig. 1). Subsequent excavations, during which approximately 18 × 10 m were cleared down to the level of the hominid bones, revealed that they were on the higher part of an uneven, eroded surface which could be traced over the whole of the exposed area. To the south-west, the surface dipped down into

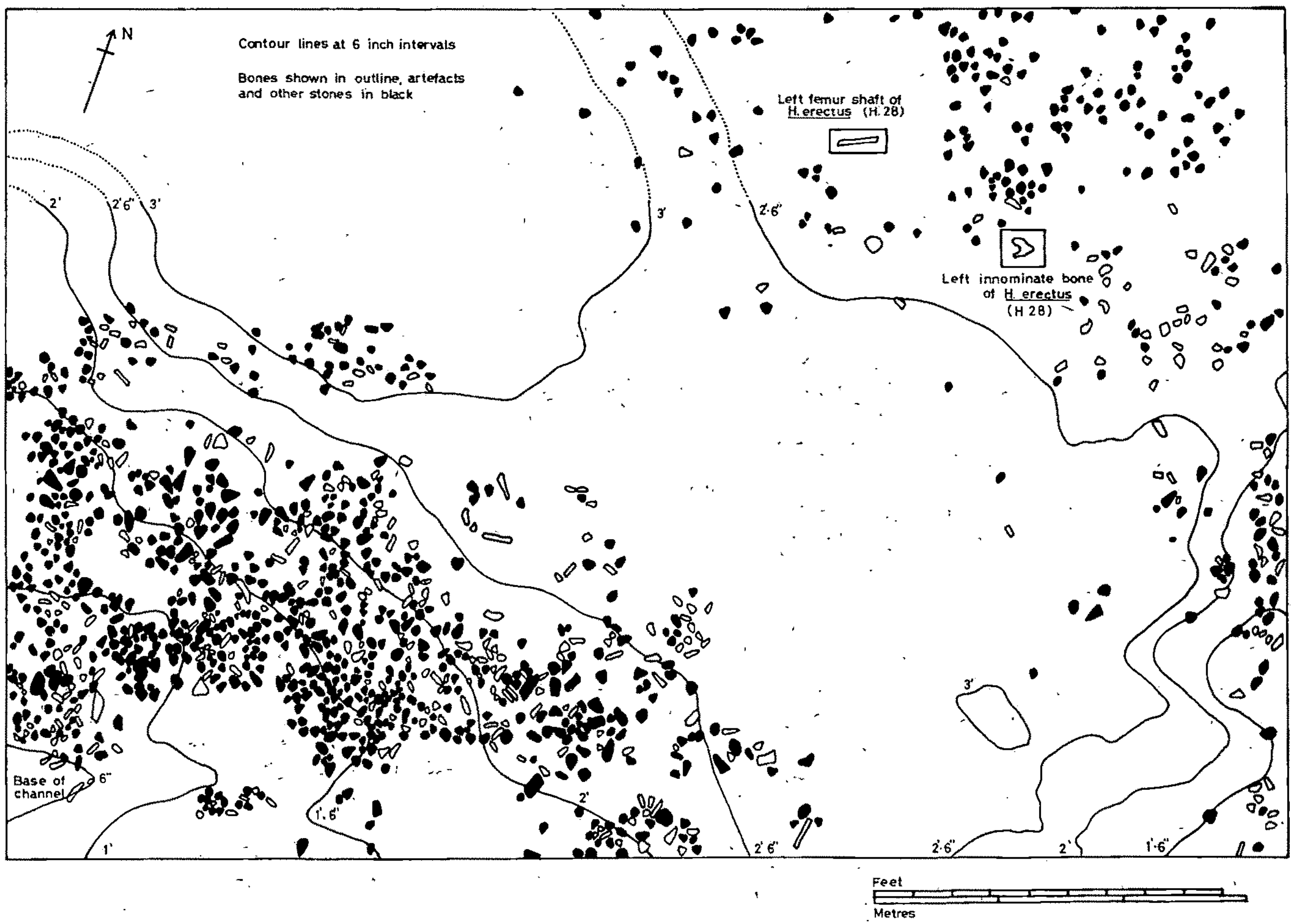

N
Contour lines at 6 inch intervals
Bones shown in outline, artefacts and other stones in black
Left femur shaft of H. erectus (H. 28)
Left innominate bone of H. erectus (H 28)
Base of channel
2'
2'6"
3'
6"
1'
1'.6"
2'6"
2'
1'6"
Feet
Metres

Fig. 1 Association of artefacts and *H. erectus* remains at site WK. Contour lines are at 6 inch intervals. Bones are shown in outline; artefacts and other stones are shown in black.

a channel approximately 1 m deep that was aligned roughly NE–SW. Several irregular depressions were uncovered in the eastern part of the area, none of which exceeded 0.75 m in depth; the bases of these depressions and of small hollows in the principal channel were clearly marked by a thin coating of silt.

Both the principal channel and the depressions contained dense accumulations of occupational debris, incorporated in a coarse sandy matrix. This level yielded a total of 2,600 specimens comprising hand axes, cleavers and other artefacts, including artificially pitted stones, faunal remains and unmodified cobbles. Abrasion is evident on some of the specimens but many are sharp and do not seem to have been weathered or transported by water for any considerable distance.

Two more horizons containing artefacts were uncovered at this site, the lowest in a conglomerate at the junction of Beds III–IV and the second approximately 1 m below the hominid level (Fig. 2).

Geology of the Site

Dr R. L. Hay of the Department of Geology, University of California, Berkeley, whose monograph on the geology of Olduvai Gorge is now in preparation, has supplied the following note on the geology of the site, with the accompanying section (Fig. 3).

Bed IV ranges from 20–22 feet thick in the vicinity of WK. It sharply overlies Bed III and is overlain by aeolian tuffs of the Mesak Beds (formerly known as Bed IVb). Bed IV is 50%–60% claystone and most of the remainder is sandstone and conglomerate, which form lenticular beds. The claystones are usually sandy, root-marked, and locally contain vertical veins of sandy claystone a centimetre thick and a few feet long that represent deep mud cracks filled by sediments during the deposition of Bed IV. Most sandstones contain both volcanic and quartzose basement debris.

A marker tuff (Tuff IVB) extends through most of this area near the middle of Bed IV. It is as much as 7 feet thick, hard and reddish-brown, and is characterized by altered mafic glass and abundant rock fragments. Most of the tuff is thin-bedded or laminated. The tuff is locally eroded away and fragments of it occur in the lowermost sandstones and conglomerates above the tuff. The hominid fossils were found at the base of a sandstone with fragments of the tuff, showing that they postdate the tuff, but probably by only a short period of time.

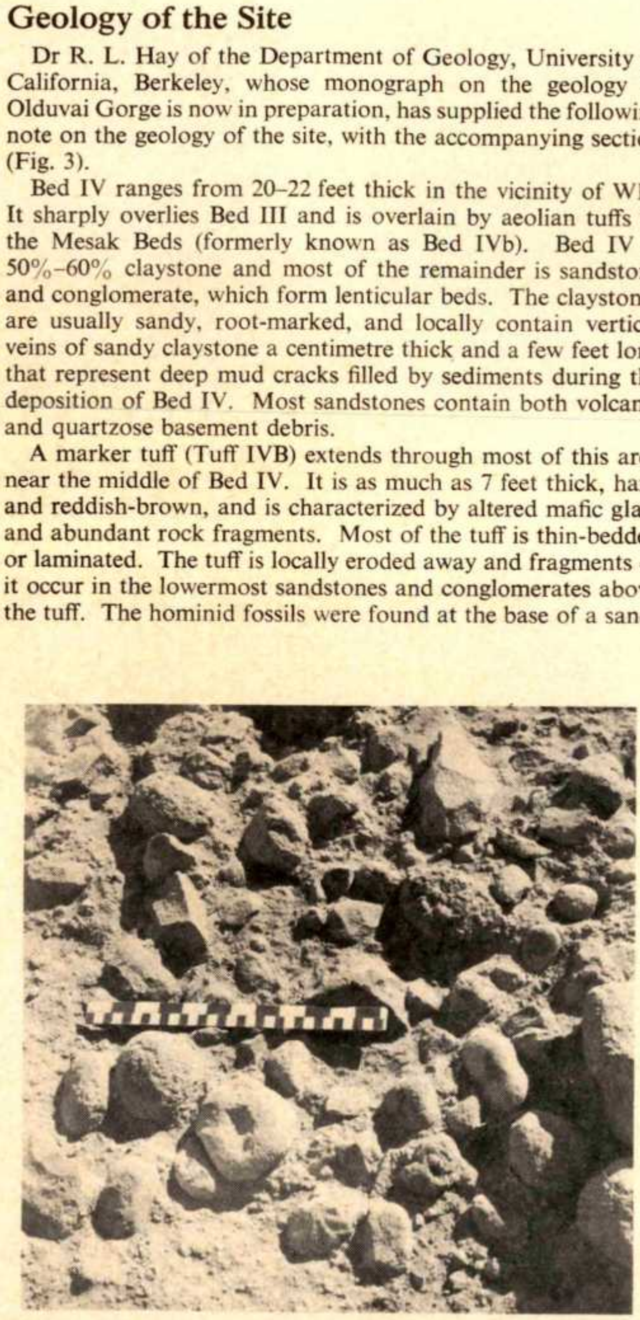


Fig. 2 Artefacts and other stones in the channel at site WK. A pitted cobble can be seen below the scale.

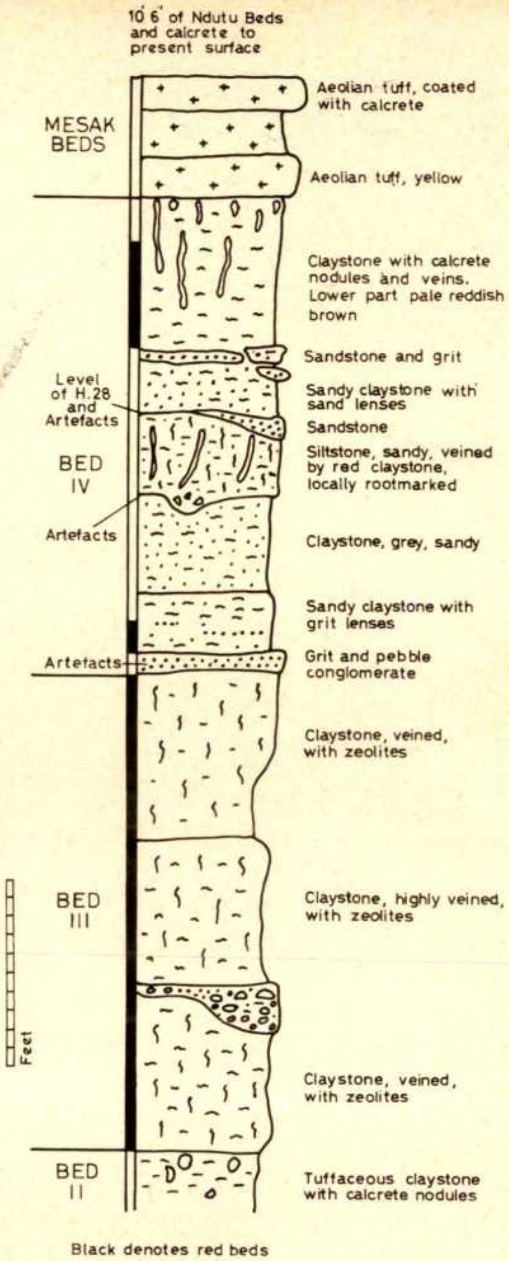

10 6' of Ndutu Beds and calcrete to present surface
MESAK BEDS
Aeolian tuff, coated with calcrete
Aeolian tuff, yellow
Claystone with calcrete nodules and veins. Lower part pale reddish brown
Sandstone and grit
Level of H.28 and Artefacts
Sandy claystone with sand lenses
Sandstone
BED IV
Siltstone, sandy, veined by red claystone, locally rootmarked
Artefacts
Claystone, grey, sandy
Sandy claystone with grit lenses
Artefacts
Grit and pebble conglomerate
Claystone, veined, with zeolites
BED III
Claystone, highly veined, with zeolites
Feet
Claystone, veined, with zeolites
BED II
Tuffaceous claystone with calcrete nodules
Black denotes red beds

Fig. 3 Stratigraphic section of site WK prepared by R. L. Hay. Black denotes red beds.

The non-tuffaceous deposits of Bed IV are fluvial; sandstones and conglomerates fill the stream channels, and the claystones represent overbank deposits. The WK area lay near the drainage axis of the Bed IV basin which received volcanic detritus from the south and east, and basement detritus from the north and west. Tuff IVB was deposited in a temporary, small, saline playa lake, at a time when the climate was relatively hot and dry.

Recent geomagnetic polarity measurements on red beds provide a basis for tentatively estimating the age of Bed IV. A. Brock (University of Nairobi) has found reversed polarity in three samples from the upper part of Bed II and one from upper Bed III; a sample from near the base of Bed IV is normal. These few data, added to data from Bed I and the lower part of Bed II[2], suggest that the boundary between the Matuyama Reversed and Brunhes Normal epochs, dated at about 0.7 m.y.[2], lies approximately at the base of Bed IV. The Lemuta (= aeolian tuff) Member of lower Bed II has an age of about 1.65 m.y.[3]. Extrapolating on the basis of stratal thickness between the

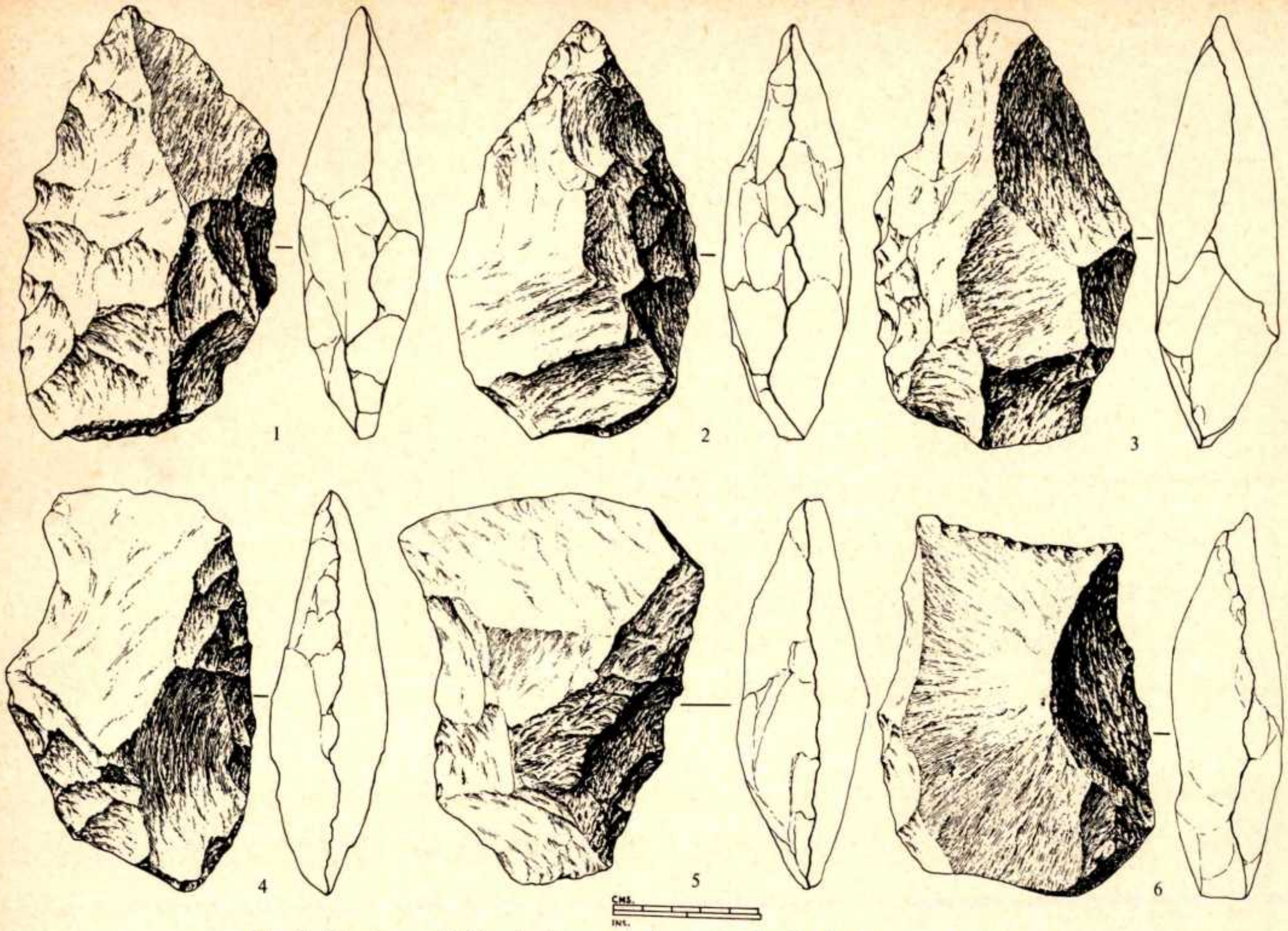


Fig. 4 Hand axes (1–3) and cleavers (4–6) associated with the *H. erectus* remains.

levels of 1.65 and 0.7 m.y., the top of Bed IV may be about 0.3 or 0.4 m.y. old, and the hominid fossils may have an age of about 0.5 m.y.

Artefacts

The sample of occupational debris reviewed here consists of 494 artefacts (Table 1), 347 unmodified cobbles and 257 largely fragmented remains of fauna. This material was obtained from one trench within the principal channel and can be considered to represent a fair sample from the hominid level.

Table 1 Artefacts recovered from Site WK

Tools	Nos.	%
Hand axes	20	15.9
Cleavers	23	18.2
Bifacial tools, broken and unfinished	7	5.5
Choppers	11	8.7
Discoids	9	7.1
Spheroids	10	8.0
Scrapers, heavy and light duty	24	19.0
Small tools, various	22	17.5
Utilized material		
Pitted cobbles	41	35.6
Cobbles, battered	20	17.4
Miscellaneous	50	43.5
Hammerstones	4	3.5
Debitage	253	

Most of the hand axes and cleavers have been made on side or end struck flakes, the former predominating. They are mostly of trachyandesite, though there are a few examples of phonolite and tabular quartzite. The striking platforms have been only roughly trimmed off and one face usually consists almost entirely of the untrimmed primary flake surface. With few exceptions these tools show a minimum degree of retouch and most of the trimming scars are large and deep. Only two specimens, both made from fine grained phonolite, exhibit some "resolved" flaking. The average number of trimming scars is low: 10.5 per specimen in both hand axes and cleavers, a figure that compares closely with that for the Early Acheulean hand axes from Middle Bed II[4]. The hand axes are roughly ovate in form but are markedly irregular. In the cleavers, square or oblique ended examples predominate and chisel ended specimens are rare. In spite of their general crude appearance, both hand axes and cleavers are more or less the same size and have similar breadth/length and thickness/length ratios; it is clear that they represent a homogeneous series (Table 2).

The forty-one pitted cobbles are a prominent feature of this industry and they also occur at other sites nearby of approximately the same stratigraphic level. These stones range in size from specimens no more than 5 cm in diameter to small boulders weighing several kilograms. The pits or hollows are also variable in size and measure from 1 to 5 cm in diameter. They sometimes occur singly, but are more often in pairs, one on either side of a stone; some examples have up to six hollows.

Table 2 Average Measurements of Hand Axes and Cleavers

	Length (mm)	Breadth (mm)	Thickness (mm)	Breadth/ length ratio (%)	Thickness/ length ratio (%)
Hand axes	142	84	41	61.3	28.9
Cleavers	133	90	45	67.5	34.2

Among the unspecified small tools are a number of thick-set quartz points, triangular or quadrilateral in cross-section, that are usually heavily utilized at the tips. These tools also occur at other sites where there are pitted cobbles and it seems possible that they were used in conjunction with the cobbles, perhaps as punches.

Artefact assemblages from three sites excavated within 1–2 km of WK, that are virtually contemporaneous with the hominid level, have still to be studied in detail, but it is clear that they are at variance with the WK industry in a number of important respects, particularly in the proportionately small numbers of hand axes, total absence of cleavers and relative abundance and variety of small tools. Large side and end struck flakes do not occur and the rare hand axes are made on small "cores" flaked over both faces. It is evident that the technique of biface manufacture was fundamentally different, although the materials used are the same and are also known to have been obtained from the same sources.

To conclude, the industry from the hominid level can be referred to the Acheulean in view of the proportionately high number of bifacial tools (39.6%), among which cleavers are unusually common. The hand axes bear strong resemblances to the Early Acheulean series from EF–HR in Middle Bed II, to which they seem closer than to contemporary series excavated from Bed IV.

Although other human remains that may be attributed provisionally to *Homo erectus* have been found at Olduvai, in upper Bed II[5] and in Beds III–IV[6], where abundant artefacts also occur, the discoveries at WK are the first occasion on which a well represented lithic industry has been directly associated. It is not certain whether this association implies that *H. erectus* was responsible for the industry, but it seems likely, because there are other lines of evidence at Olduvai suggesting a link between the Early Acheulean of Bed II and *Homo erectus*[4].

Received May 4, 1971.

[1] Day, M. H., *Nature*, **232**, 383 (1971).

[2] Grommé, C. S., and Hay, R. L., *Earth Planet. Sci. Lett.*, **10**, 179 (1971).

[3] Cox, A., *Science*, **163**, 237 (1969).

[4] Leakey, M. D., *Olduvai Gorge*, 3 (Cambridge University Press, in the press).

[5] Leakey, L. S. B., and Leakey, M. D., *Nature*, **202**, 3 (1964).

[6] Leakey, M. D., *Nature*, **223**, 754 (1969).

Postcranial Remains of *Homo erectus* from Bed IV, Olduvai Gorge, Tanzania

M. H. DAY

Department of Anatomy, Middlesex Hospital Medical School, London W1P 6DB

Anatomically, the postcranial remains of Olduvai Hominid 28 seem to have a close resemblance to the *Homo erectus* material from Choukoutien.

THE discovery of postcranial fragments of Olduvai Hominid 28 reported by Mrs Mary Leakey in the previous article[1] is of great interest because the bones throw some light on the structure and function of the lower limb skeleton of Middle Pleistocene hominines in East Africa. The only lower limb material of a hominine of this approximate age known previously consists of the femora from Choukoutien, China, and the femora claimed to be of this age from Trinil, Java.

The first Trinil femur (Femur I) was recovered by Dubois[2] and his workers allegedly from the same horizon as the calotte of "*Pithecanthropus*" *erectus* (Dubois, 1894)≡*Homo erectus erectus* (Campbell, 1964). At the turn of the century, the association of this femur with the calotte was strongly questioned, and often subsequently, on the grounds that its modern features are at variance with the more primitive features of the calotte. When Weidenreich later described[3] the Choukoutien remains of "*Sinanthropus pekinensis*" (Black, 1927)≡ *Homo erectus pekinensis* (Campbell, 1964), he would not accept that the Trinil femur should have been attributed to "*Pithecanthropus*" and stated that it ". . . must be attributed to a type more closely related to modern man" because it differed so markedly from the Choukoutien femora. Nevertheless, it has been widely assumed for 30 yr that the Trinil femur was good evidence that the lower limb of *Homo erectus*, and by inference the remainder of the postcranial skeleton, was fully evolved and virtually of *sapiens* type by the Middle Pleistocene in Java; it was thus assumed that the subsequent evolution of *Homo* was confined almost entirely to the cranium, jaws, teeth and brain.

The discovery of new femoral and pelvic material in East Africa, in a well documented layer associated with artefacts, casts serious doubts on this assumption.

The Femur

The femur is represented by most of the shaft; it lacks the head and the greater trochanter. Parts of the neck and the lesser trochanter are preserved but the shaft is broken off just above the popliteal surface (Figs. 1 and 2).

The shaft is flattened throughout its length with acute medial and lateral borders. The flattening (platymeria) of the upper end is exaggerated by the presence of a marked lateral expansion at the level of the gluteal tuberosity. The lower end of the shaft is just beginning to expand for the condyles at the point of breakage, and the upper end shows the beginning of the lateral expansion for the neck and the postero-medial expansion for the base of the lesser trochanter.

The medial border of the shaft is convex along its length and the narrowest point on the shaft (medial to lateral) is very near to the distal end. Laterally, the shaft is gently convex antero-posteriorly and gradually expands from the subtrochanteric region to the point of shaft breakage below. From the front the shaft is straight.

The muscular impressions on the bone are remarkably clear, particularly the direct attachment of the gluteus maximus which is a combination of a crista hypotrochanterica and a fossa hypotrochanterica. The third trochanter is not preserved, if it was present, but it is possible that there was a

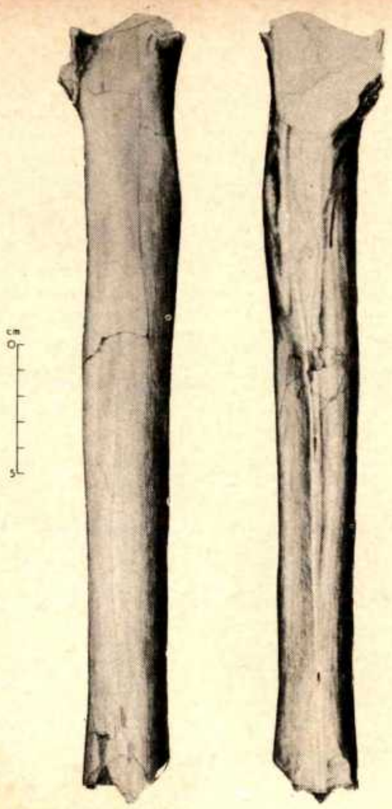


Fig. 1 Femoral shaft of Olduvai Hominid 28, anterior and posterior views. (Drawn by Audrey Besterman.)

tubercle at the top of the hypotrochanteric crest, a feature that continues up to the base of the greater trochanter.

At the upper end of the bone the spiral line is present for the attachment of vastus medialis muscle as well as a weaker pectineal line for the attachment of pectineus muscle; these lines are separated by a roughened area for the anchorage of iliacus muscle. Between the spiral line and the gluteal crest the upward extension of the ridge for adductor magnus can be discerned.

The ligaments around the hip joint seem to have been powerful because the sharp corner at the upper medial extremity of the specimen is crossed by the intertrochanteric roughening for the iliofemoral ligament; similarly, the triangular attachment of the medial band of the pubofemoral ligament is easily identified.

The linea aspera is present and divides towards the lower end into diverging medial and lateral condylar lines; there is no pilaster. Above, the attachment of vastus lateralis curves away from the linea aspera towards the lateral side and skirts the gluteal tuberosity; this permits the upper fibres of the short head of biceps femoris to mark the bone as a short ridge between the linea aspera and the gluteal tuberosity. There are three nutrient foramina, all small and placed near to the linea aspera.

X-rays show a thinning of the cortex towards the lower end of the specimen and the medial and lateral trabecular systems of the neck and upper end are clearly apparent. It would be impossible to see Ward's triangle, even if it were present, because it would have been above the upper fracture line. The medullary cavity is of moderate calibre and is generally wider than deep; the cortical thickness is greater in the coronal plane than it is in the sagittal plane at the mid-shaft. The range of modern human variability of total medio-lateral cortical thickness at the mid-shaft is large[4]. Hominid 28 shows a total thickness of 16 mm medio-laterally and 11 mm antero-posteriorly at the same point. The inferior aspect of the neck shows a well marked cortical buttress that is still 8 mm thick at the point of breakage; this is considerably thicker than is common in modern man.

The Hip Bone

The hip bone (os coxa) is represented by the major parts of its iliac and ischial portions (Figs. 3 and 4); the pubic portion is missing. The ischium lacks the tip of its spine and the ischial portion of the ischiopubic ramus, and the ilium lacks the anterior superior iliac spine. The specimen shows several cracks most of which are not displaced, but over an area of the anterior part of the iliac fossa there is a little distortion, so that there is an irregular depression of the surface and some lateral displacement of the anterior one-third of the iliac flange. This displacement is confirmed by the presence of an open crack on the medial aspect of the upper part of the anterior inferior iliac spine. Viewed from in front the ilium is canted laterally, slightly accentuating the prominence of the iliac pillar. The posterior half of the dorsum ilii also displays some cortical cracking with minor displacements and depression of the surface of the bone. There are several tooth marks on the specimen. There is no evidence of bone pathology or of the generalized effects of systemic disease.

The bone is stoutly constructed with prominent muscular and ligamentous markings and a large acetabulum indicating a large femoral head. The articular surface of the acetabular fossa is smooth and the non-articular region is thin. The ischium is relatively small by comparison with the acetabulum and is separated from it by a deep and narrow groove. This groove seems to be accentuated by the medially rotated position

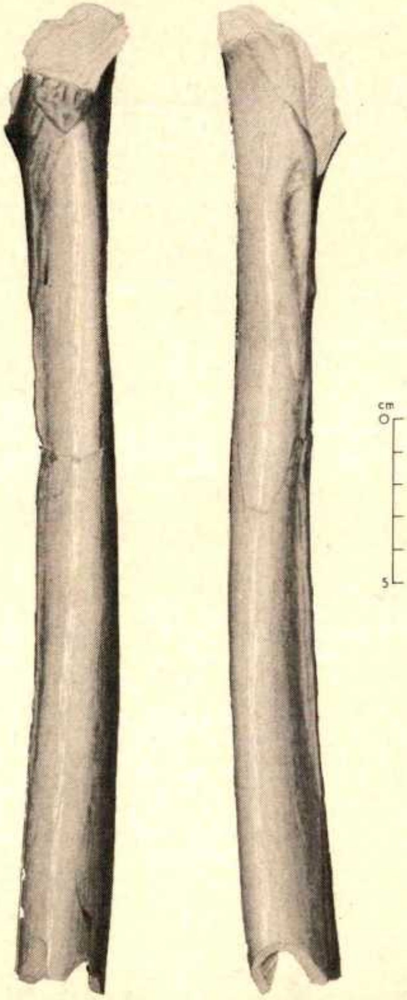


Fig. 2 Femoral shaft of Olduvai Hominid 28, lateral and medial views. (Drawn by Audrey Besterman.)

of the ischium. This also gives an appearance of shortness of the ischium that may not be fully borne out in reconstruction of the missing portion. The ischial tuberosity shows a well marked attachment for semimembranosus separated from that of the long head of biceps femoris and semitendinosus by the oblique ridge. On the lateral aspect of the ischial tuberosity the impression for quadratus femoris muscle can be seen, as well as some roughening for obturator externus muscle on the antero-lateral part of the ischial ramus. The medial surface of the ischium below the spine is smooth and rounded for the play of the tendon of obturator internus muscle.

The ilium is large and characterized by its prominent muscular markings and ligamentous impressions as well as the presence of an extremely robust vertical iliac pillar. Although it is true that this pillar may be slightly exaggerated by the distortion of the anterior part of the bone, none the less the feature is more prominent than on any modern human ilium so far examined. Correspondingly, the horizontal iliac bar which runs posteriorly from the acetabulum above the greater sciatic notch to the posterior iliac spine is very stout and well buttressed. Within the right angle enclosed by the vertical iliac pillar and the horizontal iliac bar the iliac fossa is very thin; the blade of the ilium has the characteristic hominid S-shape and it is set at a wide angle to the plane of the acetabulum.

The anterior superior iliac spine is missing but it may have been raked forward as suggested by the line of the anterior border as it runs upward from the anterior inferior iliac spine. The anterior inferior iliac spine is distinct and rounded showing a clear marking for the upper attachment of the iliofemoral ligament of the hip joint and another for the straight head of rectus femoris. To the lateral side of the spine the attachment of the reflected head of this muscle can be clearly traced above the acetabulum. Otherwise the gluteal aspect of the bone discloses a posterior gluteal line separating the attachments of gluteus maximus and gluteus medius as well as a middle gluteal line separating the attachments of gluteus medius and minimus. The pelvic aspect of the ilium shows a wide and shallow groove anteriorly for the iliacus muscle medial to the anterior inferior iliac spine; just below and behind the groove the iliopectineal line can be detected. In modern man this line is usually extended posteriorly as a ridge that separates the iliac fossa from the true pelvic wall, but in Olduvai Hominid 28 this ridge is missing; the iliac fossa runs smoothly down towards the greater sciatic notch and there is no excavation of the fossa above the horizontal iliac bar.

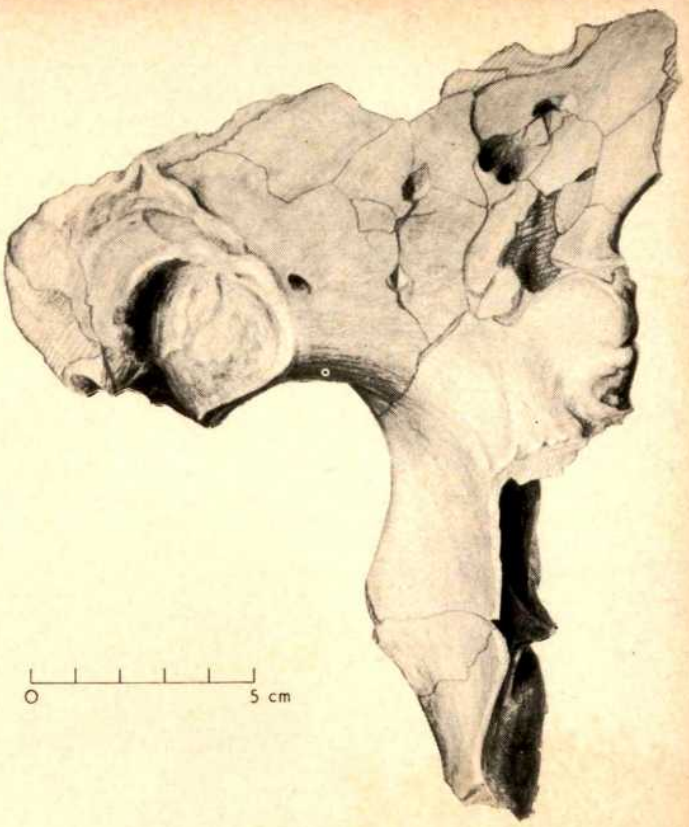


Fig. 4 Hip bone of Olduvai Hominid 28, pelvic surface. (Drawn by Audrey Besterman.)

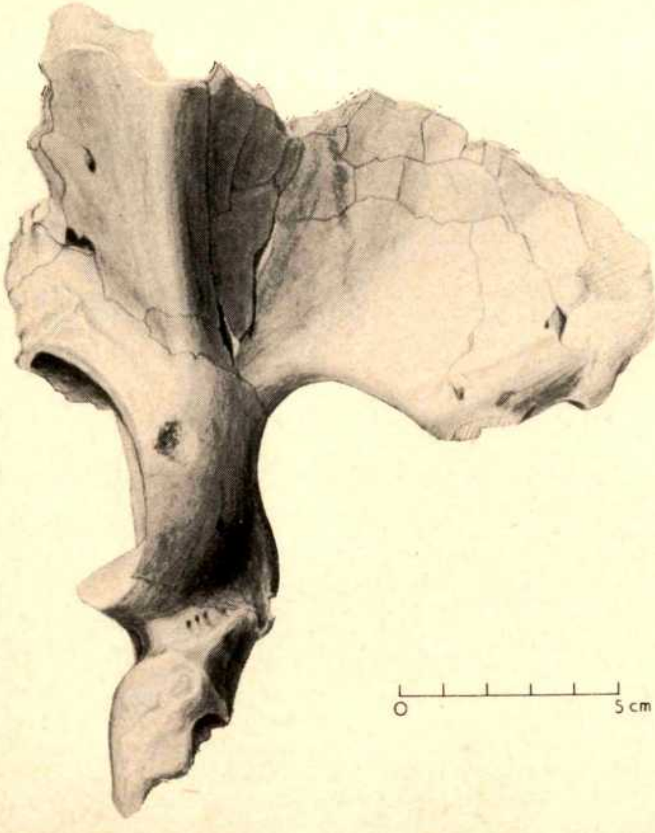


Fig. 3 Hip bone of Olduvai Hominid 28, gluteal surface. (Drawn by Audrey Besterman.)

Posteriorly the auricular surface is small, set low on the bone and is slightly obliquely placed. It is also extended laterally round the inferior border of the ilium. This extension of the auricular surface is at an angle of approximately 120° to the principal part of the surface and would seem to limit relative movements of the sacrum and ilium in a vertical direction. Behind the auricular surface the ilial tuberosity is large and very roughened for the attachment of extensive posterior sacroiliac ligaments. There is no pre-auricular sulcus.

Sexing the hip fragment proved difficult, partly because the pubis is missing. There are a series of contradictory features such as a general robusticity (male), wide sciatic notch (female), large acetabulum (male), auricular surface small and obtuse (female), but, on balance, it seems more likely that it is female though the matter is still open.

An X-ray of the pelvic fragment disclosed several interesting features, some of which confirm the anatomical findings; for example, the stout horizontal iliac bar and the ischium are reinforced by coarsely woven internal trabeculae, and the vertical iliac bar is heavily buttressed with dense bone.

Functional Considerations

The combination of features that characterize the hip bone and the femur allows some functional conclusions to be drawn. These conclusions are strengthened if the two bones are assumed to belong to the same individual; anatomically they are not incompatible and both belong to the left side, and because they were found in close association in the deposit it would seem reasonable and economical to assume that they belong with each other. It is suggested therefore that:

(1) The strength of the vertical iliac pillar, to resist the pull of gluteus medius, gluteus minimus and tensor fascia lata, coupled with the femoral expansion for the lower attachments for gluteus medius and minimus muscles, suggests that the alternating pelvic tilt mechanism of striding bipeds was well developed.

(2) The length and strength of the horizontal iliac bar, coupled with well marked attachments for gluteus maximus (on both hip bone and femur) and the hamstring muscles, indicate a powerful hip extensor mechanism for returning the trunk to the erect position and/or raising the trunk (as in walking uphill or climbing).

(3) The impressions for the iliofemoral ligament on both hip and femur suggest that the pelvic rest mechanism of advanced bipeds was fully developed and that the centre of gravity of the body was placed behind the line joining the rotation centres of the two hip joints.

(4) The rugged area for the attachments of the sacroiliac ligaments shows that the weight of the upper part of the body was being transferred to the pelvis by way of the sacral suspensory mechanism familiar in upright bipeds.

(5) The attachments of ilio-psoas and rectus femoris muscles indicate the power of hip flexion, and the rectus femoris muscle coupled with vastus medialis and lateralis muscles indicate that powerful extension of the knee was possible.

In summary there seems little doubt that Olduvai Hominid 28 was a habitual upright biped. Whether bipedalism in this form was as efficient as that of modern man will not be determined until these bones have been reconstructed and some of their special features fully explained in biomechanical terms, that is, the extremely strengthened vertical pillar, the medial rotation of the ischium and the like.

Comparative Anatomy

Comparisons have been made with a series of modern human femora and with the following.

(1) Originals: Trinil femora (I–VI); Rhodesian femora; Tabūn I femur; Olduvai Hominid 20 femur; and the Skhul IX femur.

(2) Casts and descriptions: Choukoutien femora; Krapina femora (1049 and II); Swartkrans femora; Spy II femur; Skhul IV femur; and the Kanjera femur.

Comparisons have also been made with a series of modern human hip bones and with the following pelvic remains.

(1) Originals: Rhodesian; Tabun.

(2) Casts: Swartkrans; Sterkfontein; Makapansgat; and Kromdraai.

Although some of the individual anatomical features of both the femur and pelvic fragment of Olduvai Hominid 28 can be matched in the comparative material, there is no doubt that it is only with the femora of the Choukoutien group of *Homo erectus* that the combination of features of the femur of Olduvai Hominid 28 can be closely paralleled. The combination of features of the Olduvai Hominid 28 pelvis are, however, so far unmatched in the comparative material studied.

It is established beyond reasonable doubt that the Choukoutien femora are part of the same assemblage as the Choukoutien *Homo erectus* cranial and dental remains[3] and can be assigned to the Middle Pleistocene[5]. In the meticulous descriptions of the Choukoutien postcranial remains, Weidenreich makes plain (1) that the femora must be attributed to a member of the genus *Homo*, and (2) that they are specifically distinct from the femora of *Homo sapiens*. On these grounds the bones were attributed, with the cranial and dental remains, to "*Sinanthropus pekinensis*". In his summary Weidenreich lists a number of features that he states are specific for this taxon. It is of interest to compare these features with the corresponding features of Olduvai Hominid 28 (Table 1).

Of these points, five agree completely, three are in partial agreement (mostly because of lack of evidence) and the sole point in disagreement is subject to considerable anatomical variability.

Table 1 Comparison between *Homo erectus pekinensis* and Olduvai Hominid 28

Homo erectus pekinensis	Olduvai Hominid 28
(1) Straightness of the shaft (that is, lack of marked A/P bowing)	Agrees
(2) The distal position of the least breadth of the shaft	Agrees
(3) Convexity of the medial border of the shaft	Agrees
(4) Pronounced platymeria of the subtrochanteric region combined with angularity (medial and lateral cristae)	Agrees
(5) (i) Development of a hypotrochanteric tubercle at the base of the greater trochanter	(i) Uncertain
(ii) The direct continuation of the hypotrochanteric crest into the greater trochanter	(ii) Agrees
(6) Absence of linea intertrochanterica and linea pectinea	Disagrees
(7) Extraordinary thickness of the wall of shaft and the neck combined with the narrow calibre of the medullary canal	Unusual neck thickness present, walls of moderate thickness, canal of moderate calibre
(8) Diffuse arrangement of the trajectorial system of the upper end combined with a tight-meshed and coarse cancellous substance	Specimen incomplete, but radiographic evidence is not inconsistent

Thus the group of features said by Weidenreich to be specific for Choukoutien *Homo erectus* are shared in large part by Olduvai Hominid 28 and are not found together in *Homo sapiens*. Furthermore, all the metrical features quoted by Weidenreich for Choukoutien *Homo erectus* fall within the range of variability quoted by him for *Homo sapiens*; but the important point made by him (before the advent of multivariate statistics in the assessment of affinity) was that it is the combination of features in Choukoutien *Homo erectus* that differs from the combination of features in *Homo sapiens*.

The femora that must be considered closely are the six femora from Trinil, Java[2,6–11]. It is primarily on these bones that there rests the widely held view that the lower limb of *Homo erectus* differed little from that of modern man, a view that depends in turn on the contemporaneity of the femora with the Trinil calotte, their proper attribution to a Middle Pleistocene date, and a morphological appraisal that considers these femora to be essentially sapient.

Through the kindness and courtesy of the director of the Leyden Museum I have been able to re-examine the Trinil femora both anatomically and radiologically. The preliminary results of this examination reaffirm that these femora have no significant anatomical, radiological or metrical features that lie outside the known range of variation for modern man, nor do they show any combination of features that constitutes a taxonomically or functionally significant morphological pattern. Further work is in progress, in conjunction with Miss Theya Molleson, of the British Museum (Natural History), in order to try to clarify the position by reviewing the evidence for the supposed provenance of the Trinil femora, and by reassessing their relative and absolute dating.

The Olduvai Hominid 28 femur clearly bears remarkable similarities to the well established *Homo erectus* femora from Choukoutien, anatomically, metrically (Table 2) and radiologically. At the same time the Olduvai Hominid 28 pelvic fragment shows a number of anatomical and radiological features that distinguish it from modern sapient pelves, but

Table 2 Comparison of Femora

		Subtrochanteric region			Mid shaft region					Femoral indices			
	Length (mm)	Trans.	Sagitt.	Circum.	Trans.	Sagitt.	Circum.	Torsion	Curvature	Length/thickness index	Robusticity index	Platymeric index	Pilastric index
Martin Nos. (1928)	(2)	(9)	(10)		(7)	(6)	(8)	(30)	(27)	$\frac{(8)}{(2)}\times 100$	$\frac{(6+7)}{(2)}\times 100$	$\frac{(10)}{(9)}\times 100$	$\frac{(6)}{(7)}\times 100$
Olduvai Hom. 28	456*	37.4	23.3	99.0	32.7	24.7	95.0	> 18°	2.91	20.8	14.8	62.3	75.5
Trinil I	455	—	—	—	28.0	29.0	89.0	25°	1.77	19.6	12.5	—	103.6
Trinil II	460*	32.7	26.2	95.0	27.1	26.2	85.0	—	1.75	18.5	11.6	80.1	97.0
Trinil III	—	32.2	24.2	92.0	27.7	25.4	85.0	—	1.53	—	—	75.2	91.7
Trinil IV	—	33.7	23.8	92.0	28.4	25.7	87.0	—	1.50	—	—	70.6	90.5
Trinil V	—	—	—	—	26.3	27.0	85.0	—	—	—	—	—	102.6
Peking I	*c.* 400	34.3	23.2	90.0	29.7	27.1	98.0	—	—	24.5?	14.2?	67.6	91.2
Peking II	—	—	—	—	26.4	22.8	77.0	—	—	—	—	—	86.4
Peking IV	407*	34.3	22.7	94.0	29.3	25.0	85.0	35°/36°	2.25	20.9	13.4	66.2	85.3
Peking V	—	—	—	—	29.5	23.7	83.0?	—	—	—	—	—	80.3
Peking VI	—	—	—	—	29.2	26.1	85.0	—	—	—	—	—	89.4

* Reconstructed.

there are no known examples of *Homo erectus* pelvic remains from elsewhere with which to make comparisons.

It seems reasonable therefore to suggest that the morphological affinities of Olduvai Hominid 28 lie with *Homo erectus* from Choukoutien and that it should be attributed to this specific taxon. Only a full re-examination of the morphology and dating of the Trinil femora may enable the taxonomic position of this material to be established because they appear, perhaps as they always did, to have little in common with the *Homo erectus* femora from Choukoutien.

I thank Mrs Mary Leakey for allowing me to examine this material and Dr W. G. S. Hopkirk (Nairobi Hospital) and Dr Alan Walker (Department of Anatomy, University of Nairobi) for their help.

Received May 6, 1971.

[1] Leakey, M. D., *Nature*, **232**, 380 (1971).
[2] Dubois, E., Pithecanthropus erectus-*eine Menschenaehnliche Ubergangsform aus Java* (Landesdruckerei, Batavia, 1894).
[3] Weidenreich, F., *Palaeont. Sin.*, New Ser. D, **10**, 1 (1941).
[4] Brothwell, D. R., Molleson, T., and Metrewelli, C., in *The Skeletal Biology of Earlier Human Populations* (edit. by Brothwell, D. R.), 173 (Pergamon, Oxford, 1968).
[5] Kurtén, B., and Vasari, Y., *Commentat. Biol.*, **23**, 1 (1960).
[6] Dubois, E., *Proc. Acad. Sci. Amst.*, **29**, 730 (1926).
[7] Dubois, E., *Proc. K. Ned. Akad. Wet.*, **29**, 1275 (1927).
[8] Dubois, E., *Proc. K. Ned. Akad. Wet.*, **35**, 716 (1932).
[9] Dubois, E., *Proc. K. Ned. Akad. Wet.*, **37**, 139 (1934).
[10] Dubois, E., *Proc. K. Ned. Akad. Wet.*, **38**, 850 (1935).
[11] Dubois, E., *Proc. K. Ned. Akad. Wet.*, **40**, 864 (1937).

Spatial Discrimination in the Cytoplasm during Microtubule Morphogenesis

J. B. TUCKER
Department of Zoology, The University, St Andrews, Fife

Patterns of microtubule formation and breakdown in the ciliate *Nassula* provide some indications of the ways in which the deployment of substances influencing microtubule assembly is related to control of tubule growth.

DETAILS of microtubule distribution during the fission cycle in the ciliate *Nassula*[1–6] provide striking examples of how the precise spatial and temporal controls of assembly and breakdown of several microtubular organelles can be exercised simultaneously within a single cell. These findings raise fundamental issues concerning control of the ways in which microtubule precursor protein subunits, and other substances affecting microtubule stability, encounter the sites where tubules are growing or dissociating.

It is generally supposed that structural molecules are synthesized on ribosomes distributed throughout the cytoplasm and that these, and all other molecules influencing the growth and stability of organelles, reach their sites of action by random diffusion through the cytoplasm. This supposition has never been seriously challenged. It seems especially reasonable because several structural molecules, microtubule subunits included[7], self-assemble *in vitro* to form structures identical in many respects with those developing *in vivo* when this assembly is seeded by portions of the structures in question. In addition, there are indications that, in some cells, the distribution and form of certain nucleating sites define the positioning of the microtubules which grow out from them *in vivo* by providing sites which seed tubule assembly[3,4,8]. The positioning of these nucleating sites is probably crucial to the control of the number and location of microtubules. This article is not concerned with how nucleating sites act, or how they are distributed, but with the localization of microtubule subunits, and any other molecules influencing their assembly, at the sites where assembly or dissociation of these subunits is occurring. Some of the morphogenetic events described here, which are probably paralleled in other cells, cannot be entirely accounted for by the interaction of randomly diffusing molecules with a set of nucleating sites and mature and growing microtubular organelles.

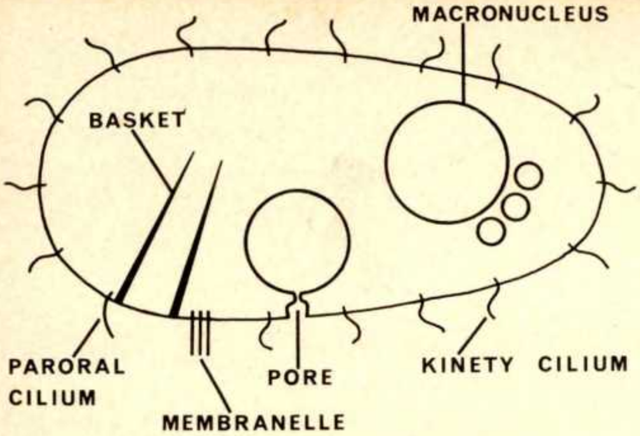


Fig. 1 Diagrammatic median longitudinal section of an interfission *Nassula*. Its anterior end is to the left and its ventral surface towards the bottom of the figure.

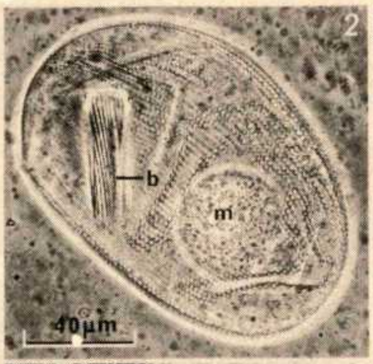
Nassula during the Cell Cycle

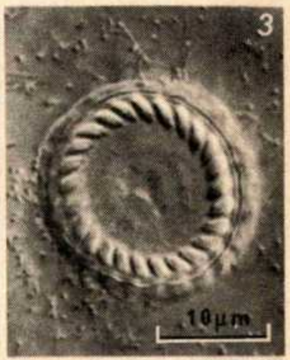
Nassula possesses a single macronucleus (Fig. 2, *m*) with three adjacent micronuclei. Most of the other large organelles are attached to the pellicle (Fig. 1). The contractile vacuole pore (Fig. 5, *c*) is a pellicular invagination connecting to the vacuole. A feeding organelle, the cytopharyngeal basket, consists largely of a circular palisade of rod-shaped microtubule bundles (Figs. 2, 3 and 4). A row of paroral cilia (*p*) flanks the oral opening (*o*) and a row of ciliary membranelles (*x*) passes just over halfway around the organism (Fig. 5). Rows of cilia called kineties extend from one end of the organism to the other; basal bodies in the same kinety are linked by bands of microtubules.

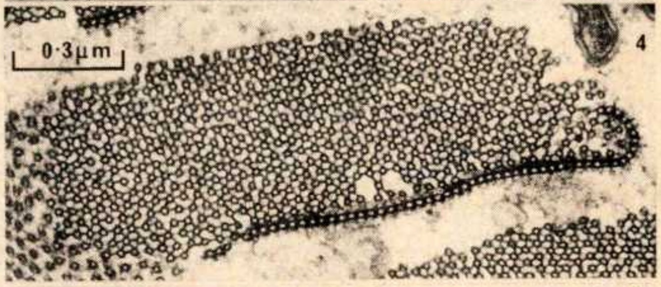
Many of the changes in microtubule organization during binary fission, summarized here with the aid of Fig. 6, occur simultaneously. At 20° C all take place within 2 h of the start of fission, except the growth of new baskets, which continues for another 2.5 h. The old basket (1) detaches from the pellicle and two new baskets (2) develop. In each region where a new basket develops, a set of new rods initially forms a series of short rows, which align to form a single long row, which then rolls up to form a circular palisade.

Spindle tubules form and break down inside the macronucleus (3) and three micronuclei (4) while nuclear membranes remain intact. Morphogenesis of all the other microtubular organelles considered here occurs in a common continuously streaming cytoplasm. No membranes partition this cytoplasm to prevent molecules diffusing between these organelles. While nuclei divide, three types of tubule arrays develop just before, and break down shortly after, they are spatially associated with changes in cell shape or movement of cytoplasmic structures. A reticulum of microtubules (5) interconnects growing cytopharyngeal rods as they roll up to form two new baskets (2). Simultaneously microtubular strands (6) contact contractile vacuole pores as pores move posteriorly for about 30 µm, while a microtubular girdle (7) forms at the base of the cleavage furrow. Also, new microtubules are augmenting the bands (8) to link permanently new and old kinety basal bodies.

If tubule subunits in each type of array differ from those in other arrays, assembly of appropriate amounts of each species of subunit at the sites, where only they can assemble, may be largely determined by the amounts synthesized. Even if subunits are synthesized more or less randomly throughout the cytoplasm, approximately equal quantities of a subunit species will by random diffusion encounter each appropriate assembly region. But if the tubules in these several arrays are of identical composition, control of the distribution of subunits to assembly sites must be more refined; at least as refined as that which controls the morphogenesis of cilia, basal bodies and cytopharyngeal microtubules. There are important instances involving these structures where initially identical tubules develop in one cytoplasmic region and simultaneously break down in another. For example, old cytopharyngeal baskets (1) gradually break down for 1 h while new ones (2) develop. Even some new cytopharyngeal rods (9) break down while the remainder continue lengthening; these are errant rods which have not been included in a new circular palisade during rolling up. Neither old baskets nor errant rods are enclosed in vacuoles while they break down. Paroral cilia and their basal bodies (10) develop about 1 h before any of the others, while only the cilia of the old membranelles (11) break down and the old kinety cilia (12) remain intact. Subsequently new membranelles (13) and kinety cilia (14), and new cilia forming on old membranelle basal bodies (11), all develop at about the same time. It is difficult to account for these events if it is assumed that tubule







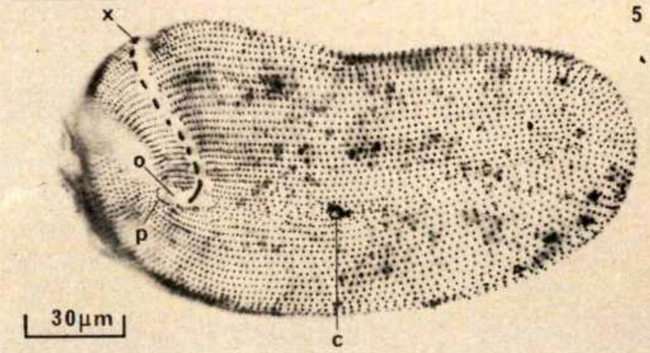


Fig. 2 An interfission *Nassula* after cold ethanol digitonin treatment[9] which dissolves most cytoplasmic components and leaves a pellicular ghost enclosing the cytopharyngeal basket (*b*) and the macronucleus (*m*). The top of the basket, which is attached to the pellicle, is towards the top of the figure. Phase contrast.

Fig. 3 The circular palisade of rods in a living *Nassula* at the top of a cytopharyngeal basket, viewed from above. Numerous cilia are also shown. Nomarski interference contrast.

Fig. 4 A microtubular cytopharyngeal rod in cross-section. Electron micrograph.

Fig. 5 The ventral surface of an interfission *Nassula* with its anterior end to the left and its left side towards the top of the figure. The pellicle is stained with silver[10] around the rim of the contractile vacuole pore (*c*) and where it is penetrated by cilia. A row of paroral cilia (*p*) flanks the oral opening (*o*). A row of ciliary membranelles (*x*) and numerous rows of kinety cilia are also shown. Bright field.

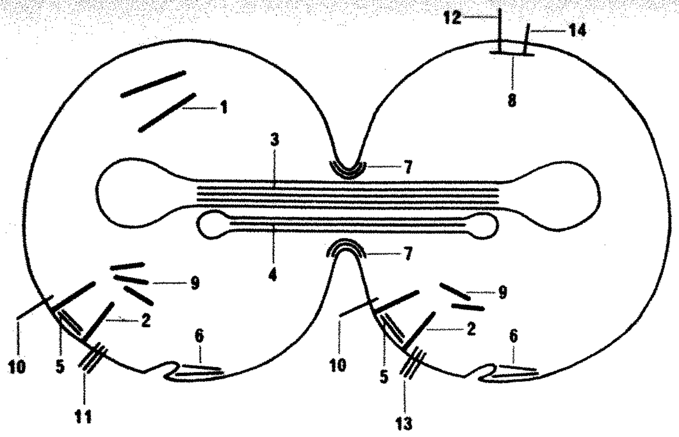


Fig. 6 Schematic diagram of a median longitudinal section of a dividing *Nassula* showing the positions of the majority of its microtubular organelles. The organism is oriented as in Fig. 1. For clarity contractile vacuoles have been omitted, and only two kinety cilia and one of the three micronuclei have been included. The lines indicating the positions of microtubular arrays and organelles also show the orientation of the longitudinal axes of the tubules but provide no indication of the numbers of tubules involved. Old cytopharyngeal basket, (1); new cytopharyngeal basket, (2); macronuclear spindle, (3); micronuclear spindle, (4); microtubular reticulum associated with rolling up of new cytopharyngeal rods, (5); microtubular strand associated with posteriorly directed movement of contractile vacuole pore, (6); microtubular girdle at the base of the cleavage furrow, (7); microtubular band linking kinety basal bodies, (8); errant rods, (9); paroral cilium, (10); old ciliary membranelle, (11); old kinety cilium, (12); new membranelle, (13); new kinety cilium, (14).

subunits are synthesized more or less randomly throughout the cytoplasm, and that they, and all agents which affect tubule stability, reach tubules and tubule nucleation sites by simple diffusion through the cytoplasm. There must be special ways of concentrating these materials in discrete cytoplasmic regions without partitioning these regions off from the remainder of the cytoplasm by membranes. Analyses of cytopharyngeal morphogenesis, now being undertaken, suggest ways in which such concentrations may be achieved.

Cytopharyngeal Microtubules

When dividing organisms are immersed in aqueous solutions of colchicine (5 mg/ml.), assembly of cytopharyngeal tubule subunits is completely inhibited. Tubules subsequently attain normal lengths after assembly has been delayed for 1 h if organisms are returned to their normal culture medium (water). Immersion in aqueous solutions of cycloheximide (5 μg/ml.) greatly reduces the rate of subunit assembly, and when assembly ceases the tubules are much shorter than is normally the case. In some experiments assembly was delayed by using colchicine for 1 h just after assembly had started, and then colchicine was leached out of the organisms while simultaneously cycloheximide was applied. Tubules finally attained the same lengths as they did in organisms in which subunit assembly had proceeded normally for the first hour of development before cycloheximide was administered. Thus there is an increase in the amount of tubule subunit assembly occurring in the presence of cycloheximide following a 1 h delay of assembly induced by colchicine. Hence cycloheximide probably does not interfere directly with assembly in a marked fashion, and is almost certainly inhibiting assembly by blocking synthesis of proteins, possibly tubule subunits, which is occurring while subunits assemble. Cycloheximide acts in this manner during flagellar regeneration in *Chlamydomonas*[11]. While assembly is most sensitive to cycloheximide in *Nassula*, ribosome-bearing cisternae of endoplasmic reticulum are clustered around the tips of tubules where subunit assembly occurs. Thus tubule subunits, or materials which stabilize their assembly, may be synthesized on particular ribosomes and not released until the ribosomes are very close to the tubule assembly points.

Errant rods move away from new baskets before breaking down. If organisms are immersed in aqueous solutions of vinblastine sulphate (10 μg/ml.) this movement does not occur and errant rods remain intact. The agents provoking breakdown have probably not been inactivated, for the more lengthy breakdown of the much larger old baskets still proceeds under these conditions. Thus it seems that these agents are strictly localized in particular cytoplasmic regions. To account for the spatial discrimination involved in selective breakdown of this kind there is little alternative but to postulate the operation of sophisticated procedures. For example, breakdown of errant and old rods, but retention of new baskets, could be accomplished if the agents promoting breakdown have a relatively short active life, and are only present and bound to the nuclear envelopes for about 90 min from the start of fission. Errant and old rods may contact the envelopes of the centrally positioned nuclei but new short baskets attached to the pellicle cannot do so (Fig. 6). It is also necessary to postulate that the agents are only released from nuclear envelopes when contacted by tubules, to which they attach, and then progressing along tubules cast off subunits behind them, while remaining attached to the contiguous tubule remnants until their deactivation is induced by fragmentation of the final portions of tubules. It is significant that *in vivo* cytopharyngeal tubules break down progressively from their tips[3].

Experiments to test these hypotheses are in progress. In the meantime it is worth bearing in mind the possibility that the distribution of molecules involved in organelle morphogenesis may often be extensively governed by their being bound to larger structures up to the point at which they interact with a mature or growing organelle.

Other Cells

To what extent are the events described here typical of cells generally? The large size of some of the organelles involved is certainly unusual, but the information required to specify that a bundle of about 10 microtubules about 10 μm long grows out from a particular site at a particular phase of the cell cycle need not be greatly augmented to ensure that the development of a bundle of about 1,000 tubules about 100 μm long is equally precisely controlled. It is also worth noting that very precisely controlled patterns of simultaneous microtubule formation and breakdown are known to occur in the cells of certain multicellular organisms, for example[12], and for spindles, at least, we know that control of tubule deployment is very exact.

Egg cytoplasm differentiates into regions of different composition sometimes characterized by distinct groupings of ribosomes and other organelles[13]. Patterns of egg cytoplasm differentiation are spatially related to patterns of cell differentiation in embryos. Mechanisms defining cytoplasmic patterns in eggs may be similar to those controlling microtubule arrangement in cells generally.

I thank the Science Research Council for a research grant, Miss C. T. Graff for conducting some of the experiments, and the *Journal of Cell Science* for permission to reproduce Fig. 3.

Received May 13; revised June 30, 1971.

[1] Tucker, J. B., *J. Cell Sci.*, **2**, 481 (1967).
[2] Tucker, J. B., *J. Cell Sci.*, **3**, 493 (1968).
[3] Tucker, J. B., *J. Cell Sci.*, **6**, 385 (1970).
[4] Tucker, J. B., *J. Cell Sci.*, **7**, 793 (1970).
[5] Tucker, J. B., *J. Cell Sci.*, **8**, 557 (1971).
[6] Tucker, J. B., *J. Cell Sci.* (in the press).
[7] Stephens, R. E., *Quant. Rev. Biophys.*, **1**, 377 (1969).
[8] Tilney, L. G., and Goddard, J., *J. Cell Biol.*, **46**, 564 (1970).
[9] Child, F. M., and Mazia, D., *Experientia*, **12**, 161 (1956).
[10] Chatton, E., and Lwoff, A., *CR Séanc. Soc. Biol.*, **118**, 1068 (1935).
[11] Rosenbaum, J. L., Moulder, J. E., and Ringo, D. L., *J. Cell Biol.*, **41**, 600 (1969).
[12] Ledbetter, M. C., in *Formation and Fate of Cell Organelles* (edit. by Warren, K. B.), 55 (Academic Press, London, 1967).
[13] Mahowald, A. P., and Hennen, S., *Devel. Biol.*, **24**, 37 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Atmospheric Total Ozone Increase during the 1960s

NAMIAS[1] has postulated that sea-air interactions on a regional basis, and not necessarily air pollution, volcanic activity, or solar intensity variations, may have caused the cooling trend that was observed in numerous areas of the world during the past decade[2,3]. In particular, he attributed the United States temperature anomaly, that is, lower than normal winter temperatures in the eastern two-thirds of the county, and higher than normal winter temperatures in the west, to intensification of the Rossby wave at the 700 mbar level. This intensification strengthens the ridge over western North America and deepens the trough to the east, thus increasing the frequency of deployment of Arctic air masses into the eastern United States. Namias attributed the change in the character of the Rossby wave to abnormal cyclonic activity that occurred in the winter atmospheric circulation over the North Pacific, the surface waters of which were unusually warm during the 1960s.

Because the effects of a deepened 700 mbar trough over the eastern United States might reasonably be expected to extend to higher altitudes, we considered the possibility that the increased southward flow of air in the troposphere and low stratosphere led to an average increase in total ozone amount over central and eastern North America during the 1960s. Ozone is transported poleward from the equatorial stratosphere source region in the winter hemisphere, before it descends to lower levels[4]. To determine whether an increase in total ozone occurred, we analysed data obtained during the decade 1961 to 1970 at Edmonton, Goose Bay, and Toronto, in Canada, and at Boulder, Bismarck, Green Bay, Caribou, Bedford, Nashville, and Tallahassee, in the United States. The periods of record ranged from 7 to 10 years for all stations except Tallahassee, where observations were made for only 5 yr. At eight of the ten stations, we found statistically significant increases in ozone that varied between 4.4% per decade at Goose Bay, to 8.8% per decade at Nashville, with an average increase for the eight stations of 5.6% per decade. At Green Bay and Toronto, although the calculated trends in ozone were not found to be statistically significant, they produced small positive values, 2.0% and 3.5% per decade, respectively.

To ascertain whether the trough deepening described by Namias extended into the low stratosphere, the geopotential heights of the 200, 100, and 50 mbar pressure surfaces at two stations—Caribou and Nashville—were investigated for the period January 1961 to August 1970. The results of the analysis suggest to us that trough intensification occurred at both stations, particularly during February and March and to a lesser extent in September, October, and November. During other months of the 10 year period, progressive weakening of the trough occurred.

Our total ozone data analyses also yielded statistically significant ozone increases during the 1960s at Dobson spectrophotometer stations distant from the North American continent. At Mauna Loa Observatory in Hawaii, the upward trend was 2.5% per decade, while at Huancayo, Peru, the trend was 6.0%. Subsequent analyses of data from several foreign stations (Fig. 1) confirmed that increases in ozone were not confined to the North American region, but were also characteristic of other areas of the world.

Fig. 1 shows mean monthly total ozone deviations from monthly normals, based on the periods of record indicated, for seven stations in the northern and southern hemispheres. The ozone monthly means were calculated, in most instances, from observations during 25 or more days per month; in isolated cases, however, the means were determined from as few as 10 days of observations. The ozone trend lines were fitted to the data by the least squares method. Table 1 summarizes the results.

Note that the calculated rates of ozone increase range from 2.4±0.6% per decade at Mauna Loa Observatory in Hawaii, to 10.0±0.4% per decade at Kodaikanal in India. Errors given with the trend figures are computed probable errors. In calculating the trends we have assumed that the calibration levels of the spectrophotometers, operated at the stations listed in Table 1, remained constant in time. Using the criterion that the sign of the ozone trend is significant, provided that it does not change within the range of ±3 probable errors, the

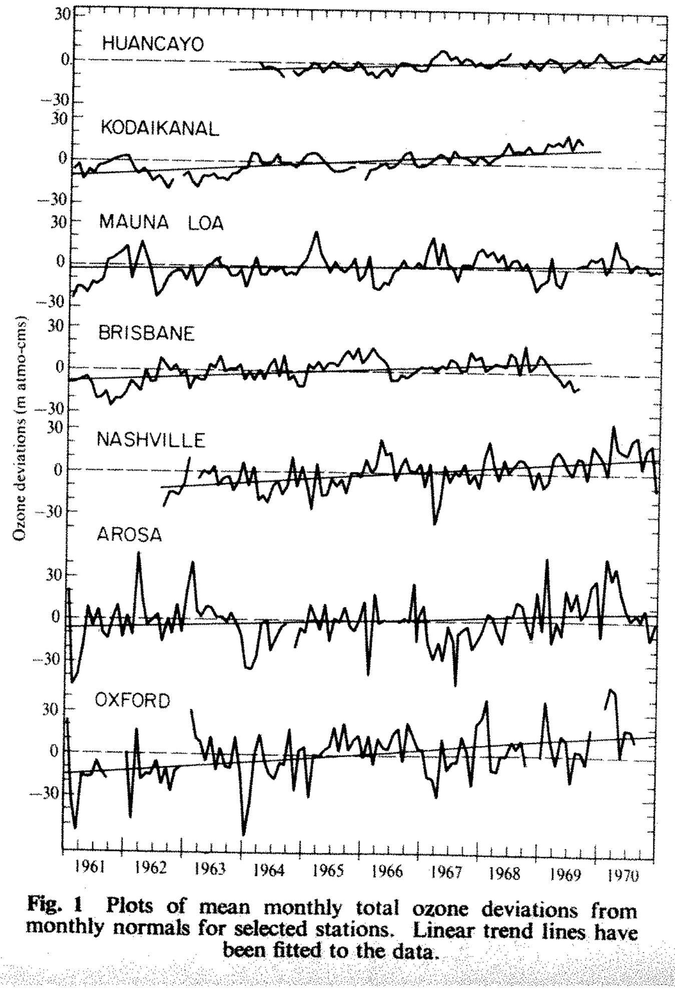


Fig. 1 Plots of mean monthly total ozone deviations from monthly normals for selected stations. Linear trend lines have been fitted to the data.

data clearly indicate that statistically significant increases in total ozone amount were detected at the stations listed in Table 1.

Table 1 Summary of the Data Analyses presented in Fig. 1

Station	Lat., long.	Mean O_3 in m atmo-cms†	% O_3 increase per decade*
Huancayo, Peru	12° 03′ S, 75° 19′ W	262	6.0 ± 0.4
Kodaikanal, India	10° 14′ N, 77° 28′ E	257	10.0 ± 0.4
Mauna Loa, Hawaii	19° 32′ N, 155° 35′ W	276	2.4 ± 0.6
Brisbane, Australia	27° 28′ S, 153° 02′ E	289	6.6 ± 0.6
Nashville, USA	36° 15′ N, 86° 34′ W	332	8.8 ± 0.7
Arosa, Switzerland	46° 46′ N, 09° 40′ E	333	4.1 ± 1.0
Oxford, England	51° 45′ N, 01° 11′ W	354	8.9 ± 1.0

* Extrapolated from the periods of record shown in Fig. 1.
† The unit "m atmo-cms" or "milli-atmo-cms" is used to denote the total amount of ozone in a vertical column of the atmosphere, expressed as the thickness of the ozone layer at STP, assuming that the ozone is separated from the rest of the air.

Although additional data analyses yielded increases in ozone at most stations investigated, the results were not always statistically significant. For example, a small but statistically insignificant increase in ozone of $1.1 \pm 0.9\%$ per decade was calculated for Aspendale, Australia (38° 02′ S, 145° 06′ E). We also analysed data obtained at Tateno in Japan (36° 03′ N, 140° 08′ E), and Vladivostok in Siberia (45° 07′ N, 131° 54′ E). Both these stations lie near a region that, according to Namias, experienced a deepening of the 700 mbar trough during the past decade similar to the trough intensification that occurred over eastern North America. Although a positive trend in total ozone of $6.2 \pm 3.3\%$ per decade was computed for Vladivostok, we cannot classify this as significant under the criterion that it did not change sign within the range of ± 3 probable errors. For Tateno, on the other hand, a downward trend in ozone was calculated, also statistically insignificant, and of magnitude $-3.4 \pm 1.3\%$ per decade.

The tentative linear analyses of the North American total ozone data, as well as the analyses of the data from foreign stations shown in Fig. 1, suggest that an increase in total ozone occurred in many areas of the world during the past decade. This increase cannot be interpreted as an apparent ozone increase that has arisen because of errors in measurement associated with changing atmospheric turbidity. The fundamental method of measuring ozone with Dobson ozone spectrophotometers[5], using wavelength pairs A(3055 Å, 3254 Å) and D(3176 Å, 3398 Å), is highly insensitive to scattering of solar radiation by particulate matter because, through the subtraction of two nearly equal terms, the scattering term that appears in the equation from which total ozone amounts are calculated is rendered negligibly small. The possibility that the computed rates of ozone increase are due to long term drift in spectrophotometer calibrations should, however, not be discounted. We recently compared the calibration level of our Caribou spectrophotometer with that of a standard spectrophotometer maintained at Boulder, Colorado, and found a drift in the calibration of the Caribou instrument. The magnitude and direction of the drift are such as to account for about one-half of the 5.9% calculated rate of ozone increase per decade at Caribou. In June 1970, on the other hand, the Nashville spectrophotometer was brought to Boulder and compared with our standard instrument. The intercomparison tests showed that the calibration level of the Nashville spectrophotometer did not change by more than 2% during 8 years of operation. Thus, the deduced 8.8% per decade increase in ozone at Nashville cannot be attributed, in large measure, to drift in instrument calibration.

A more comprehensive analysis than that presented here is needed to determine whether a global increase in atmospheric total ozone occurred during the past decade, or whether ozone increases in certain areas of the world were compensated by decreases in other areas. Such an analysis should not only involve data from the worldwide network of total ozone observatories, but should also take into account the calibration histories of the instruments with which observations were made. We are currently recalibrating our Dobson ozone spectrophotometers in an effort to determine the influence of drift in instrument calibrations on the accuracy of the North American ozone measurements.

The Huancayo station is operated in cooperation with the Instituto Geofisico del Peru.

W. D. Komhyr
E. W. Barrett
G. Slocum
H. K. Weickmann

Air Resources Laboratory,
Boulder,
Colorado 80302

Received May 10, 1971.

[1] Namias, J., *Science*, **170**, 741 (1970).
[2] Mitchell, jun., J. M., in *Global Effects of Environmental Pollution* (edit. by Singer, S. F.) (Springer, New York, 1970).
[3] Wahl, E. W., and Lawson, T. L., *Mon. Weather Rev.*, **98**, 259 (1970).
[4] Dütsch, H. U., in *World Survey of Climatology*, **4** (edit. by Landsberg, H. E.) (Elsevier, Amsterdam, 1969).
[5] Dobson, G. M. B., and Normand, C. W. B., in *Annals of the International Geophysical Year*, **16**, 161 (Pergamon, Oxford, 1962).

Do Neutron Stars have an Ocean Surface?

This article draws attention to one of the more important unsolved problems concerning the qualitative nature of the outer layers of a neutron star. We refer to the likelihood of there being a vapour-liquid type phase transition, involving a major density discontinuity by a factor that could be as high as three or more, between the crust (in which starquakes are thought to take place) and the neutron superfluid below. The idea that such a phase discontinuity might exist is not new[1], but the question does not seem to have been taken seriously in recent years. This is due to the absence of discontinuities not only in the highly simplified but historically important equations of state of Harrison and Wheeler[2] and of Tsuruta and Cameron[3] which played such an important role in discussions of neutron star structure in the period extending up to the mid sixties, but also in the much more sophisticated equations of state which have been published in recent years, such as those of Langer, Rosen, Cohen and Cameron[4], Bethe, Borner and Sato[5], and Wang, Rose and Schlenker[6].

We wish to make clear that the issue is still far from closed and that the existence of a major discontinuity is not only possible but even probable. The general problem of vapour-liquid type phase transitions in many fermion systems has recently been the subject of a reappraisal by Baker and Kahane[7]. They give naive but cogent reasons for expecting the existence of a discontinuous phase transition in any system of fermions where the interparticle forces are predominantly attractive at medium ranges but repulsive at short ranges: conditions which are believed to hold in a real neutron gas. This simple reasoning cannot, of course, be conclusive (the chief element of doubt being in the question of how, when the forces have a complicated anisotropic form, one defines "predominantly"), but on the other hand, according to Baker and Kahane, the accuracy and convergence properties of the Brueckner-type expansion calculations, on which most of the present day continuity-free equations of state are based, are necessarily questionable in the neighbourhood of a real discontinuity. Furthermore, the most recent calculation along traditional lines, that of Baym, Bethe and Pethick[8], predicts a first order phase transition (although their density is discontinuous by a very small fraction, quite unlike the order of magnitude change which we have in mind). What is clear is that considerably more elaborate techniques will be needed to decide conclusively whether or not neutron star matter has a large first order phase discontinuity between sub-nuclear and nuclear densities.

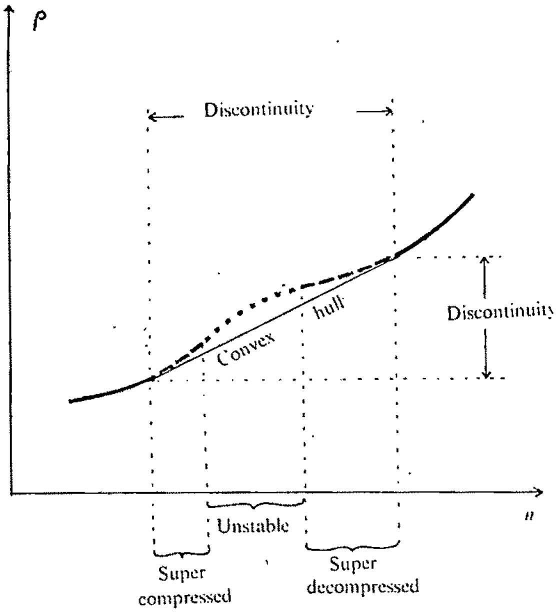


Fig. 1 Typical form of an equation of state with a first order phase transition.

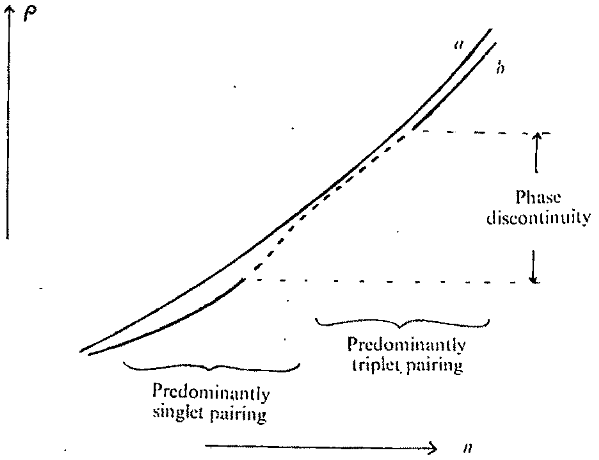


Fig. 2 Sketch illustrating (on an exaggerated scale) the qualitative form of the correction to the equation of state which can be expected to result from taking Cooper pairing energy into account. The convex curve *a* represents the uncorrected equation; the partly concave curve *b* represents the corrected equation.

It is worth emphasizing that we are not suggesting that the best present day equations of state are grossly unreliable in the sub-nuclear to nuclear density range (10^{11} to 10^{14} g cm^{-3}) as they are at higher densities where hyperons and other high energy phenomena are important. The point is that quite a small alteration in the basic equation of state—which consists of an expression for the ground state energy density ρ as a function of baryon number density *n*—can introduce or remove a large density discontinuity. To make this clear we recall the standard principle (see, for example, ref. 2) that the necessary and sufficient condition for the absence of a discontinuity is that the curve of ρ against *n* be everywhere convex downwards. States where the curve is concave are locally unstable, and states where the curve is convex, but within and not on the convex hull, are only metastable (corresponding to supercompressed or superdecompressed states). Hence no states off the convex hull can exist in a stationary situation and nearly all methods of approach predict that the ρ–*n* curve is almost straight in the density range from 10^{11} to 10^{14} g cm^{-3} so the distinction between convexity and concavity is a fine one. For example, the ρ–*n* curve of Baym, Bethe and Pethick deviates from a straight line over the range 1.3×10^{14} to 3.9×10^{14} g cm^{-3} by an amount corresponding to at most about 1.2 MeV per baryon. This means that energy corrections of this order of magnitude, which corresponds to about 10% of the total non-rest mass energy, would be sufficient to introduce a density discontinuity over a comparable range.

No one would claim that even the best present day equation of state calculations are reliable to within one MeV per baryon. Corrections of this order can come from many subtle effects such as three body interaction energies and Cooper pairing energies. Moreover, it would appear that the contribution from this latter effect is indeed of such a form as to introduce a concavity into the ρ–*n* curve, because the curve of pairing energy density against baryon number density can itself be expected to have a concavity due to the changeover from relatively strong singlet pairing at sub-nuclear densities to relatively weak triplet pairing at nuclear densities[9]. It may well be that this effect is not quite large enough, or that it is cancelled out by other fine corrections. Nevertheless it seems at least very likely that this effect really does introduce a significant phase discontinuity.

Such a discontinuity would be in many ways analogous to the terrestrial ocean surface, and as such might admit gravity waves, provided that the mechanism (such as a starquake) by which they were generated could release sufficient energy to dissolve the crystal structure at the base of the crust.

We thank James B. Hartle for useful discussions.

B. CARTER
H. QUINTANA *

Institute of Theoretical Astronomy,
Cambridge

Received May 26; revised July 5, 1971.

* On leave from Facultad de Ciencias, Universidad de Chile, Santiago.

[1] Salpeter, E. E., *Ann. Phys.*, **11**, 393 (1960).
[2] Harrison, B. K., Thorne, K. S., Wakano, M., and Wheeler, J. A., *Gravitation Theory and Gravitational Collapse* (University of Chicago Press, 1965).
[3] Tsuruta, S., and Cameron, A. G. W., *Canad. J. Phys.*, **43**, 2056 (1965).
[4] Langer, W. D., Rosen, L. C., Cohen, J. M., and Cameron, A. G. W., *Astrophys. Space Science*, **5**, 259 (1969).
[5] Bethe, H. A., Borner, G., and Sato, K., *Astron. Astrophys.*, **7**, 279 (1970).
[6] Wang, C. G., Rose, W. K., and Schlenker, S. L., *Astrophys. J. Lett.*, **160**, L17 (1970).
[7] Baker, G. A., and Kahane, J., *J. Appl. Math.*, **10**, 1647 (1969).
[8] Baym, G., Bethe, N. A., and Pethick, C. J., *Nuclear Phys.* (in the press).
[9] Hoffberg, M., Glassgold, A., Richardson, R., and Ruderman, M., *Phys. Rev. Lett.*, **25**, 775 (1970).

The Hubble Constant and X-rays from Galaxy Clusters

X-RAY measurements and limits on the Coma and Virgo clusters of galaxies have been said to limit the mass of hot gas in them to 2% of the amount required for stabilization according to the virial theorem[1]. Such a conclusion is strongly dependent on the value of the Hubble constant H_0. The linear diameter of the cluster D and the cluster mass M, estimated by the virial theorem, each vary as H_0^{-1}. Therefore, the density ρ has the dependence $\rho \sim H_0^2$. The predicted X-ray flux F from this gas is $\sim \rho M/R^2$, where R is the distance to the cluster. R varies as H_0^{-1}. We therefore have the relation

$$F \sim H_0^3 \tag{1}$$

Meekins *et al.*[1] appear to have used the value $H_0 = 95$ km s^{-1} Mpc^{-1} found by van den Bergh[2], because they took $R = 70$ Mpc. If Abell's value[3] $H_0 = 47$ is used, the predicted flux is reduced by a factor of 0.12. A reduction of H_0 to a value as low as 20 is not inconceivable. Although clumpiness increases F/M^2, the presence of helium as a mass fraction f reduces F for fixed M in the ratio $(1-\frac{1}{2}f)$.

Meekins *et al.* measured a flux of 0.03 photons cm^{-2} s^{-1} almost entirely in the range 3–17 Å, and from other wavelength channels were able to state that the gas temperature exceeds 8×10^6 K. For a flat counter response I have determined from standard bremsstrahlung formulae[4] that it would take 2.6×10^{47} g of hydrogen at this temperature, uniformly distributed in a sphere of diameter 4 Mpc at the adopted distance of 70 Mpc, to produce this flux. This is four times the mass limit given by Meekins *et al.*[1], and is in clear disagreement because they gave a diameter of 5 Mpc and the actual counter response rises as the bremsstrahlung spectrum falls. A recalculation by Dr Meekins (private communication) based on the original assumptions and actual counter efficiency has confirmed that the mass estimate is higher than originally stated and in fact is increased to 7×10^{47} g. On this basis, a value

$$H_0 = 38 \text{ km s}^{-1} \text{ Mpc}^{-1} \tag{2}$$

would suffice to give agreement with the virial theorem for the Coma cluster. Those who are sure, as I am not, that clusters of galaxies are stabilized by ionized gas may interpret the X-ray measurement of Meekins *et al.* as a measurement of H_0.

I thank the National Science Foundation for partial support.

PETER D. NOERDLINGER*
New Mexico Institute of Mining and Technology,
Socorro,
New Mexico 87801

Received June 14, 1971.

* Present address: NASA Ames Research Center, Theoretical Studies Branch, Moffett Field, California 94035.

[1] Meekins, J. F., Fritz, G., Chubb, T. A., Friedman, H., and Henry, R. C., *Nature*, **231**, 107 (1971).
[2] van den Bergh, S., *Bull. Amer. Astron. Soc.*, **2**, 223 (1970).
[3] Abell, G. O., and Eastmond, S., *Bull. Amer. Astron. Soc.*, **2**, 179 (1970). See also de Vaucouleurs, G., *Ap. J.*, **159**, 435 (1970).
[4] Allen, C. W., *Astrophysical Quantities*, second ed., 100 (The Athlone Press, London, 1962). (A factor Hz^{-1} must be supplied in the units for j_ν.)

Mercury in Water of the Tidal Thames

WE have developed a method for the determination of traces of mercury, and have applied it to the tidal reaches of the River Thames. This article reports our observations of the concentrations of mercury and its partition between dissolved forms and that adsorbed on suspended particulate matter.

There is a background concentration of about 0.02 μg/kg mercury in natural waters[1–3], and some recent results[4] showed values of 0.009–0.021 μg/l. for essentially dissolved mercury in sea and river water. In regions of mercury mineralization[5], however, up to 100 μg/kg has been reported. Similar amounts are found as a result of industrial pollution[1]. For this study we chose sampling sites ranging from river water at Teddington to seawater at Southend with intermediate stations at Woolwich and Gravesend. The effluent from the Northern Outfall Sewage Works which provides a major input into the Thames[6] was also examined. To achieve uniform sampling conditions all samples were collected by officers of the Port of London Authority on one day (May 24, 1971) 0.5 h after the afternoon high water at each station. Mercury was determined in whole and in filtered water and the mercury associated with the particulate matter found by difference.

The concentration of mercury in filtered water was low in the river water at Teddington and the seawater at Southend, and higher at Woolwich and Gravesend (Table 1). The level of dissolved mercury in the Northern Outfall effluent (0.515 μg/kg) suggests that some mercury may be held in solution as organic complexes. At all stations most of the mercury was associated with the particulate matter. In waters of high chloride concentration 96–98% of the total mercury was in this form, the proportion being lower in the river water (87%) and in the sewage effluent (82%). The concentration of mercury in the suspended particulate matter varied significantly with 1.8 mg/kg in the river water at Teddington, 9.6 mg/kg in the seawater and 4.3 and 4.9 mg/kg at Woolwich and Gravesend. The seawater may have been affected by effluent from the Southend outfall. Particulate matter in the Northern Outfall effluent contained 35 mg/kg mercury. The total concentration of mercury in the effluent (2.85 μg/kg) was about sixty times that in the river water at Teddington but is comparable with the slightly below 1 μg/l.[7] reported for the Hyperion outfall, Los Angeles. Thames estuary water is only slightly stratified[6] indicating a high degree of mixing, and the outfall is sited and operated to achieve a rapid dilution of the effluent.

Surface water was collected in a plastic container and transferred immediately to acid washed screw cap polypropylene bottles. For total mercury determination about 200 g was used. For dissolved mercury about 1 kg of water was filtered by pressure through a Whatman GFC filter, the collecting bottle

Table 1 Concentration of Mercury in Thames River Water

Miles from London Bridge	Station	Cl$^-$ (mg/l.)	Particulate matter (mg/kg)	Mercury found (μg/kg)			Calculated levels of mercury on particulate matter	
				Total	Dissolved	Difference	mg/kg	% of total
−18.5	Teddington	39	21.6	0.045	0.006	0.039	1.8	87
9	Woolwich	3,610	73.1	0.328	0.013	0.315	4.3	96
11.5	N. Outfall	142	66.0	2.85	0.515	2.34	35.5	82
27	Gravesend	10,030	89.9	0.446	0.009	0.437	4.9	98
43	Southend	16,480	8.2	0.082	0.003	0.079	9.6	96

washed free of particulate matter with distilled water, and the filtered sample replaced. (Only 200 ml. of the filtered Outfall water was used.) Both sizes of sample were weighed, made normal in sulphuric acid by adding 50% v/v H_2SO_4, and 0.05 normal in permanganate by adding 5% w/v $KMnO_4$ in water, then shaken for 1 h. This treatment dissolved elemental mercury, mercury salts, and organo-mercury compounds as Hg^{2+}. Excess permanganate and any MnO_2 formed were reduced by adding 30% H_2O_2 solution. A silver wire coil[8] freed of mercury by heating was added to each bottle and the bottles shaken overnight. Up to this stage the samples remained in their collecting bottles, thus minimizing contamination and losses of mercury. Each coil was removed from the solution, washed with water and acetone and dried in air. Optimum conditions for the recovery of mercury were determined using ^{203}Hg tracer. Mercury amalgamated with the silver was driven off by heating the coil to roughly 250° C in a silica tube. The mercury vapour was passed in a stream of nitrogen through a cell of 15 cm light path, in an atomic absorption spectrophotometer. The mercury atomic absorption peak was recorded on a strip-chart recorder and the amount of mercury present determined by comparison with the peak heights of calibration standards. Blanks of distilled deionized water and standards of Hg^{2+} added to distilled deionized water were run through the complete procedure with each set of samples. Interference by non-atomic absorption was found to be negligible during experiments using an intermediate distillation onto gold, but a deuterium arc background corrector was used in the determinations. For 1 kg samples the standard deviation of the blank was 0.0014 μg/kg and the coefficient of variation at the 0.050 μg/kg level was 1.8%. Particulate matter was determined from the increase in weight of the GFC filters used to filter the 1 kg samples after drying at 110° C. Chlorosity was determined by titration of a separate sample of water using silver nitrate.

Examination of additional samples taken at the Victoria Embankment (Cleopatra's Needle steps) at low water showed that the suspended particulate matter contained 5.0 mg/kg mercury and the deposited sediment 0.20 mg/kg mercury. Both samples were in contact with water containing 0.017 μg/kg dissolved mercury, the difference reflecting the proportions of fine particles in the two materials. Only 4% of the sediment passed through a 63 μm sieve. The level of 0.20 p.p.m. mercury is similar to values we have found in sediment samples from the outer Thames estuary, and to reported values for shales[1].

This study indicates that 82–97% of the mercury in tidal waters of the Thames is held on suspended particulate matter and that significant amounts of mercury are introduced in sewage effluent. Among aquatic organisms, filter feeders such as molluscs and tunicates are especially exposed to mercury in the particulate form, but as dissolved mercury is readily adsorbed onto particulate matter, the effects of mercury on photosynthesis and productivity of phytoplankton may be more important. Both these aspects of mercury accumulation warrant further investigation.

We thank N. M. Baker and J. A. Tetlow of the Port of London Authority for water samples.

J. DAVID SMITH
R. A. NICHOLSON
P. J. MOORE

Institute of Geological Sciences,
Geochemical Division,
64 Grays Inn Road,
London WC1

Received July 8, 1971.

[1] *US Geol. Survey Professional Paper 713* (1970).
[2] Jonasson, I. R., *Geological Survey of Canada*, Paper 70–57 (1970).
[3] Keckes, S., and Miettinen, J. K., *FAO Tech. Conf. Marine Pollution*, Rome (1970).
[4] Burton, J. D., and Leatherland, T. M., *Nature*, **231**, 440 (1971).
[5] Dall'Aglio, M., in *Origin and Distribution of the Elements* (edit. by Ahrens, L. H.), 1065 (Pergamon, Oxford, 1963).
[6] Water Pollution Research Technical Paper No. 11, *Effects of Polluting Discharges on the Thames Estuary* (HMSO, London, 1964).
[7] Klein, D. H., and Goldberg, E. D., *Env. Sci. Technol.*, **4**, 765 (1970).
[8] Hinkle, M. E., and Learned, R. E., *US Geol. Survey Professional Paper 650D*, 254 (1969).

The Oxfordshire Coalfield

THE discovery of eight coal seams of workable quality in the Geological Survey Apley Barn Borehole[1,2], sunk in 1960–61 near Witney, prompted the recent sinking of a second borehole (1970–71) near Steeple Aston, Oxfordshire. This borehole is situated between Bicester and Chipping Norton some 14 miles north-east of the Apley Barn Borehole (Fig. 1). The exact site is 825 yards (750 m) at 254° from St Peter's Church, Steeple Aston (National Grid Reference SU 4687 2586). The geophysical Bouguer anomaly gravity contour map shows that the borehole lies in the eastern part of a low density area where a thick sequence of light sedimentary rocks could be expected. It was thought possible that this anomaly could be due to a thickening of the Coal Measures sequence as a whole and that perhaps even Middle Coal Measures (with valuable coal seams) could be developed. In the event, however, this hope has not been realized, and although Upper Coal Measures were proved the borehole has shown that the low density anomaly feature must be due to a thickening of Devonian and/or older sediments.

The sequence proved in the Steeple Aston Borehole is as follows: Jurassic and Permo-Triassic rocks to 751 feet 3 inches (228.98 m), Upper Coal Measures to 2,003 feet 6 inches (610.67 m), intrusive igneous rocks to 2,545 feet 4 inches (775.82 m), Upper Devonian rocks to 3,172 feet 3 inches (966.90 m) then further intrusive rocks to a final depth of 3,199 feet 3 inches (975.13 m). Previously the Apley Barn Borehole had proved 820 feet 10 inches (250.19 m) of Jurassic and Permo-Triassic rocks, Upper Coal Measures (including eight seams totalling 27 feet of coal) to 3,968 feet 7 inches (1,209.62 m), Upper Devonian to 4,408 feet 4 inches (1,343.66 m) and Lower Devonian to a final depth of 4,941 feet 8 inches (1,506.22 m). In spite of the thinner sequence of Upper Coal Measures contained in the Steeple Aston Borehole, seven coal seams of workable quality more than 2 feet thick were encountered: (1) a 2 feet 2 inches seam base at 896 feet 6 inches, (2) a 2 feet 10 inches seam base at 925 feet 10 inches, (3) a 3 feet 5½ inches seam base at 957 feet 4½ inches, (4) a 2 feet 1½ inches seam base at 987 feet, (5) a 2 feet 8½ inches seam base at 1,479 feet 1½ inches, (6) a 7 feet seam base at 1,533 feet 5½ inches and (7) a 5 feet 5½ inches seam base at 1,997 feet 9 inches. These seams, which total 25 feet 9 inches in thickness, are being analysed in detail by the National Coal Board. A good correlation exists between the two boreholes, the measures from 751 feet 3 inches (228.98 m) to 1,130 feet 6 inches (344.28 m) in the Steeple Aston Borehole being correlated with the Burford Coal Group which occurs between 1,475 feet (449.28 m) and 1,928 feet 5 inches (587.78 m) in the Apley Barn Borehole, the measures from 1,130 feet 6 inches (344.28 m) to 1,393 feet 3 inches (424.36 m) being correlated with the Crawley Group at 1,928 feet 5 inches (587.78 m) to 2,600 feet (792.48 m) in Apley Barn, the measures from 1,393 feet 3 inches (424.36 m) to 1,533 feet 5½ inches (467.40 m) with the Witney Coal Group at 2,600 feet (792.48 m) to 2,872 feet 4 inches (875.49 m) in Apley Barn and the measures from 1,533 feet 5½ inches (467.40 m) to 2,003 feet 6 inches (610.67 m) with the Arenaceous Coal Group at 2,872 feet 4 inches (875.49 m) to 3,968 feet 7 inches (1,209.62 m) in the Apley Barn Borehole[2]. Farther afield the Burford Coal Group correlates with the Radstock Group of the Somerset Coalfield, the Witney Coal Group with part of the Farrington Group of Somerset and the Arenaceous Coal Group with the Mangotsfield Group of Somerset and the

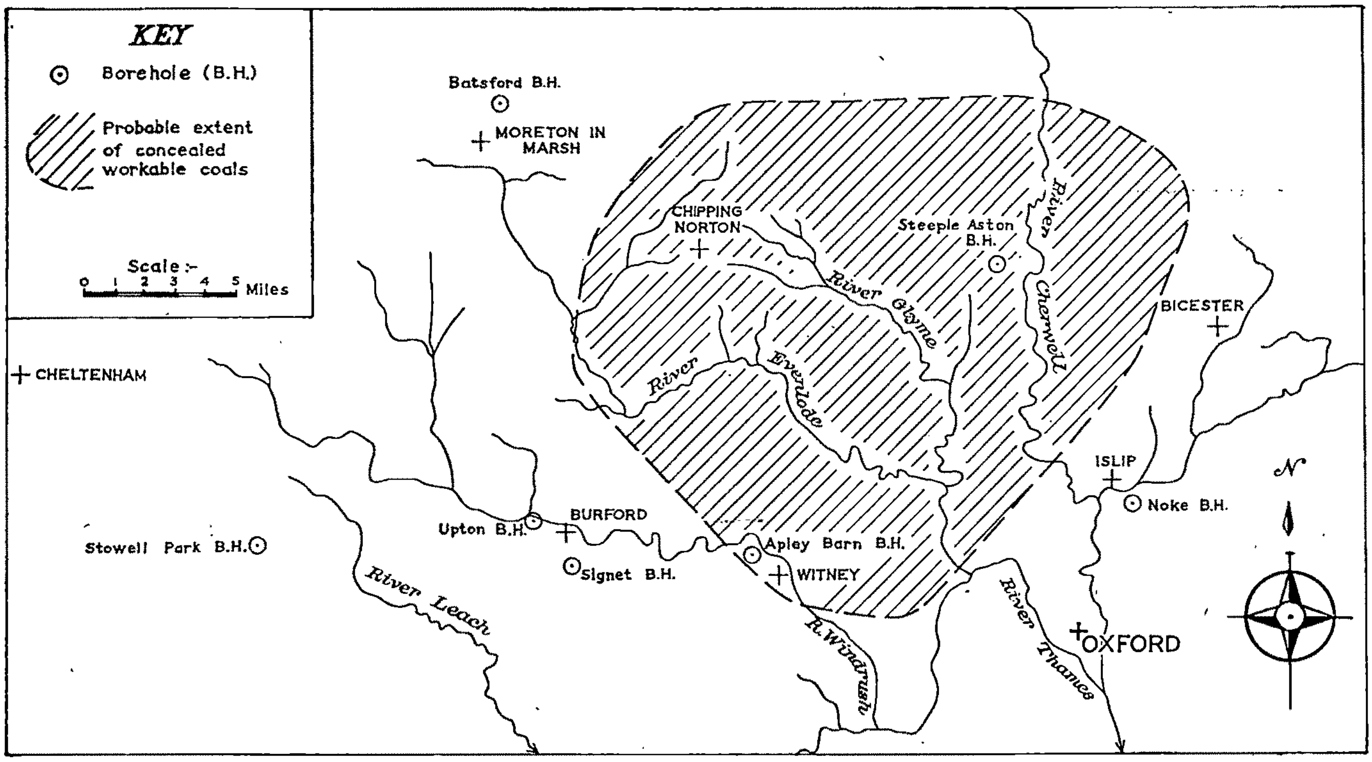


Fig. 1 Probable area of concealed workable coal in Oxfordshire.

upper part of the Pennant Group of the Forest of Dean Coalfield[2].

In the Apley Barn Borehole, the Upper Coal Measures rested unconformably on Upper Devonian sandstones, conglomerates and red and green mudstones but in the Steeple Aston Borehole a thick suite of basic intrusive rocks and agglomerates occurred beneath the Arenaceous Coal Group at 2,003 feet 6 inches (610.67 m) down to 2,545 feet 4 inches (775.82 m). Below these rocks a unique sequence of Upper Devonian marine and estuarine sediments was proved down to 3,172 feet 3 inches (966.90 m). These beds, which are yielding highly significant palynological and palaeontological results, are intruded by six thin basalt sills and the borehole was terminated in yet another basalt intrusion at 3,199 feet 3 inches (975.13 m). Natural gas was encountered in some of the sandstones of the Arenaceous Coal Group and the Devonian which was almost certainly produced by the thermal metamorphism of these carbonaceous formations. If the intrusions are of widespread lateral extent then it is possible that a small gas field may exist in the vicinity of the borehole; this would have to be proved by further drilling and pumping tests.

The probable area of concealed workable coals is shown in Fig. 1. The absolute northern limit is unknown but it almost certainly does not reach as far as Banbury, where the gravity anomaly map shows igneous or dense basement rocks at a comparatively shallow depth. Similarly it does not extend as far to the south-east as the Noke[3] Borehole (some 5 miles north-east of Oxford) where Upper Old Red Sandstone underlies Mesozoic rocks at a depth of only 380 feet (115.82 m). The north-easterly limit is very uncertain and might well extend some distance beyond Bicester. The Apley Barn and Upton[4] boreholes effectively prove the southerly and south-westerly limits of working on grounds of depth of burial. The absolute westerly limit is undetermined, but to the north-west the Batsford Borehole[5] proved 524 feet 6 inches (159.87 m) of Upper Coal Measures but no workable seams. Thus the estimated total area where workable seams may reasonably be expected to occur may be as great as 200 to 250 square miles and may contain some 6,000 million tons of coal. The apparent dip in the Upper Coal Measures in both the Apley Barn and Steeple Aston Boreholes was low (3° to 4°) and the area is probably one of simple structure. It should be emphasized, however, that the seams are thin, probably uneconomic at the present time, and that a great deal of further exploration would be necessary before a working colliery could be established in the area.

E. G. POOLE

*Institute of Geological Sciences,
London*

Received July 9, 1971.

[1] Stubblefield, C. J., and Trotter, F. M., *Nature*, **191**, 953 (1961).
[2] Poole, E. G., *Bull. Geol. Surv. Great Brit.*, **29**, 1 (1969).
[3] Falcon, N. L., and Tarrant, L. H., *Quart. J. Geol. Soc.*, **106**, 141 (1951).
[4] Worssam, B. C., *Bull. Geol. Surv. Great Brit.*, **20**, 107 (1963).
[5] Strahan, A., *Sum. Prog. Geol. Surv.*, **1912**, 90 (1913).

Formation of 2,3,7,8-Tetrachlorodibenzodioxin by Thermal Decomposition of Sodium 2,4,5,-Trichlorophenate

IN the course of investigating an explosion which occurred in the Coalite Company's 2,4,5-trichlorophenol plant in 1968, a white crystalline solid of high melting point and extremely high toxicity was isolated which proved to be 2,3,7,8-tetrachlorodibenzodioxin. The manufacture of 2,4,5-trichlorophenol involves the hydrolysis of 1,2,4,5-tetrachlorobenzene using methanol and caustic soda at elevated pressure or ethylene glycol and caustic soda at atmospheric pressure. The plant in which the accident occurred used the latter process.

Small scale experiments in which 1,2,4,5-tetrachlorobenzene hydrolysates were allowed to heat up to higher temperatures than the normal 180° C showed a significant exothermic reaction after residual ethylene glycol had distilled off. Hydrolysates of all isomers of trichlorobenzene and mixtures of caustic soda and ethylene glycol all had the exothermic reaction in common starting in the 230° C region and proceeding rapidly and uncontrollably to around 410° C. This exotherm, which we attributed to the decomposition of sodium 2-hydroxyethoxide ($NaOCH_2CH_2OH$), was invariably accompanied by a huge volume of white vapours. Full analyses of the evolved gases were never possible although the presence in trace amounts of ethylene oxide was established. In the course of one such exothermic reaction of a 1,2,4,5-tetrachlorobenzene, distillate was collected which contained the usual di, tri and higher ethylene glycols and a white microcrystalline solid, melting point 298°–300° C, of elemental analysis 43.93%, 1.88% H, 36.70% Cl with the residue oxygen; this was designated C8.

A small sample of C8 recrystallized from benzene was examined by spectroscopic and gas liquid chromatographic techniques. Mass spectrographic studies of pure C8 and an authentic sample of 2,3,7,8-tetrachlorodibenzodioxin showed them to have identical fragmentation patterns, and the former to have molecular weight 322. The nuclear magnetic resonance spectrum of C8 showed one singlet proton signal at $\tau = 3.7$; no response was observed in the aliphatic region of the spectrum; this is consistent with the symmetrical structure of 2,3,7,8-tetrachlorodibenzodioxin. Gas liquid chromatographic traces of authentic 2,3,7,8-tetrachlorodibenzodioxin and C8 run in precisely the same conditions gave single peaks of the same retention time. On this evidence it was concluded that purified C8 and 2,3,7,8-tetrachlorodibenzodioxin were identical and the elemental analysis quoted earlier which corresponded more closely to $C_{14}H_7Cl_4O_4$ than the required $C_{12}H_4Cl_4O_2$ reflected its relative lack of purity.

The high toxicity and chloracnogenic effects of 2,3,7,8-tetrachlorodibenzodioxin have been previously reported[1-6]; checks were therefore made on C8 using rabbits. Single oral doses of 1 or 10 μg/kg were given to the animals; the higher dose level killed them all, and the lower dose caused serious liver damage and chloracne. This demonstrates the exceptional toxicity of this material.

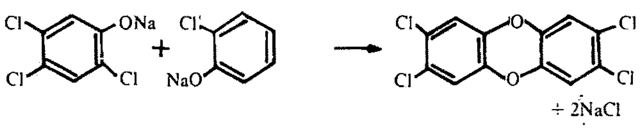


Fig. 1

The 2,3,7,8-tetrachlorodibenzodioxin (dioxin) was formed by mutual reaction of sodium 2,4,5-trichlorophenate molecules (Fig. 1) under the influence of the highly exothermic decomposition of sodium 2-hydroxyethoxide and sublimed from the reaction mixture. In preparative experiments using this method, very low yields of dioxin were isolated, chiefly because of its low tendency to sublime, but there is strong evidence of its presence in the residue after thermal decomposition. For example 218 g hydrolysate (containing 91 g of sodium 2,4,5-trichlorophenate) with a dioxin content of 17 p.p.m. was heated at 230°–260° C for 2 h, and gave a sublimate of only 6.4 mg dioxin, but the residue had a dioxin content of 1,600 p.p.m. Heating 1,2,4,5-tetrachlorobenzene hydrolysates at 180° C (normal reaction temperature) even for prolonged times gave only minimal dioxin contents.

Experiments on the thermal decomposition of hydrolysate of 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzenes (which collectively contained all six isomers of dichlorophenols), in identical conditions to those which produced C8, while undergoing exothermic reactions gave no visible or gas liquid chromatographic evidence of lower chlorinated dioxins. Distillates and residues formed in these thermal decompositions when tested for toxicity and chloracne on rabbits showed no harmful effects at all at doses as high as 1 g/kg. It is reasonable to conclude that in these conditions no lower chlorodioxins are formed.

A practical outcome of the isolation of the highly toxic dioxin was the development of a gas liquid chromatographic technique of analysis and monitoring of dioxin at all stages of 2,4,5-trichlorophenol manufacture. A revised reaction scheme minimizing the dioxin content of the product was worked out for a new 2,4,5-trichlorophenol plant which was commissioned in late 1969, and the safest possible method devised to dispose of dioxin contaminated residues. Currently produced 2,4,5-trichlorophenol contains < 1 p.p.m. dioxin.

We thank Mr R. Davies of the Huntingdon Research Centre for investigations on the rabbits.

M. H. MILNES

Coalite and Chemical Products Ltd,
Post Office Box No. 21,
Chesterfield,
Derbyshire

Received June 19; revised September 4, 1970.

[1] Bauer, H., Schultz, K. H., and Speigelberg, U., *Arch. Gowerbepath. Gowerbehyg.*, **18**, 538 (1961).
[2] Farquharson, M. D., Gage, J. C., and Northover, J., *Brit. J. Pharmacol. Chemother.*, **13**, 20 (1957).
[3] Hofmann, H. Th., *Arch. Exp. Pathol. Pharmakol.*, **232**, 228 (1957).
[4] Jones, E. L., and Krizek, H., *J. Invest. Derm.*, **39**, 511 (1962).
[5] Kimmig, J., and Schultz, K. H., *Dermatologica*, **115**, 540 (1957).
[6] Higginbotham, G. K., Huang, A., Firestone, D., Verrett, J., Ress, J., and Campbell, A. D., *Nature*, **220**, 702 (1968).

BIOLOGICAL SCIENCES

Identification of Primate Sexual Pheromones and the Properties of Synthetic Attractants

OLFACTORY communication is now known to be important in determining the interaction between the sexes in a microsmatic higher primate, namely, the rhesus monkey[1,2]. Vaginal secretions of ovariectomized, oestrogen-treated female rhesus monkeys contain "copulins" that stimulate the mounting activity and ejaculatory behaviour of male rhesus monkeys[3]. The behavioural effects of these secretions were blocked in males made temporarily anosmic, but became apparent when olfactory acuity had been restored; they thus seem to possess the properties of olfactorily acting pheromones. We have therefore investigated the chemical nature of these sex attractants.

Behaviourally active constituents of vaginal secretions can be extracted into ether[4]. A solution prepared by washing ether extracts with N/100 sodium hydroxide, acidifying the alkaline layer and re-extracting into ether, retained behavioural activity: results which suggest that these active constituents were acidic. We report here the further fractionation of vaginal secretions by chromatography on the anion-exchanger, diethylaminoethyl (DEAE)-cellulose, and on the identification of behaviourally active constituents by gas chromatography and mass spectrometry. To confirm our results, we made a synthetic mixture of authentic compounds and tested it on ovariectomized rhesus monkeys for its capacity to attract and stimulate males.

Procedures for testing the behavioural activity of samples have been described previously[3]. Essentially, tests consisted of applying a sample directly to the sexual skin of ovariectomized, untreated female rhesus monkeys. These had been ovariectomized at least 6 months before and were unreceptive to males which generally showed little sexual activity with such females. Samples were applied to these females immediately before behavioural tests with active males, each test lasting 1 h, during which animals were observed from behind one way vision mirrors. Sixty-six tests were conducted with four pairs of animals and changes in the interactions of the pair were measured and filmed using behavioural criteria described before[5,6]. Particular attention is given here to changes in the numbers of attempts at mounting of females by males and to the changes occurring in the numbers of ejaculations. Two further ovariectomized rhesus monkeys, each receiving 10 μg oestradiol monobenzoate subcutaneously daily, were used to provide a source of secretions: these were collected twice daily by lavage with a vaginal pipette using 1 ml. distilled water and were immediately extracted into 2 ml. of diethyl ether. A pool of forty extracts was washed with 5 ml. N/100 sodium hydroxide, titrated to *p*H 7.4 and eluted through a column of DEAE-cellulose (Whatman DE-52) with 50 mM (*p*H 7.4) sodium phosphate buffer. The eluate was automatically collected into fractions which were then combined according to their elution volume relative to that of a phenolic component, detected by continuous monitoring for ultraviolet absorbance at 280 nm (Fig. 1), and now identified as *p*-hydroxyphenylpropionic acid (HPPA). Fractions were acidified, extracted into ether, and tested for behavioural activity. That corresponding to 30–50% of the elution volume of HPPA was found to be highly active (Fig. 2*A*). The number of attempts at mounting females made by the two males increased from six during twelve pre-treatment tests, when ether was applied for control purposes, to fifty-two during twelve treatment tests when the fraction was applied (χ^2 test, $P < 0.01$): there were no ejaculations during the pre-treatment period compared with twelve during treatment (χ^2 test, $P < 0.001$).

Ether extracts of the behaviourally active 30–50% fraction were further examined by analytical gas chromatography using 'Carbowax 20M'-terephthalic acid columns in Pye 104 and Perkin–Elmer F 11 gas chromatographs. The retention times of the five most volatile components, 86, 131, 152, 203 and 251 U, were compared with those of the following acids: acetic (87 U), propionic (131 U), *iso*-butyric (152 U), *n*-butyric (203 U), *iso*-valeric (251 U). A synthetic mixture of these acids co-injected with an equal volume of the natural extract showed no separation between the two mixtures. Confirmation of the identity of the five components was obtained by preparative gas chromatography and mass spectrometry. Five aliquots (2 μl. each) of the active ether extract were separately injected onto the same column fitted with a preparative attachment (10/1 effluent split). The effluent corresponding to each of the five peaks was collected individually in melting point U-tubes at −70° C: these were cut and installed in the direct insertion probe of an AEI-MS 9 mass spectrometer. The resultant mass spectra were compared with those of authentic samples obtained in the same way to avoid differences resulting from the presence of solvent or column impurities. These spectra established the identification of the five volatile constituents of the active fraction.

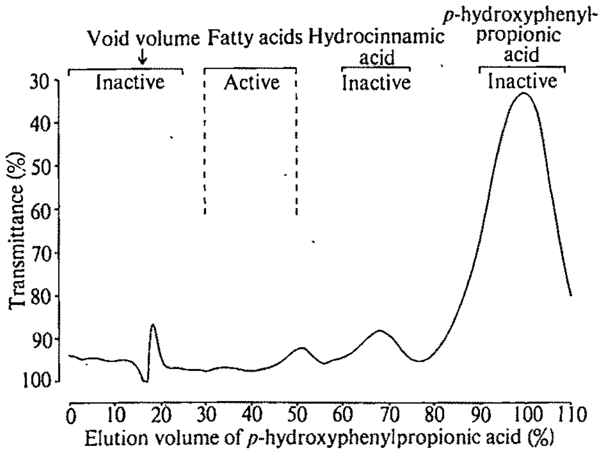


Fig. 1 Elution profile at 280 nm on DEAE-cellulose of alkaline re-extracts of vaginal secretions from ovariectomized, oestrogen-treated rhesus monkeys. The fractions were tested for behavioural activity; that containing fatty acids was found to be active, and was subjected to further analysis by gas chromatography and mass spectrometry.

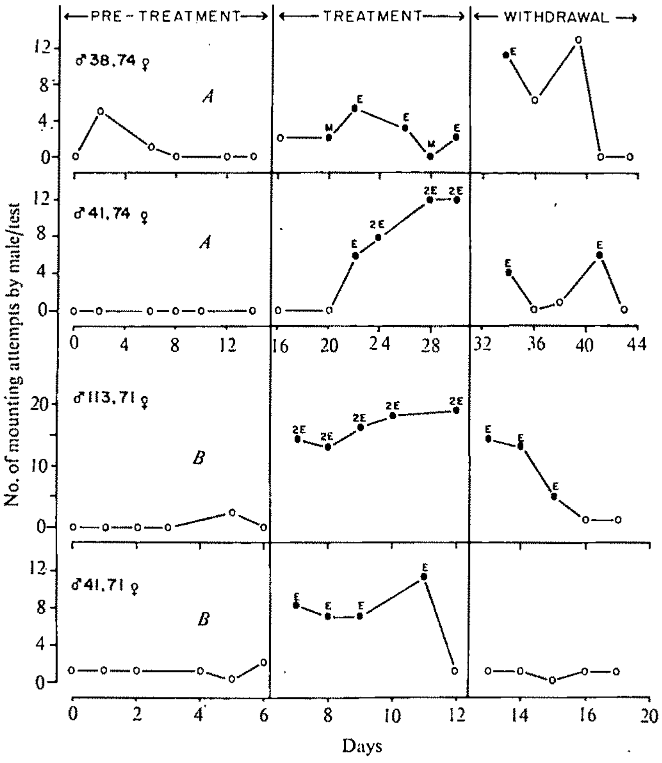


Fig. 2 Sexual stimulation of male rhesus monkeys by: *A*, the 30–50% fraction of vaginal secretions separated on a DEAE-cellulose column as indicated in Fig. 1; *B*, a synthetic mixture of the five short-chain fatty acids specified in the text.

To ascertain if the five aliphatic acids in the behaviourally active fraction were responsible for its sex attractant properties, a mixture of authentic acids was made up to match the concentrations present in a pool of twenty-four washings collected from three ovariectomized females treated with oestrogen. The synthetic mixture contained 9.2 μg acetic, 8.8 μg propionic, 4.2 μg *iso*-butyric, 12.8 μg *n*-butyric and 8.3 μg *iso*-valeric acids/ml. of ether. A 2 ml. sample of this mixture, containing about 1.5 times the acid content of a single vaginal washing, was applied to the sexual skin of each of two ovariectomized females and tested for behavioural activity (Fig. 2*B*). The number of mounting attempts made by the two males increased from eight during twelve pre-treatment tests, when ether was applied for control purposes, to 114 during ten tests when applications of the synthetic mixture were made (*t* test, $P < 0.001$): there were no ejaculations during the pre-treatment period compared with fourteen during treatment χ^2 test, $P < 0.001$). The persistence of mounting and ejaculation in some tests after withdrawing treatment was attributed to the influence of a recently rewarding sexual experience which exerted a reinforcing effect on subsequent behaviour[4].

The identification of these volatile aliphatic acids as naturally occurring constituents of vaginal secretions in the rhesus monkey, and the demonstration that a synthetic mixture of authentic acids possesses equally powerful sex attractant properties, together provide strong evidence that the attractant properties of oestrogen-stimulated vaginal secretions depend, at least in part, on their acid content. This view is strengthened by the absence of acids, or their presence in very low concentrations, in the secretions of ovariectomized, untreated females[7]. We have already established that the behavioural effects of the unfractionated, parent sections are mediated by olfaction[1], so we assume that fractions of these secretions act similarly. These findings identify and chemically characterize male sex attractants produced by a female primate, but this in no way precludes the existence of other unidentified substances with related effects. Their production by other than vaginal sources —for example, the glands of the skin—is currently being investigated.

This work was supported by grants from the Foundations' Fund for Research in Psychiatry and the Population Council.

R. F. CURTIS
J. A. BALLANTINE

Department of Chemistry,
University College of Swansea,
Singleton Park,
Swansea

E. B. KEVERNE
R. W. BONSALL
RICHARD P. MICHAEL*

Primate Behaviour Research Laboratories,
Institute of Psychiatry,
Bethlem Royal Hospital,
Monks Orchard Road,
Beckenham,
Kent

Received May 4, 1971.

*Reprint requests to R.P.M.

[1] Michael, R. P., and Keverne, E. B., *Nature*, **218**, 746 (1968).
[2] Michael, R. P., in *Recent Advances in Primatology*, 1 (edit. by Carpenter, C. R.) (S. Karger, Basel, 1969).
[3] Michael, R. P., and Keverne, E. B., *Nature*, **225**, 84 (1970).
[4] Keverne, E. B., and Michael, R. P., *J. Endocrinol* (in the press).
[5] Michael, R. P., and Saayman, G. S., *J. Endocrinol.*, **41**, 231 (1968).
[6] Michael, R. P., and Welegalla, J., *J. Endocrinol.*, **41**, 407 (1968).
[7] Michael, R. P., Keverne, E. B., and Bonsall, R. W., *Science*, **172**, 964 (1971).

p-Chlorophenylalanine and Copulatory Behaviour in the Male Rat

EVIDENCE has accumulated that copulatory behaviour in the female and male rat is inhibited by increased central nervous serotoninergic tone[1-4]. One approach to the study of the specificity of the antagonistic relationship between serotonin and copulatory behaviour has been selectively to increase serotonin by the combination of a monoamine oxidase inhibitor and the serotonin precursor 5-hydroxytryptophane (5-HTP). B. J. M. has shown that this treatment inhibits the copulatory response (lordosis reflex) in the female rat[4] and we have also shown[3] it to decrease the number of male rats mounting oestrous females. Analogous experiments with the catecholamine precursor dihydroxyphenylalanine (DOPA) revealed no inhibitory effect of increased catecholamine concentrations. According to the studies of Shillito[5,6] and Tagliamonte *et al.*[7], a selective decrease of serotonin by the serotonin synthesis inhibitor *p*-chlorophenylalanine[8] augmented sexual and social interaction between male rats, and in our work this agent also facilitated sexual receptivity in oestrogen-treated ovariectomized females[9]. Increased copulatory behaviour after *p*-chlorophenylalanine treatment has recently been shown in the male and female cat[10,11], although contradictory results are also available[12-14]. The purpose of the experiments described here was to study the effect of *p*-chlorophenylalanine on heterosexual mounting behaviour in castrated males, which had been given a dose of testosterone adjusted to maintain a submaximal mounting response.

The subjects were 140 male Wistar rats (350–420 g, specific pathogen free, Möllegaard, Ejby, Denmark) castrated as adults and with proven sexual experience before castration. Copulatory tests took place during the dark period of a reversed day–night cycle (11 h dimmed light, 13 h bright light). The male was allowed 5 min of adaptation in the test cage before a receptive female (spayed female treated with 50 μg of oestradiol benzoate 53 h before the test and 1 mg of progesterone 5 h before the test) was introduced. The time allowed from the introduction of the female to the first mount was restricted to 3 min. If the male mounted within 3 min the test continued for another 3 min or until ejaculation had occurred. In this account we are concerned chiefly with the readiness of the males to mount. Tests were carried out regularly once a week throughout the investigation.

Three to five weeks after castration the number of animals which mounted the receptive female had decreased to about 50%. After less than 50% of a batch of animals had mounted the female in two consecutive weekly tests, testosterone propionate was given to the whole of the batch. Treatment was once a week and the animals were tested 3 or 4 days after the hormone treatment. The dose (0.14–0.20 mg kg^{-1} given subcutaneously) was adjusted to give a submaximal response as to the number of males with mounting behaviour within the 3 min observation period. After the response rates at the weekly tests had become stable, the main experiment began.

The experimental group was given a neuropharmacological agent and a control group received a blank solution: this was our "experimental test". As a comparison, the last no-drug test—the "pre-experimental test"—was used.

DL-*p*-Chlorophenylalanine methylester HCl (PCPA), 100 mg kg^{-1}, was given intraperitoneally for 4 days and the animals were tested 6 h after the last injection. There was a significant increase in the number of animals which mounted the female and also the number of mounts performed each minute (Table 1*A*). By giving more testosterone, the response rate was increased to about 70–80% of males mounting, and the stimulatory effect of PCPA on the readiness to mount a receptive female was no longer obvious (Table 2). If the subject was retested after 0.5–1 h, however, this time with a castrated male introduced instead of a receptive female, there was a significant increase in mounting behaviour compared with animals not treated with PCPA. Previously[13] no stimulatory effect was seen on the heterosexual copulatory behaviour of intact vigorous males after PCPA treatment, but rather an effect on the mounting of intact males by intact males[5-7]. Our data indicate that the stimulatory effect of PCPA on male copulatory behaviour is only visible if the males are on a testosterone treatment which is submaximal as to activation of mounting behaviour. The fact that it was still possible to detect an effect when a male instead of a female was used as mounting partner might be a result of the fact that the male was less of an incentive as a mounting object.

To test whether the augmented mounting response could be attributable to a non-specific increase in exploratory behaviour, locomotor activity was measured by an 'Animex' activity meter (Farad Electronics, Hägersten, Stockholm). Movement of the animals across a tuned oscillator coil system results in change of the tuning which is recorded as a count. One subject at a time was placed in the apparatus. Each recording lasted 10 min and was carried out 0.5–1 h after the copulation test (Table 1*A*, 1–2). PCPA treatment decreased spontaneous locomotor activity. To rule out the possibility that this result was caused by fatigue after the test, another batch of six plus six animals was tested in the 'Animex' after the same drug treatment but without a copulation test and at the time at which the copulation test would have been performed. The result was very similar (Table 1*A*, 3–4). Thus, PCPA increased the number of males mounting and the number of mounts per minute, although the animals became sluggish in the locomotor test. This emphasizes the specificity of the augmenting effect of PCPA on the readiness of the male to mount a receptive female.

The effect of PCPA on copulatory behaviour was also investigated in twenty-six adrenalectomized males. All received

0.9% NaCl in their water bottles and once a week dexamethasone, 0.5 mg per animal, was given subcutaneously. Experimental animals were given PCPA intraperitoneally, 100 mg kg^{-1} on the first day and 50 mg kg^{-1} each day for another 3 consecutive days, and they were tested 6 h after the last injection. Compared with the pre-experimental test values and the saline-treated controls there was an obvious increase in the mounting performance of subjects treated with PCPA (Table 1*B*). This stimulatory effect on the readiness to mount a receptive female in castrated and adrenalectomized males rules out the possibility that PCPA increases the mounting rate by stimulating endogenous testosterone production. The number of mounts per minute (pre-experimental test) was less than in rats not adrenalectomized, probably because in spite of the dexamethasone treatment the animals became less alert after adrenalectomy. The limited number of subjects (three) which mounted both in the pre-experimental and experimental test makes it difficult to say whether there is a true increase in mounts per minute.

It has been demonstrated[3] that the general increase of monoamine concentrations achieved by the monoamine oxidase inhibitor pargyline (40 mg kg^{-1}, given intraperitoneally) significantly inhibited mounting behaviour in males. This is confirmed in this study (Table 3*A*). We investigated the possible prevention of this inhibition by decreased biosynthesis of serotonin caused by PCPA. When the animals were pretreated with PCPA, 4×100 mg kg^{-1}, given intraperitoneally, the inhibitory effect of pargyline on the mounting behaviour was not obtained (Table 3*B*) (see *Note added in proof*). A comparison of the data in the experimental test with the results of the pre-experimental trial reveals that there was still an evident increase in the percentage of animals mounting. The increase in mounting was about the same as if pargyline had not been given (compare Table 1*A*). In contrast to animals treated only with PCPA, however, there was no significant change in the number of mounts per minute. The inhibitory effect of PCPA on serotonin biosynthesis (tryptophane hydroxylase inhibition) does not involve the step from the precursor amino-acid 5-HTP to serotonin. Thus, by treating with 5-HTP it is possible to increase serotonin concentrations although PCPA has been given. DL-5-HTP was injected 30 min before the mating test in an otherwise analogous PCPA plus pargyline experiment, which gave a clearcut inhibitory effect on the copulatory behaviour (Table 3*B*, 2). Overt behaviour was no more affected in these animals than in those which had PCPA alone.

These data suggest that PCPA in the male rat increases the hormone-activated copulatory response. This effect is probably related to the ability of PCPA to decrease the serotoninergic tone, although the possibility that PCPA interacts with further biochemical events which would contribute to its effect on copulatory behaviour cannot be excluded. In the female rat, the results of similar experiments led to the hypothesis that there are serotoninergic pathways mediating inhibition of copulatory behaviour[1,2,4,9,15]. It has been suggested that decreased serotoninergic activity would remove this inhibition and thereby enhance the hormone-activated copulatory response. This hypothesis can be extended to male copulatory behaviour.

One may ask whether this inhibitory system is restricted to the sex-specific copulatory behaviour pattern or if female behaviour in the male and male behaviour in the female is under the same serotoninergic control. Previous data suggest the latter: decreased monoamine concentrations achieved by amine depletors (reserpine and tetrabenazine) facilitate female copulatory behaviour (lordosis response) in oestrogen-treated males castrated as adults[16]. The specificity of serotonin in this respect needs further investigation, however.

We thank Miss Ann-Kristin Söderlund for technical assistance. Oestradiol benzoate and progesterone were donated by Pharmacia, Uppsala, Sweden; testosterone propionate by

Table 1 Effect of PCPA on Testosterone Propionate Activated Heterosexual Mounting Behaviour and Locomotor Activity for Castrated Male Rats

Treatment	Mounting (%) Pre-experimental test	Mounting (%) Experimental test	No.	Mounts per min* Pre-experimental test	Mounts per min* Experimental test	No.†	Locomotor activity 'Animex' activity meter counts per 10 min*	No.
A 1 PCPA, 4×100 mg kg^{-1}, i.p.	49	90‖	39	3.4±0.3	5.1±0.3**	19	357±33‖	12
2 Saline, 4×0.4 ml., i.p.	45	45	40	3.9±0.3	3.5±0.3§	15	691±24	12
3 PCPA, 4×100 mg kg^{-1}, i.p.	—‡	—					336±62‖	6
4 Saline, 4×0.4 ml., i.p.	—	—					916±34	6
B Adrenalectomized animals								
1 PCPA, 100+3×50 mg kg^{-1}, i.p.	23	92**	13	1.4±0.3	3.1±0.8§	3		
2 Saline, 4×0.4 ml., i.p.	46	46	13	1.9±0.4	2.5±0.4§	3		

* Mean ±s.e.

† The estimate of mounts per minute is based on subjects which mounted in both the pre-experimental and experimental tests.

‡ No copulatory test performed. *P* of difference in % of animals mounting between the pre-experimental and experimental tests was tested by the Sign test. Student's *t* test was used for mean difference in mounts per minute and to test the difference between PCPA and saline treatment on locomotor activity.

§ NS, $P>0.05$. ** $P<0.01$. ‖ $P<0.001$.

PCPA and saline were given once daily for 4 consecutive days. The last injection was given 6 h before the experimental test. Adrenalectomized animals received dexamethasone 0.5 mg per animal subcutaneously once a week.

Table 2 Effect of PCPA on Testosterone Propionate Activated Mounting of Female and of Male Partner, with Castrated Male Rats

Treatment	Mounting of female (%) Pre-experimental test	Mounting of female (%) Experimental test*	*P*	Mounting of male (%) Experimental test	*P*†	No.
PCPA, 4×100 mg kg^{-1}, i.p.	67	83	>0.05	67	<0.01	12
Saline, 4×0.4 ml., i.p.	75	83	>0.05	17		12

PCPA and saline was given once a day for 4 consecutive days. The last injection was given 7 h before the first experimental test. The test for mounting a male was performed 0.5–1 h after the test for mounting a female.

* Difference between pre-experimental and experimental was tested by the Sign test.

† Difference between PCPA and saline treatment was tested by the χ^2 test.

Table 3 Effect of Pargyline Alone and in Combination with PCPA and PCPA+5-HTP on Testosterone Propionate-Activated Mounting

Treatment	Mounting (%)			Mounts per minute*		
	Pre-experimental test	Experimental test	No.	Pre-experimental test	Experimental test	No.†
A 1 Pargyline, 40 mg kg^{-1}	78	6‖	18	1.8±0.3	—	(14)
2 Saline, 0.4 ml.	76	82	17	3.0±0.2	2.8±0.4§	12
B 1 PCPA, 4×100 mg kg^{-1} and pargyline, 40 mg kg^{-1}	38	75**	24	2.3±0.5	3.1±0.7§	
2 PCPA, 4×100 mg kg^{-1} and pargyline, 40 mg kg^{-1} and 5-HTP, 5.0 mg kg^{-1}	50	4**	24	3.0±0.5	—	(12)
3 Saline, 4×0.4 ml.	58	50	24	2.5±0.4	3.2±0.4	10

* Mean ±s.e. Castrated male rats were used.
† The estimate of mounts per minute is based on subjects which mounted in both the pre-experimental and experimental tests.
Other footnotes are as in Table 1.
P of difference was calculated as in Table 1. PCPA (*B*1,2) and saline (*B*3) were given once daily for 4 consecutive days. The last injection was given 10 h before the experimental test. Pargyline was injected 6 h and 5-HTP 0.5 h before the experimental test. All injections were given intraperitoneally.

Erco, Stockholm, Sweden, and pargyline by Abbott Labs, Chicago, Illinois. Supported by a grant from the National Institute of Child Health and Development and a grant from the Swedish Medical Research Council.

Note added in proof. After this paper had been submitted for publication, data on the effect of pargyline and pargyline+PCPA on the copulatory behaviour in the male rat were reported by A. Tagliamonte, P. Tagliamonte and G. L. Gessa (*Nature*, **230**, 244; 1971). Their results are in good agreement with our earlier[3] and present data.

CARL-OLOF MALMNÄS
BENGT J. MEYERSON

Department of Pharmacology,
University of Uppsala

Received December 14, 1970; revised March 10, 1971.

[1] Meyerson, B. J., *Acta Physiol. Scand.*, **63**, 241 (1964).
[2] Meyerson, B. J., *Ann. Med. Exp. Fenn.*, **46**, 394 (1968).
[3] Malmnäs, C.-O., and Meyerson, B. J., *Acta Pharmacol. Toxicol.*, **28**, Suppl. 1 (1970).
[4] Meyerson, B. J., *Arch. Intern. Pharmacodyn.*, **150**, 4 (1964).
[5] Shillito, E. E., *Brit. J. Pharmacol.*, **36**, 193 (1969).
[6] Shillito, E. E., *Brit. J. Pharmacol.*, **38**, 305 (1970).
[7] Tagliamonte, A., Tagliamonte, P., Gessa, G. L., and Brodie, B. B., *Science*, **166**, 1433 (1969).
[8] Koe, B. K., and Weissman, A., *J. Pharmacol. Exp. Ther.*, **154**, 499 (1966).
[9] Meyerson, B. J., and Lewander, T., *Life Sci.*, **9**, 661 (1970).
[10] Hoyland, V. J., Shillito, E. E., and Vogt, M., *Brit. J. Pharmacol.*, **40**, 659 (1970).
[11] Ferguson, J., Henriksen, S., Cohen, M., Mitchell, G., Barchas, J., and Dement, W., *Science*, **168**, 499 (1970).
[12] Segal, D. S., and Wahlen, R. E., *Psychopharmacologia*, **16**, 434 (1970).
[13] Wahlen, R. E., and Luttge, W. G., *Science*, **169**, 1000 (1970).
[14] Zitrin, A., Beach, F. A., Barchas, J. D., and Dement, W. C., *Science*, **170**, 868 (1970).
[15] Meyerson, B. J., *Psychopharmacologia*, **6**, 210 (1964).
[16] Meyerson, B. J., *Nature*, **217**, 683 (1968).

p-Chlorophenylalanine facilitates Copulatory Behaviour in Male Rats

IT has been proposed that the inhibition of male copulatory patterns by anterior portions of the nervous system[1] is mediated by brain monoamines[2–4], for reserpine facilitates certain aspects of the male pattern in rats[3–5] although monoamine oxidase inhibitors retard copulatory behaviour (D. A. D., H. N. Davis and P. E. Jansen, results submitted for publication). Meyerson has proposed[6] that an analogous mechanism may control female oestrous behaviour in rats.

These data suggest that *p*-chlorophenylalanine (PCPA), which depletes brain serotonin[7], should facilitate male copulatory behaviour as it does female oestrous behaviour[8]. Evidence that this drug facilitates mounting behaviour in male rats[2,9–12] supports this hypothesis. But another report[13] indicates that the species typical copulatory pattern of male rats is not affected by PCPA. We have now demonstrated that PCPA can substantially facilitate the normal pattern of heterosexual behaviour in the male rat.

Twenty-three male Long–Evans rats 95 days old were pretested—each was handled, followed by two successful tests of copulatory behaviour. Twenty-eight females of the same age and strain served as partners. Tests were conducted in 'Plexiglas' arenas 76–86 cm in diameter and 61–66 cm high.

Each male received three experimental tests of copulatory behaviour at intervals of 4 weeks. Tests followed administration of isotonic saline, 50 mg kg^{-1} or 100 mg kg^{-1}, of a saline suspension of DL-*p*-chlorophenylalanine (*p*H 1.8). Injections were given intraperitoneally on four successive nights between midnight and 0100 h and tests were initiated on the day after the fourth injection at 0300 h—5 h after the beginning of the dark phase of the light–dark cycle. Subgroups were tested in each of the six possible running orders in a balanced design.

The tests began with the introduction of a receptive female into the test arena 5 min after the introduction of the male, and were terminated after the first intromission following the third ejaculation by the male. Females were brought into behavioural oestrus with intramuscular injections of 0.1 mg of oestradiol benzoate 3 days before testing and 1.0 mg of progesterone approximately 3 h before testing. Only receptive females were used.

Copulatory behaviour in rats consists of bouts or series of mounts (without intromission) and vaginal intromissions, with each complete series terminated by an ejaculation. We considered the following standard measures: mount latency (ML)—the period from the introduction of the female to the first mount; intromission latency (IL)—the period until the first intromission; ejaculation latency (EL)—the period from the first intromission of a series to ejaculation; intromission frequency (IF)—the number of intromissions in a series; mount frequency (MF)—the number of mounts in a series; mean inter-intromission interval (MIII)—the mean interval separating the intromissions of a series; and post-ejaculatory interval (PEI)—latency from ejaculation to the next intromission. Individual series are designated by a hyphen and the appropriate number (for example, IF-3).

We used multivariate analyses of variance with repeated measures[14] on our data. There was a significant overall effect of drug condition across series on all five measures characterizing series (EL, MIII, PEI, IF, MF). The mean scores and

Table 1 Mean Scores and Significance Levels of Male Copulatory Behaviour as a Function of Drug Condition

	Mean score			Significance level*			
Measure	Saline	50 mg kg^{-1}	100 mg kg^{-1}	Total	Saline versus 50 mg kg^{-1}	Saline versus 100 mg kg^{-1}	50 mg kg^{-1} versus 100 mg kg^{-1}
Latency measures (s)							
ML	14.43	7.57	6.87	NS	NS	NS	NS
IL	47.30	10.30	12.35	NS	NS	NS	NS
EL-1	342.26	210.00	168.87	<0.01	NS	<0.05	NS
EL-2	170.65	98.65	98.09	<0.01	<0.05	<0.01	NS
EL-3	248.65	109.09	125.13	<0.05	(<0.10)	(<0.10)	NS
MIII-1	39.90	24.12	20.12	<0.01	NS	<0.05	NS
MIII-2	40.43	27.29	25.85	(<0.10)	NS	(<0.10)	NS
MIII-3	46.38	27.55	29.99	(<0.10)	(<0.10)	NS	NS
PEI-1	339.00	322.83	322.30	NS	NS	NS	NS
PEI-2	416.35	377.57	379.13	<0.01	NS	<0.01	NS
PEI-3	486.52	430.52	446.09	<0.05	(<0.10)	NS	NS
Frequency measures							
IF-1	8.87	8.74	8.78	NS	NS	NS	NS
IF-2	4.74	3.78	3.87	<0.01	<0.05	<0.05	NS
IF-3	5.17	3.87	4.04	<0.01	<0.01	<0.05	NS
MF-1	5.30	2.39	2.43	<0.05	(<0.10)	<0.05	NS
MF-2	1.65	1.35	0.70	<0.05	NS	(<0.10)	NS
MF-3	3.61	1.65	1.30	<0.05	NS	(<0.10)	NS

* Overall tests with multivariate repeated measures analyses (d.f. = 2,21); multiple comparisons with Scheffé's tests (d.f. = 2,21). NS, Not significant.

significance levels for all the measures for individual series are presented in Table 1. It is evident that PCPA substantially facilitated most aspects of the copulatory pattern. The means of all measures for all series have consistent differences indicative of this facilitation. Many of these differences were significant at the 0.05 level or better. In several cases, changes in group means in the drug treatments were accompanied by changes in group variances; in general, the drug treatments produced decreases in variances, although there were exceptions to this trend. Ejaculations were usually attained sooner, and after fewer mounts and intromissions, than after saline administrations, and post-ejaculatory refractory periods were shorter. Both dose levels facilitated the behaviour at least to some extent, but there were more significant differences after administration of the higher dose.

The demonstration of a facilitation effect of PCPA on male rat heterosexual copulatory activity is consistent with its facilitation of mounting behaviour[2,9–12]. The effects of PCPA on copulatory behaviour of male cats are unclear[15,16]. Our data are inconsistent with a study[13] in which there was no effect of PCPA on heterosexual copulation in male rats. Differences between the two experiments which might account for our positive results include: our use of the reported[2,12] optimal injection-test interval; our use of a larger number of subjects; our confinement of tests to a part of the photoperiod in which copulatory behaviour is relatively fast and variability relatively low[17]; our use of a balanced testing order with different subjects receiving treatment in all possible orders; and differences in the form and mode of injection of PCPA.

These data, together with others from our laboratory[3,4], are consistent with the hypothesis that brain monoamines, and particularly serotonin, inhibit male copulatory behaviour.

This research was supported by a grant from the National Science Foundation. We thank P. L. Perlman of the Schering Corporation for hormones, Dr N. Belcher of Pfizer and Co. for PCPA and Dr C. Becker of the University of Florida for preparing the suspension.

Patricia J. Salis
Donald A. Dewsbury

Departments of Psychiatry, Psychology and
Center for Neurobiological Sciences,
University of Florida, Gainesville,
Florida 32601

Received February 17, 1971.

[1] Beach, F. A., *Physiol. Rev.*, **47**, 289 (1967).
[2] Tagliamonte, A., Tagliamonte, P., Gessa, G. L., and Brodie, B. B., *Science*, **166**, 1433 (1969).
[3] Dewsbury, D. A., and Davis, H. N., *Physiol. Behav.*, **5**, 1331 (1970).
[4] Dewsbury, D. A., *Psychon. Sci.*, **22**, 177 (1971).
[5] Soulairac, A., and Soulairac, M. L., *CR Soc. Biol.*, **155**, 1010 (1961).
[6] Meyerson, B. J., *Acta Physiol. Scand., Suppl.*, **63**, 241 (1964).
[7] Koe, B. K., and Weissman, A., *J. Pharmacol. Exp. Ther.*, **154**, 499 (1966).
[8] Meyerson, B. J., and Lewander, T., *Life Sci.*, **9**, 661 (1970).
[9] Gessa, G. L., *Ann. Internal Med.*, **73**, 622 (1970).
[10] Gessa, G. L., Tagliamonte, A., Tagliamonte, P., and Brodie, B. B., *Nature*, **227**, 616 (1970).
[11] Shillito, E. E., *Brit. J. Pharmacol.*, **38**, 305 (1970).
[12] Sheard, M. H., *Brain Res.*, **15**, 524 (1969).
[13] Whalen, R. E., and Luttge, W. G., *Science*, **169**, 1000 (1970).
[14] Morrison, D. F., *Multivariate Statistical Methods* (McGraw-Hill, New York, 1967).
[15] Ferguson, J., Henriksen, S., Cohen, H., Mitchell, G., Barchas, J., and Dement, W., *Science*, **168**, 499 (1970).
[16] Zitrin, A., Beach, F. A., Barchas, J. D., and Dement, W. C., *Science*, **170**, 868 (1970).
[17] Dewsbury, D. A., *Commun. Behav. Biol.*, **1**, 373 (1968).

Mate-seeking by Female *Amblyomma maculatum* (Acarina: Ixodidae) on a Bovine

Little information is available concerning the attraction between sexes of ticks, and the literature contains no reports of sex pheromones in these arachnids. In some tick species the males are known to seek the attached females[1–4], though male bont ticks, *Amblyomma hebraeum* Koch, in Africa probably attract females[5]. When, however, I investigated the mating behaviour of the Gulf Coast tick, *Amblyomma maculatum* Koch, on bovines restrained inside environmentally controlled stalls (24 ± 3° C; 70 ± 7% relative humidity; 12 h photophase: 12 h scotophase per 24 h photocycle), I found that the females were attracted to the males.

In preliminary tests, female *A. maculatum* (AM) did not attach to a bovine, either alone, or in the presence of males of another species in the same genus (*Amblyomma americanum* (L.) (AA)). This effect is unusual because females of other tick species do attach to hosts in the absence of males[6–8].

Therefore, in three replicates, stanchioned bovines were fitted with mating chambers (sleeves)[9] and infested with ten ticks of each sex in the following combinations on the indicated day (counting day 1 as the time of first infestation): (1) AA males on day 1 and AM females on day 6; (2) AA males and AM females both on day 1; (3) AM females on day 1; (4) AM males on day 1 and AM females on day 6; (5) AM males and AM females both on day 1.

After the scheduled infestation was complete, the sleeves were inspected every 24 h for 8 days (counting from the day of infestation with females), and the number of attached AM females was recorded. Attachment of all ten females occurred only in sleeves that contained the AM males (treatments 4 and 5; Table 1), and 100% attachment of females occurred 5 days sooner in sleeves that contained already-attached AM males (treatment 4) than in sleeves that received the AM females and AM males at the same time (treatment 5). Apparently the males must feed for several days before most females will attach. Few females introduced into sleeves without males were stimulated to attach, and those that did attach remained no longer than 24 or 48 h. Rates of attachment of females in sleeves containing AA males were intermediate between those of females in sleeves with males of the same species and those of females in sleeves without males. Possibly the presence *per se* of the males or an interspecific pheromone induced moderate attachment of AM females in sleeves with AA males.

Table 1 Rates of Attachment to Bovines of AM ♀♀ placed Inside Sleeves Alone or with One of Four Groups of ♂♂

Treatment No.	AM ♀♀ placed inside sleeves	Replicate No.	No. AM ♀♀ attached at indicated day post infestation							
			1	2	3	4	5	6	7	8
1	With previously attached AA ♂♂	1	0	1	0	0	0	1	0	0
		2	1	1	0	1	3	2	8	7
		3	1	3	1	4	4	4	2	3
2	At the same time as AA ♂♂	1	0	0	0	0	0	0	0	1
		2	0	0	2	0	0	1	0	2
		3	1	0	2	4	3	4	6	4
3	Alone	1	0	0	0	0	0	0	1	0
		2	0	1	0	0	0	0	0	0
		3	0	1	0	0	0	2	3	1
4	With previously attached AM ♂♂	1	9	10*	10	10	10	10	10	10
		2	10	10	10*	10	10	10	10†	9
		3	8	10	10*	10	10	10	10†	9
5	At the same time as AM ♂♂	1	0	1	3	6	8	10	10	10*
		2	2	3	10	9	6	6	10	10*
		3	0	0	5	9	10	10	10	10

Each sleeve contained ten ticks of each sex.

* First mating observed.

† First detachment of mated, engorged females observed.

The females that attached in sleeves containing attached AM males (treatment 5) were mated beginning 2 or 3 days after the females were introduced, and detached when replete. Females introduced into sleeves at the same time as AM males (treatment 4), however, were not mated until 8 days post-infestation, because the completion of spermatogenesis by many male ixodid species does not take place until after they feed[1]. Females released into sleeves containing conspecific males engorged partially were mated by the males, and eventually detached when replete. Females that attached in sleeves by themselves (treatment 3) or in sleeves with AA males (treatments 1 and 2) imbibed no discernible amounts of blood, and cross-mating did not occur between AA males and AM females.

Because each AM female that was introduced into a sleeve containing attached AM males actively sought the site of the male and attached immediately adjacent to it, the behaviour of AM females in seeking AM males was studied further in another test in which the two sexes were not confined in close proximity. The methods were similar to those used in a study of the migration of AA males to AA females on bovines[4] except that the AM females, not the males, were marked and released.

Each AM female was marked on the dorsum with 1 µl. of saturated acetone solutions of fluorescent powders (Series A pigments; Day-Glo Color Division, Switzer Bros., Inc., Cleveland, Ohio). Four days before the marked females were to be released on stanchioned bovines, the animals were infested with seventy-five AM males which were confined on an area of the upper shoulder by the stockinette-sleeve technique[4]. After 4 days, the sleeve was removed, and twenty-five females were released onto each of three areas (the hocks of one front and one hind leg, and the belly of the animal) on the sides of the animals opposite the sides on which the males were attached. Each group of twenty-five females was marked with a different colour for each site of release. Many females did not cling firmly to the hair of the animals and fell to the floor of the stall immediately after release and were subsequently lost.

At 24 h after release, the marked females were located on the animals with a portable ultraviolet lamp, and the site of attachment of each tick was recorded on a diagram of the animal. For a further 11 days, the cattle were inspected daily for paired adult ticks. Females found within 1 cm of a male were removed, examined under the ultraviolet lamp, and then discarded. I was therefore able to determine how long the females were on the hosts before they located the males. The test was replicated six times.

By 24 h after release, eighty of the ninety-two detected females had preferred to attach within 1 cm of males rather than attach at any other site on the animals' bodies; the second most preferred site of attachment (eight out of ninety-two) was the ears, even though no males were present in them. Thus, most females located males within 24 h regardless of the site of initial infestation (the last female took 10 days to reach the males). Of a total of 450 marked females originally released on the animals, 111 (25%) were paired with males when collected.

I thank R. O. Drummond and C. C. Dawkins for their assistance.

William J. Gladney

Entomology Research Division,
Agricultural Research Service,
US Department of Agriculture,
PO Box 232,
Kerrville, Texas 78028

Received March 19, 1971.

[1] Balashov, Y. S., *Dokl. Akad. Nauk SSSR*, **110**, 1113 (1956).

[2] Arthur, D. R., *Ticks and Disease* (Row, Peterson and Co., Evanston, Illinois, and Elmsford, New York, 1962).

[3] Sactor, B., Hutchinson, M., and Granett, P., *J. Econ. Entomol.*, **41**, 296 (1948).

[4] Gladney, W. J., and Drummond, R. O., *J. Econ. Entomol.*, **63**, 1214 (1970).

[5] Lounsbury, C. P., *Agr. J. Cape Good Hope*, **15**, 728 (1899).

[6] Gladney, W. J., Drummond, R. O., Whetstone, T. M., and Ernst, S. E., *J. Med. Entomol.*, **7**, 92 (1970).

[7] Pervomaiskii, G. S., *Tr. Vses. Entomol. Obshchestoa.*, **44**, 62 (1954).

[8] Srivastava, S. C., and Varma, M. G. R., *J. Med. Entomol.*, **1**, 154 (1964).

[9] Gladney, W. J., and Drummond, R. O., *Ann. Entomol. Soc. Amer.*, **63**, 1036 (1970).

Systemic Action of an Insect Feeding Deterrent

Many phytophagous insects are restricted in host range because of the presence of naturally occurring distasteful chemical substances in otherwise acceptable plants[1,2]. In the search for possible substitutes for toxic pesticides, however, comparatively little attention has been directed towards the application of non-toxic antifeeding compounds or rejectants as a means of deterring insects from attacking crops. To be of practical value, a rejectant should not only be persistent but also absorbed and translocated to the growing points of treated plants. Otherwise the new plant growth developing after treatment, being uncontaminated, will be selectively attacked while the older treated leaves remain distasteful.

The neem tree *Azadirachta indica* has many uses, one of which is that a suspension of crushed seeds or leaves will deter some insect species from feeding when applied directly to plant foliage or stored products. A compound which is a potent rejectant for the desert locust, *Schistocerca gregaria* Forsk[3,4], has been isolated from seeds and termed azadirachtin[5] though its structure has yet to be elucidated. We have now obtained results which show that azadirachtin, and possibly other substances present in neem extracts, can be absorbed by the roots and translocated within bean plants to act as a systemic rejectant.

Three products were tested against *Schistocerca* adults. These were (1) azadirachtin, the pure chemical; (2) an alcoholic concentrate, refined from a 95% ethanol extract of neem seed, containing 3.8% by weight of the seed; and (3) an aqueous suspension prepared by grinding seed kernels in water until the material passed through a 100-mesh sieve.

These products were diluted with water containing 0.05% 'Teepol' and applied in a range of seven concentrations to the soil around young bean plants at the six to eight leaf stage. The bean plants were transplanted singly to plastic cups containing 150 g of a soil consisting of a sieved and sterilized mixture of 3 parts loam, 2 parts sand and 1 part peat, and the different treatments were applied in 10 ml. as part of the first watering. The efficacy and persistence of the treatments were assayed by placing the treated plants, together with controls, randomly in a $0.31\ \mathrm{m}^3$ cage with 200 adult desert locusts which had been starved for 8–10 h. After 12 h of exposure to the hungry locusts, the damage suffered by each plant was assessed using a visual ranking index system. The same treated plants plus fresh controls were exposed in this way to starved locusts for 12 h after 1.5, 4, 7, 15, 21 and 25 days. The experiment was repeated three times using three replicates of each treatment and controls on each occasion. The locusts were still hungry at the end of each exposure and were ready to attack fresh control plants.

Table 1 Effects of Azadirachtin and Neem Preparations on *Schistocerca* attacking Young Bean Plants

Azadirachtin			Alcoholic preparation			Aqueous preparation		
p.p.m.	Day		p.p.m.	Day		p.p.m.	Day	
	1.5	15		1.5	15		1.5	15
10	0	1	100	0	1	1,000	0	1
1.0	0	2	10	0	3	100	0	3
0.5	0	3	2.5	0	4	80	0	4
0.2	0	4	1.0	1	5	40	1	5
0.1	1	5	0.5	1	5	20	1	5
0.05	1	5	0.2	1	5	10	2	5
0.01	1	5	0.1	1	5	1	4	5
Controls	5	5		4	5		5	5

Plant damage was assessed by a ranking index: 0, no feeding; 1, very slight nibbling, apical bud intact; 2, leaves partly eaten, some damage to apical bud; 3, apical buds and 1/3 of plant eaten; 4, a few lower buds remaining on stem only; 5, all the buds and most or all of the plant eaten. Treatments are expressed as parts per million of dry soil.

The results obtained shortly after treatment and after 15 days are given in Table 1. After 25 days the plants grown in the most heavily treated soil (10, 100, 1,000 p.p.m. of azadirachtin, alcoholic and aqueous preparations respectively) were still only slightly damaged by the hungry locusts.

Similar results have been obtained using older established plants of both tall and dwarf varieties, up to the fruiting stage. As soil composition is known to have an important bearing on the systemic uptake of chemicals by plants, experiments have been carried out with five different types of soils. The results indicate that high organic content and alkalinity in soils reduce the systemic anti-feeding activities of azadirachtin and the two crude neem preparations.

In other trials, bean seedlings grown from seeds soaked for 24 h in 0.01, 0.1 and 1% solutions of azadirachtin, alcohol extract and kernel water extract, respectively, were protected against damage by *Schistocerca* adults for one week after germination in cage feeding tests.

Plant nematodes of the genus *Pratylenchus* are also deterred by seedling treatments. When barley seedlings were dipped in a 1% aqueous suspension of crushed neem kernels for 2 h, then grown in a naturally infested soil for ten days, there was a decrease (>50%) in the number of nematodes invading the developing root system. Further experiments are in progress to establish whether active compounds other than azadirachtin are present in neem extracts, using several species of test insect.

We thank Dr E. D. Morgan for azadirachtin and Dr R. Skrentny for soil samples and useful advice. J. S. G. thanks the Food and Agriculture Organization for a research fellowship and Professor T. R. E. Southwood and Dr P. T. Haskell for research facilities.

J. S. GILL
C. T. LEWIS

Department of Zoology and Applied Entomology,
Imperial College,
London SW7

Received April 14, 1971.

[1] Thorsteinson, A. J., *Ann. Rev. Entomol.*, **5**, 193 (1960).
[2] Fraenkel, G., *Entomol. Exp. Appl.*, **12**, 473 (1969).
[3] Pradhan, S., Jotwani, M. G., and Rai, B. K., *Indian Fmg.*, **12**, 7 (1962).
[4] Haskell, P. T., and Mordue, A. J., *Entomol. Exp. Appl.*, **12**, 591 (1969).
[5] Butterworth, J. H., and Morgan, E. D., *Chem. Commun.*, **23** (1968).

HL-A Antigens on Human Trophoblast Cells

IN an outbred population, the placenta is essentially an allograft and many hypotheses have been put forward to explain why it is not rejected by the mother during pregnancy. One possibility is that placental tissue is devoid of transplantation antigens or these antigens are prevented from being expressed.

In mice, transplantation antigens are present on trophoblast cells, but this effect is masked by a coating of inert sialomucin material[1,2]. There is only circumstantial evidence[3], however, that an analogous situation pertains in humans. By the use of monolayer cultures of human trophoblast cells, we have been able to show in cytotoxicity *in vitro* tests that HL-A antigens are present.

Cultures of trophoblast and foetal skin cells were obtained as before[4]: cells were removed with 0.1% trypsin, and resuspended in Hanks solution to a concentration of 1×10^6 cells/ml.

Cytotoxic tests were performed on siliconed Clay–Adams ceramic ring slides using 0.025 ml. unit volumes. Cells were tested against: serum of the mother from whom the placenta was obtained; a multispecific anti-HL-A serum (Burke) which gives a strong cytotoxic reaction with the lymphocytes of more than 70% of Caucasian populations; a monospecific anti-HL-A2 serum (Wolfe).

Controls were set up by incubating the cells with: serum from a group AB rhesus positive nulliparous woman (Mar-

Table 1 Reaction of Trophoblast and Foetal Cells with Anti-HL-A Serum

Grade	Average % dead
—	0
(w)	5
w	15
(+)	30
+	50
(++)	75
++	100

chand) who has had no blood transfusions; Hanks only; Hanks and complement; the multispecific anti-HL-A serum without the addition of complement.

The mixtures were incubated at 37° C for 1 h in a moist container. Each test was then stained with 0.025 ml. of 0.4% trypan blue and read with a phase contrast microscope.

Both serum Burke and serum Wolfe were derived from group A rhesus positive individuals. After testing against a 'Selectogen' red cell panel, natural anti-B antibody was found in both sera but there was no evidence of antibody towards any of the following red cell antigens: rhesus C.D.E. $\bar{c}$.e.f.c.^{w}, Kell, Duffy, Kidd, Lewis, MNS, P, Lutheran, Vell, and Xg^{a}.

Trophoblast cells from four different placentae (T9, T13, T15, T16) were used. In one of these (T13), blood groups of both parents were ascertained to be group O Rhesus positive. The placenta in this case would therefore also be group O. This mother had had two previous pregnancies. The three other mothers were primigravidae. Corresponding cultures of foetal skin cells (S9, S13, S15) were also used.

Table 2 Reactions of Trophoblast and Foetal Skin Cells with anti-HL-A Sera, Maternal Sera and Controls

	Multi-specific serum+ complement	Multi-specific serum+ Hanks	Anti-HL-A2 serum+ complement	Control AB serum+ complement	Maternal serum+ complement	Hanks+ complement	Hanks
Trophoblast (T9)	(++)	—	ND	W	W	—	—
Trophoblast (T13)	(++)	—	W	W	(W)	(W)	W
Trophoblast (T15)	(++)	—	W	(W)	W	W	(W)
Trophoblast (T16)	(++)	—	(++)	—	ND	(W)	(W)
Foetal skin (S9)	(++)	—	ND	W	(W)	—	—
Foetal skin (S13)	(++)	—	ND	—	(W)	—	—
Foetal skin (S15)	(++)	—	ND	(W)	W	(W)	—

ND, Not done.

The tests were scored according to the method described by Joysey[5] as shown in Table 1.

Both trophoblast and foetal skin cells reacted strongly with the multispecific anti-HL-A serum Burke, the cytotoxic activity being complement dependent (Table 2). The monospecific anti-HL-A2 serum Wolfe was cytotoxic to one out of three trophoblasts tested. None of the maternal sera had any cytotoxic activity towards their respective trophoblast or foetal skin cells.

Currie and Bagshawe[6] demonstrated that maternal as well as allogeneic lymphocytes had a cytotoxic effect on human trophoblast cells and concluded that trophoblast must have antigenic groupings foreign to the mother. Hulka *et al.*[7] found that fluorescein-labelled serum globulin obtained from post-partum women would localize in placental tissue. These experiments provide evidence for the existence of antigens on human trophoblast cells, but do not indicate whether these are HL-A determinants.

Van Rood[8] reported that serum containing anti-leucocyte antibodies was absorbed by placental tissue but Seigler and Metzgar[9] were unable to confirm the presence of HL-A antigens on whole trophoblast.

The results of our experiment have shown that a serum containing strong multispecific anti-leucocyte antibody has cytotoxic activity towards human trophoblast cells *in vitro*, and it seems likely that this activity is directed against HL-A determinants. Incompatibility due to ABO groups can be definitely excluded in one case where both parents are group O. Furthermore, both serum Burke and serum Wolfe were found to possess natural anti-B only: no antibodies against other red cell antigens could be demonstrated. The monospecific anti-HL-A2 serum Wolfe was cytotoxic to one out of three trophoblasts tested, the frequency of the HL-A2 phenotype being about 49% in the Cambridge panel.

The finding of HL-A antigens on trophoblast cells *in vitro* does not nullify the concept that these determinants may be masked *in vivo*. There have been numerous reports on the presence of a covering layer around human trophoblast cells which may fulfil this function[10-13].

In some cases, however, foeto-maternal immunization certainly occurs as indicated by the presence of anti-leucocyte antibodies in the serum of multiparous women[14,15]. It seems likely that the trophoblast may at least partly be the source of the antigenic stimulation especially as these cells are widely deported in the maternal circulation during human pregnancy[16,17]. There is a positive correlation between maternal sensitization and parity[18]. In our series, there was no demonstrable activity of maternal sera towards their respective trophoblast and foetal skin cells.

We thank the US National Institutes of Health for serum Burke. The work is supported by a grant from the Cancer Research Campaign (Y. W. L. and R. B.) and the Medical Research Council (V. C. J.).

Y. W. Loke

Department of Pathology

Valerie C. Joysey

Tissue Typing Laboratory,
John Bonnett Clinical Laboratory

R. Borland

Department of Pathology,
University of Cambridge

Received February 9; revised May 5, 1971.

[1] Kirby, D. R. S., Billington, W. D., Bradbury, S., and Goldstein, D. J., *Nature*, **204**, 548 (1964).
[2] Currie, G. A., Van Doorninck, W., and Bagshawe, K. D., *Nature*, **219**, 191 (1968).
[3] Billington, W. D., *Immunology and Development* (Spastics International Medical Publications, London, 1969).
[4] Loke, Y. W., and Borland, R., *Nature*, **228**, 561 (1970).
[5] Joysey, V. C., *Proc. Symp. Transpl. Antigens Tissue Typing* (1968).
[6] Currie, G. A., and Bagshawe, K. D., *Advance in Transplantation* (Munksgaard, Copenhagen, 1967).
[7] Hulka, J. F., Brinton, V., Schaaf, J., and Baney, C., *Nature*, **198**, 501 (1963).
[8] Van Rood, J. J., *Leukocyte Grouping* (Drukkerij Pasmans, Den Haag, 1962).
[9] Seigler, H. L., and Metzgar, R. S., *Transplantation*, **9**, 478 (1970).
[10] Wynn, R. M., *Amer. J. Obstet. Gynecol.*, **97**, 832 (1967).
[11] Tighe, J. R., Garrod, P. R., and Curran, R. C., *J. Pathol. Bacteriol.*, **93**, 559 (1967).
[12] Boyde, J. D., Hamilton, W. J., and Boyde, C. A. R., *J. Anat.*, **102**, 553 (1968).

[13] Bradbury, S., Billington, W. D., Kirby, D. R. S., and Williams, E. A., *Amer. J. Obstet. Gynecol.*, **104**, 416 (1969).
[14] Payne, R., and Rolfs, M. R., *J. Clin. Invest.*, **37**, 1756 (1958).
[15] Van Rood, J. J., Eernisse, J. G., and Van Leeuwen, A., *Nature*, **181**, 1735 (1968).
[16] Douglas, G. W., Thomas, L., Carr, M., Cullen, N. M., and Morris, R., *Amer. J. Obstet. Gynecol.*, **78**, 960 (1959).
[17] Attwood, H. D., and Park, W. W., *J. Obstet. Gynaecol. Brit. Cwlth*, **68**, 611 (1961).
[18] Terasaki, P. I., Mickey, M. R., Yamazaki, J. N., and Vredevoe, D., *Transplantation*, **9**, 538 (1970).

Transplantation of Radiation-induced Canine Myelomonocytic Leukaemia

An important variant of leukaemia in man is the myelomonocytic form[1], the exact nature of which is still unclear, for the only available model for studies of this disease is the mouse[2]. We have developed a canine model which shows promise for studies of certain aspects of the pathogenesis of myelomonocytic leukaemia (MML) as related to changes that develop in a single animal. Because dogs live longer and are larger than mice, they are well suited to serial samplings of blood, bone marrow and other tissues, facilitating dynamic studies of changes in the haematopoietic system occurring with time.

Beagles continuously exposed to high ^{90}Sr concentrations developed a myeloproliferative syndrome (MPS)[3]. Several further cases of MPS were observed subsequently, including two of myelomonocytic leukaemia, and studies were undertaken to develop a canine model to aid studies of the aetiology, pathogenesis, diagnosis and possible control of the disease. This report concerns the transplantation of cells from one of the dogs with MML; similar studies utilizing cells from the other case produced comparable results.

The donor dog (D50F52), fed ^{90}Sr daily from birth, had accumulated about 5,000 rad of β-radiation to the bone marrow during her life. The diagnosis of MML (made when the dog was about 5 yr old) was based on haematological changes and confirmed by terminal pathological findings. There was a persistent significant increase in peripheral white blood cell numbers, especially granulocytes (including occasional blast forms) and monocytes. There was also an extreme increase in the percentage of granulocytes relative to other cell types in all samples of bone marrow examined (Table 1). Approximately 30% of the granulocytes in the terminal marrow samples were primitive, often atypical blast forms. Terminal lesions consisted of extensive invasion of haematopoietic organs (bone marrow, liver, spleen and lymph nodes), often with complete obliteration of the normal structure. In all the organs except the spleen, the tumour infiltrates appeared as fleshy green masses (chloroma). Most other organs and tissues contained focal accumulations of tumour cells.

Primary transplantation was accomplished with bone marrow cell preparations. Subsequent transplantations were done with cells from solid tumours or tumour ascites. The number of viable cells used in the inoculum varied from 1×10^7 to 2×10^8 cells ml.$^{-1}$. The pregnant beagles used as recipients were not related to the donor dogs. Transplantation was effected by performing a laparotomy at 46–53 days of gestation and inoculating the foetuses intraperitoneally through the uterine wall[4]. Except in passage 5, at least one foetus in each litter served as a control and was inoculated with a radionuclide marker only, for identification[4].

Animals were usually killed about a week after enlarged lymph nodes or solid tumours became evident and haematologic abnormalities were observed, which occurred 8–71 days after birth (Fig. 1).

Lesions observed in all five passages closely resembled those in the original donor dog, but were more pronounced. The population of neoplastic cells in the recipients consistently had more primitive cell types than the donor population. A further prominent feature was the occurrence, in all recipients, of large retroperitoneal masses of chloromatous tumour tissue in the sublumbar region, comprising up to 10% of the host's body weight. Peripheral blood, serum lysozyme and bone marrow values for the donor and one passage 3 recipient which manifested a severe case of MML, are presented in Table 1. All control pups had normal haemograms, and no lesions were seen in the nine control pups that were killed.

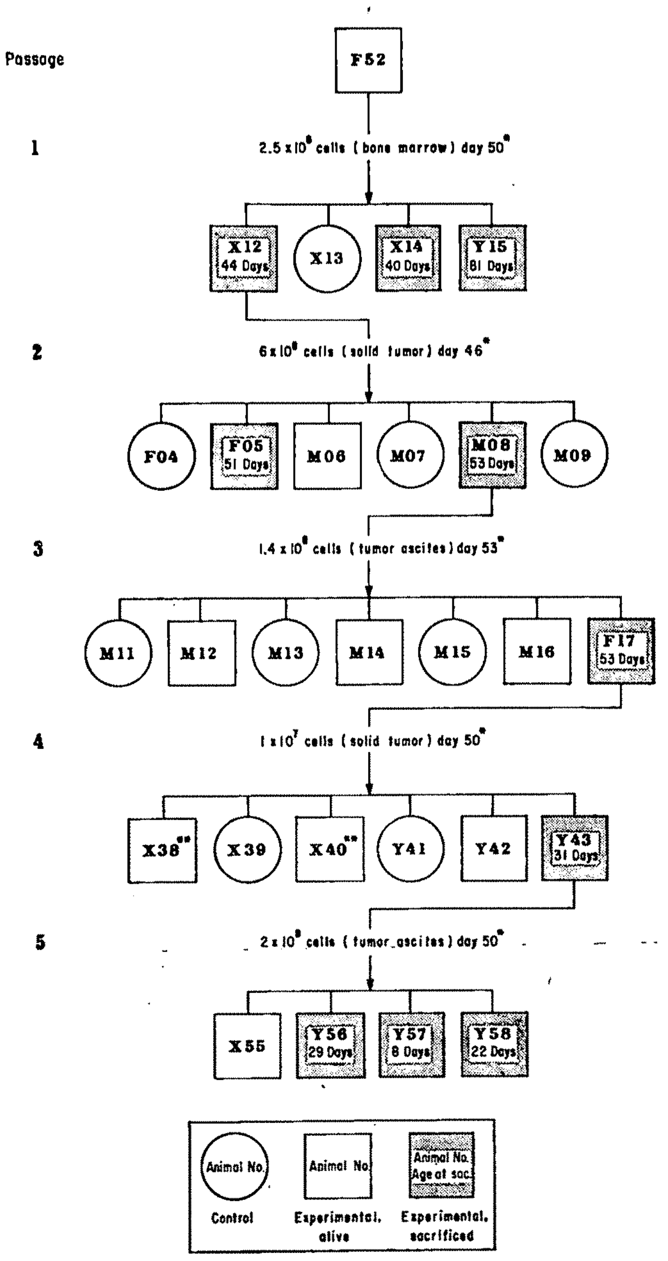


Fig. 1 Transplantation of myelomonocytic leukaemia. *, Day of gestation inoculated; **, died at birth.

Serum lysozyme concentrations, assayed by the method of Osserman and Lawlor[5], were high in the primary donor and in all recipients that developed MML, whereas lysozyme was not detected in pups that did not manifest the disease (Table 1). Increased lysozyme concentrations, primarily in urine, are found in cases of human MML, which is considered to be of value in diagnosis[5]. Electron microscopic examination of tumour tissues has not revealed any virus particles. Further work is in progress to determine the possibility of a viral aetiology for the disease.

Chromosome analysis was performed on direct preparations of bone marrow, of peripheral and mesenteric lymph nodes

Table 1 Terminal Values for Peripheral Blood, Serum Lysozyme and Bone Marrow in Donor Dog and One Recipient with a Marked Case of Myelomonocytic Leukaemia

Parameter	Dog No. D50F52 (donor)	Dog No. T14F17 (passage 3)	Normal values (mean)*
Haematocrit (%)	32.1	26.4	30.0
Total leucocytes ($\times 10^3$ mm^{-3})	142.3	105.7	16.7
Leucocyte differential (in absolute numbers in thousands as percentage of total)			
Immature granulocytes †	5.7 (4)	30.7 (29)	0.6 (3.6)
Segmented neutrophils	82.5 (58)	24.3 (23)	7.8 (46.8)
Lymphocytes	19.9 (14)	14.8 (14)	6.0 (35.9)
Monocytes	17.1 (12)	4.2 (4)	1.8 (10.8)
Eosinophils	0 (0)	0 (0)	0.5 (2.9)
Unclassified cells	17.1 (12)	31.7‡ (30)	
Serum lysozyme (μg ml.$^{-1}$)	250	130	0
Marrow granulocytic series (percentage of total granulocytes) §			
Myeloblasts	32	30	0.7
Promyelocytes	9	14	1.2
Myelocytes	30	19	6.8
Metamyelocytes	15	13	23.1
Bands	9	21	32.6
Segmented	3	2	35.6
Mitotic figures	2	1	0
Myeloid : erythroid ratio (M/E)	57	56	1.2

* Peripheral blood: mean values of fifteen normal 38 to 52 day old pups. Marrow: mean values of multiple samples of rib marrow from twelve young adult beagles.

† Promyelocytes, myelocytes, metamyelocytes, and bands.

‡ Data are presented as percentage of granulocytes rather than of total cells because few cells were seen other than granulocytes, as evident from the M/E ratio.

§ Unclassified cells consist of atypical blast cells and abnormal cells with the morphological characteristics of both granulocytic and monocytic elements.

and of solid and ascitic tumour material. Cytogenetic evaluation of the recipient puppies, by analysis of the males' sex chromosomes, showed conclusively that the tumour tissues consisted of the original female donor cells. Most of the cells examined from all the recipient puppies contained a very small, abnormal, acrocentric autosome which may be a disease-specific marker comparable with the Philadelphia chromosome that is characteristic of chronic granulocytic leukaemia in man.

To sum up, we have demonstrated, by the transplantation of malignant cells, the feasibility of using the dog as a model for the study of myelogenous leukaemia.

This work was supported in part by the US Atomic Energy Commission, in part by an NIH health sciences advancement award and in part by the National Cancer Institute. We thank S. Jean Galligan, Dr Gary Colgrove and Boyd Harrold for technical assistance, Robert Davis for excellent animal care, Ellen K. Haro for editorial assistance and Dr Leo K. Bustad for help and encouragement.

MOSHE SHIFRINE
MARIE S. BULGIN
NANCY E. DOLLARHIDE
H. G. WOLF
NANCY J. TAYLOR
FLOYD D. WILSON

Radiobiology Laboratory,

D. L. DUNGWORTH

Department of Veterinary Pathology,

YUAN-CHUNG ZEE

Department of Veterinary Microbiology,
University of California, Davis, California 95616

Received November 23, 1970; revised April 2, 1971.

[1] Linman, J. W., *J. Chronic Dis.*, **22**, 713 (1970).
[2] Warner, N. L., Moore, M. A. S., and Metcalf, D., *J. Nat. Cancer Inst.*, **43**, 963 (1969).
[3] Dungworth, D. L., Goldman, M., Switzer, J. W., and McKelvie, D. H., *Blood*, **34**, 610 (1969).
[4] Stone, J. M., and Shifrine, M., *J. Immunol.*, **104**, 1562 (1970).
[5] Osserman, E. F., and Lawlor, D. P., *J. Exp. Med.*, **124**, 921 (1966).

Persistent Corpora Lutea of Mice in a Cold Environment

IF laboratory mice, *Mus musculus*, are bred in permanently mated pairs in an environment kept at −3° C, their litters are usually smaller at birth than those of controls at 21° C[1]. Cold adaptation is also accompanied by differences in the relative weights of many organs and tissues[2-4]. In particular, the gonads, at least of unmated mice, tend to be lighter in a cold environment.

Smaller litters in a cold environment could be due to a lower ovulation rate or to higher losses of embryos before or after implantation. During an investigation of this and allied questions, we have made an apparently novel observation on the ovaries of cold-adapted mice. The laboratory mice were of several types, mated in separate pairs at the age of 5 weeks and allowed to rear their first litters. The males were removed shortly before the birth of the second litters, which were killed within 24 h of parturition. The males were then replaced, and the females watched for the appearance of a vaginal plug. Fifteen days after a plug was recorded, that is, on the sixteenth day of gestation, each female was killed and dissected. The ovaries were quickly removed, cleaned, weighed to the nearest 0.1 mg, fixed, serially sectioned and stained; the corpora lutea were then counted. The number of foetuses and abnormal implantations was also recorded. Some wild house mice were studied[5]: they were similarly treated, except that the males were not removed and the females were killed when visibly in late pregnancy.

The first anomalous finding was that the ovaries of the cold-adapted, pregnant mice were heavier than those of the controls. Table 1 illustrates this from mice of strain A2G/Tb, for which we also have weights of the ovaries of virgin mice at both temperatures; the ovaries of the virgins, unlike those of the pregnant females, were lighter at −3° C. The discrepancy in ovarian weights in pregnancy was due to a difference in the number of corpora lutea in the two temperatures. Table 2

Table 1 Mean Weights (mg) ±s.e. of Ovaries of A2G/Tb Mice

	21° C N	21° C	−3° C† N	−3° C†	P< ‡
Virgin*	16	21.9±1.2	13	16.4±2.2	0.01
Pregnant	25	19.1±0.9	21	29.4±1.3	0.001

* From Barnett and Widdowson[2].
† First generation reared in cold.
‡ Student's *t* test.

Table 2 Corpora Lutea and Foetuses of Mixed Stock

	21° C	21→−3° C	−3° C*	−3° C†
No. of mice	32	35	25	28
Corpora lutea	19.1±0.9	34.2±1.5	34.8±1.3	27.2±1.5
Foetuses	10.6±0.6	9.8±0.5	12.4±0.6	10.3±0.7

Figures are means ±s.e.
* Generation 1.
† Generations 27 to 31.

Table 3 Corpora Lutea and Foetuses of Inbred and F_1 Mice

	21° C	21→−3° C	−3° C
C57BL/Tb			
No. of mice	25	23	—
Corpora lutea	12.2±0.8	18.4±0.9	—
Foetuses	8.4±0.5	8.0±0.8	—
A2G/Tb			
No. of mice	25	22	21*
Corpora lutea	16.2±0.7	29.6±1.0	29.4±1.8
Foetuses	9.0±0.6	8.6±0.6	9.7±0.7
F_1			
No. of mice	23	28	—
Corpora lutea	16.1±0.8	23.5±1.0	—
Foetuses	13.1±0.4	12.6±0.4	—
Wild			
No. of mice	10	—	12†
Corpora lutea	8.4±0.4	—	12.8±1.2
Foetuses	6.4±0.4	—	9.2±1.1

Figures are means ±s.e.
* First generation reared in the cold.
† Ninth generation reared in the cold.

gives figures for three classes of cold-adapted mice of a mixed, outbred stock. Mice which had been transferred to the cold environment as young adults before mating (21→−3° C), and those of the first generation reared at −3° C, had about 80% more corpora lutea than the controls. Mice of the same origin after many generations in the cold environment had about 42% more corpora lutea. Table 2 also shows that the number of viable foetuses was not correspondingly higher in the cold.

The effect of cold on the ovary was not a peculiarity of one type of mouse. Table 3 gives the figures for corpora lutea of mice of two inbred strains, A2G/Tb and C57BL/Tb, the F_1 generation produced by crossing them, and of wild house mice. All showed a similar effect of cold. The findings from the mixed stock (Table 2) and from strain A2G/Tb show that the effect on the ovary of transfer to −3° C at mating was the same as that of being reared (first generation) in the cold. All differences between temperatures in number of corpora lutea are statistically significant ($P<0.01$; Student's t). The wild mice, unlike the laboratory mice, also had larger litters in the cold environment ($P<0.05$).

The number of corpora lutea is usually regarded as a reliable index of the number of ova shed at the most recent ovulation. If it were so for our laboratory mice, we should have to assume exceptionally high ovulation rates in the cold environment, and corresponding losses before implantation. To test this, more pairs of the mixed stock were transferred to −3° C; pairs from the same litters were also mated at 21° C. When a vaginal plug indicated the beginning of the third pregnancy, the female was killed. The ovaries and fallopian tubes were removed, fixed and stained, and the eggs shed at that ovulation were counted. The mean number of ova per tube from nine females at 21° C was 5.6±0.5; the corresponding figure for six females at −3° C was 5.7±0.7. Hence the excess of corpora lutea at −3° C does not represent a raised ovulation rate in these mice. A methodological conclusion follows: the number of corpora lutea, at least in a mouse ovary, should not be assumed always to correspond to the effective ovulation rate. This applies especially in unusual environments.

In the eleven classes of laboratory mice the mean number of abnormal conceptions ranged from 0.87 to 2.69. The wild mice had almost none. Evidently, therefore, losses after implantation were few. There was also little or no effect of cold on the litter sizes of the laboratory mice (that is, number of normal foetuses at 16 days). This differs from previous findings[1] because the mice of the present study were never rearing a litter of sucklings during gestation, as were some of those studied in earlier work.

In experimental work on the cold-adaptation of mammals, exposure to cold is rarely longer than a few weeks, and effects on reproduction have usually been neglected. Attention has been concentrated on the changes which take place while an isolated small laboratory mammal is adjusting to a sudden lowering of environmental temperature[3]. In contrast, our mice at −3° C were presumably in metabolic and hormonal equilibrium. Nevertheless, this probably entailed pituitary and adrenal cortical activity above that at 21° C. Adrenal weights are given in Table 4; except the F_1 mice, which had heavy adrenals even in the warm environment, all classes at −3° C had heavier adrenals than at 21° C.

The large numbers of corpora lutea in the cold were probably due to persistence of the corpora lutea from a previous ovulation, resulting from the altered endocrine activity. This requires further study. So does the larger litter size of wild mice at −3° C. If these phenomena regularly accompany the cold-adaptation of other mammals, the question arises whether they occur also in other adverse conditions, such as exceptional crowding. It would be interesting to know more about the corpora lutea of species which, in their natural environment, continue to reproduce in varying conditions of climate, feeding and other environmental features.

Table 4 Adrenal Weights

	Mixed stock		C57BL/Tb		A2G/Tb		F_1		Wild	
	N		N		N		N		N	
21° C	11	4.7±0.3	22	4.7±0.3	23	4.7±0.3	22	5.5±0.2	9	5.0±0.6
21→−3° C	32	6.2±0.4†	19	7.0±0.5‡	20	5.5±0.2*	27	5.6±0.3	—	—
−3° C	20	5.8±0.5	—	—	19	5.9±0.4*	—	—	12	6.8±0.6*

Figures are means ±s.e. * $P<0.05$ (Student's t test). † $P<0.01$ (Student's t test). ‡ $P<0.001$ (Student's t test).

S. A. Barnett
Kathleen M. H. Munro

Departments of Zoology and
Pathology (Western Infirmary),
University of Glasgow,
Glasgow W2

Received May 28, 1971.

[1] Barnett, S. A., *Quart. J. Exp. Physiol.*, 49, 290 (1964).
[2] Barnett, S. A., and Widdowson, E. M., *Proc. Roy. Soc.*, B, 162, 502 (1965).
[3] Barnett, S. A., and Mount, L. E., in *Thermobiology* (edit. by Rose, A. S.) (Academic Press, London, 1967).
[4] Barnett, S. A., and Widdowson, E. M., *J. Reprod. Fert.* (in the press).
[5] Barnett, S. A., Smart, J. L., and Stoddart, R. C., *J. Zool.*, 163, 443 (1971).

Molecular Pathology of Human Haemoglobin: Stereochemical Interpretation of Abnormal Oxygen Affinities

WHEN the atomic model of oxyhaemoglobin had just been completed, we showed that the haemoglobin molecule is insensitive to replacements of most amino-acid residues on its surface but extremely sensitive to quite small alterations of internal non-polar contacts. And replacements near the haems and at the contacts between α and β subunits affect the respiratory function[1].

Many amino-acid substitutions in abnormal haemoglobins produce clinical symptoms by altering the oxygen affinity. Now that the atomic structures of both deoxy and oxyhaemoglobin are known[2,3] and a cooperative mechanism has been proposed[4], we can try to interpret these abnormal oxygen affinities in stereochemical terms. In the first instance, the oxygen affinity depends on the conformation of the globin pocket surrounding the haem group and on residues known to be involved in the Bohr effect or the binding of 2,3-diphosphoglycerate. But in other cases of abnormal oxygen affinity the substituted residues are far removed from any of the binding sites for either oxygen, hydrogen ions or 2,3-diphosphoglycerate. We have found that several of these substitutions alter the oxygen affinity by their effect on the allosteric equilibrium between either the two alternative tertiary structures of the individual subunits or the two alternative quaternary structures of the haemoglobin tetramer.

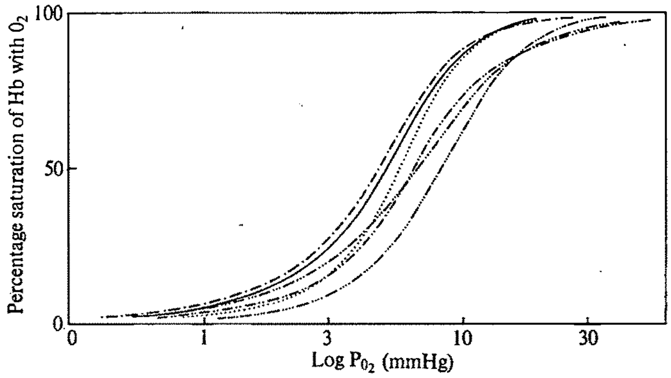


Fig. 1 Oxygen equilibrium curves of haemolysates containing unstable abnormal haemoglobins.

	Concentrations of abnormal haemoglobins	Methaemoglobin concentration before	Methaemoglobin concentration after
· From normal person	——	1.5%	4.0%
– A+Borås	10%	5.5%	7.5%
– · – A+Shepherd's Bush	16%	8.0%	12.0%
– · · – A+Hammersmith	30%	6.5%	12.0%
– · · · – A+Bucuresti	15%	11.5%	13.0%
– · · · · – Lepore Boston+ Peterborough	27% (Lepore) 70% (Peterborough)	2.5%	4.5%

Except for haemoglobins A, Peterborough and Lepore Boston, haemolysates were prepared without the use of organic solvents, with water and dilution appropriate buffers. Haemoglobin Peterborough (Fig. 2) was separated from Lepore and its purity checked by starch gel electrophoresis. Haemoglobin Borås was also separated by starch block electrophoresis; it showed a high oxygen affinity and a Bohr effect, but quantitative data were not obtained. For haemoglobin Shepherd's Bush see Table 2. Oxygen equilibrium curves were determined by the method of Imai *et al.*[7] which involved the use of monochromatic light at 480 nm with haemoglobin at a concentration of 6×10^{-5} M/haem in 0.1 M K_2HPO_4 KH_2PO_4 and 0.5 mM EDTA (pH 7.43±0.02) at 20° C. The concentration of methaemoglobin was checked spectrophotometrically before and after the measurement, using the optical density ratio at 508 nm and 576 nm. The spectra of abnormal methaemoglobins were assumed to be the same as the spectrum of the normal form, and the ratio of the absorption coefficients was assumed to be 3.20 when the methaemoglobin concentration was zero. The concentrations of the abnormal haemoglobins were calculated from optical densities of protein (280 nm) and haem (540 nm) of the cyanomet form in the supernatant after incubating the haemolysate for one hour in 0.05 M Tris-HCl buffer of pH 7.4. The concentrations of haemoglobins Lepore Boston and Peterborough were determined by chromatographic separation. Since carbon tetrachloride was used for preparing the haemolysates for the estimations of fractional concentration, the concentration of the abnormal haemoglobins in the fresh blood may be higher than in the haemolysates.

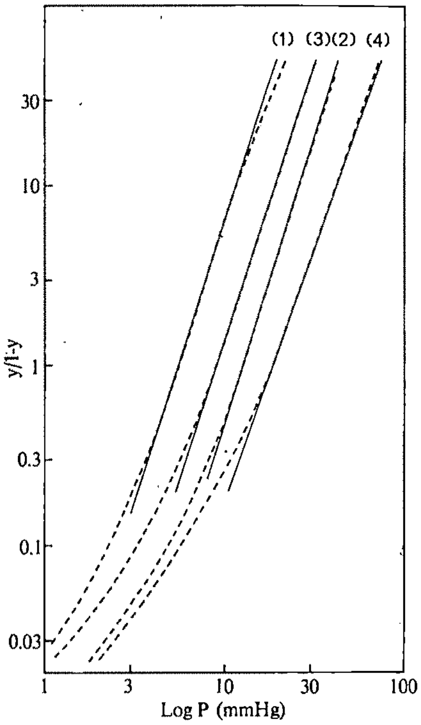


Fig. 2 Hill's constants of haemoglobins A (haemolysate) and Peterborough.

	pH	Hill's constant	Methaemoglobin concentration before	Methaemoglobin concentration after
(1) Haemoglobin A	7.41	3.1	1.0%	2.5%
(2) Haemoglobin A	6.56	3.2	1.0%	2.5%
(3) Haemoglobin Peterborough	7.42	3.0	2.0%	3.5%
(4) Haemoglobin Peterborough	6.56	2.8	4.0%	8.0%

y, Fractional saturation of haemoglobin with oxygen; P, oxygen partial pressure. Conditions as for Fig. 1. The Bohr effect of haemoglobin Peterborough is seen to be normal.

We have considered some cases of abnormal oxygen affinity recorded in the literature, and others recently detected here, which are shown in Figs. 1 and 2. Table 1 lists four substitutions which alter the conformation of the haem pocket: haemoglobins Zürich and Bristol throw light on the part played by the distal histidine E7 which is present in all mammalian haemoglobins (though absent in *Chironomus*[5] and *Aplysia*[6]). Model building suggests that oxygen may not be able to get into or out of the haem pocket unless that histidine swings out of the way; in haemoglobin Zürich it is replaced by arginine which cannot be accommodated in the haem pocket and leaves it open. This gives rise to a very high oxygen affinity. In haemoglobin Bristol, on the other hand, the histidine must be held firmly in position by a salt bridge connecting it to an internal aspartate (E11); this lowers the oxygen affinity, though it is not clear how the oxygen gets in and out at all in these conditions. The substitution of phenylalanine CD1 by serine in haemoglobin Hammersmith and by leucine in Bucuresti may lower the oxygen affinity because the benzene ring of the phenylalanine in haemoglobin A acts as a spacer that stabilizes the haem group

Table 1 Substitution of Residues Directly Affecting the Oxygen Binding Site

References	Designation	Residue		Replacement		Variability	Clinical symptoms	Oxygen affinity (Hill's constant; Bohr effect)	Effect of replacement on equilibrium between deoxy and oxy forms
		No.	Position	from	to				
8, 9	Hammersmith	β42	CD1	Phe	Ser	I	Severe, haemolytic anaemia, splenomegaly. Heinz bodies after splenectomy	Low	The haem group changes its tilt from a more upright position in deoxy to a more inclined one in oxyhaemoglobin. Phe CD1 acts as a spacer that keeps it in the more inclined position; its removal would facilitate the transition to the more upright one in the deoxy form
37	Bucuresti	β42	CD1	Phe	Leu	I	Mild haemolytic anaemia. Spleen palpable	Low	As above
10–12	Zürich	β63	E7	His	Arg	I	Haemolytic inclusion body anaemia on treatment with sulphonamides	Very high (1.8; nearly normal)	The arginine cannot be accommodated in the haem pocket, leaving a large cavity at the ligand site of the iron atom. The haem pocket is wide open
13, 14	Bristol	β67	E11	Val	Asp	I	Haemolytic anaemia with splenomegaly, methaemoglobin formation	Low	Asp at E11 forms a salt bridge with His E7 which would keep the haem pocket tightly closed. Also the carboxyl group would sterically interfere with the bound oxygen
15, 16	Borås	β88	F4	Leu	Arg	I	Mild haemolytic anaemia with splenomegaly. Tendency to methaemoglobin formation	High	The replacement would leave a gap in the haem pocket which apparently makes the haem group more susceptible to autoxidation. This may be the cause of the high oxygen affinity

The positions of all these residues are shown in Fig. 2 of ref. 1. In this and the following tables, Hill's constant and Bohr effect, if determined, are indicated in brackets after the oxygen affinity. References refer to structure, oxygen affinity, and clinical symptoms, in that order. Variability refers to mammalian species only.

Table 2 Substition of Residues Affecting the Balance Between the Two Alternative Tertiary Structures

References	Designation	Residue		Replacement		Variability	Clinical symptoms	Oxygen affinity (Hill's constant; Bohr effect)	Effect of replacement on equilibrium between deoxy and oxy forms
		No.	Position	from	to				
17–19	E	β26	B8	Glu	Lys	I	None in heterozygotes. Mild haemolytic anaemia in homozygotes under stress	Low (normal; normal)	Glu B8β is bound to His G19β in both oxy and deoxy. Its replacement by Lys would introduce a positively charged side chain in oxy where Arg B12β, His G18 and 19β are already closely clustered. In deoxy, on the other hand, the lysyl side chain could be much further away from this cluster. Therefore, the replacement would stabilize the oxy, but not the deoxy, structure
20	Shepherd's Bush	β74	E18	Gly	Asp	V	Mild haemolytic anaemia with splenomegaly	High, P_{50} 3.7 at *p*H 7.4 with P_{50} 6.3 for HbA control; (reduced, 2.5 at *p*H 7.4 with 3.4 for HbA control; normal)	In oxy the side chain of the Asp is easily accommodated, but in deoxy it comes too close to Phe Fl, which would make the tertiary deoxy structure unstable
21, 22	Seattle	β76	E20	Ala	Glu	V	Mild anaemia	Very low (2.1; nearly normal)	Unexplained
23, 24	Agenogi	β90	F6	Glu	Lys	I	None	Low (normal; normal)	Lys F6β could form a salt bridge with the C-terminal carboxyl of the same β-chain in oxyhaemoglobin. This would push tyrosine HC2 into its pocket between helices F and H, which is its deoxy position, and thus lower the oxygen affinity of the β-chains
38	Peterborough	β111	G13	Val	Phe	V	Mild anaemia	Low (normal; normal)	In the deoxy form Phe G13 can be accommodated in the $\alpha_1\beta_1$ contact by displacing the side chain or Gln H5β without further disturbance of the structure. In the oxy form, this is not possible. If oriented towards the $\alpha_1\beta_1$ contact, the side chain of Phe G13 would severely disturb it. If oriented within the β subunit it would make a short contact with Val H12 and produce dislocations. Hence, the replacement would destabilize the oxy, but not the deoxy, structure

The positions of the residue substituted in haemoglobins E and Agenogi are illustrated in Figs. 3 and 4 of ref. 1.

Table 3 Substitution of Residues Affecting the Balance Between the Two Alternative Quaternary Structures

References	Designation	Residue No.	Residue Position	Replacement from	Replacement to	Variability	Clinical symptoms	Oxygen affinity (Hill's constant; Bohr effect)	Effect of replacement on equilibrium between deoxy and oxy forms
25,39	Hirose	β37	C3	Trp	Ser	I	None	Very high (1.5; quarter)	Trp C3β_2 forms a vital part of the $\alpha_1\beta_2$ contact in both forms of haemoglobin. It is in contact with Tyr HC2α_1 in deoxy, and occupies a position such that it would oppose the expulsion of that tyrosine from its pocket between helices F and H which occurs on oxygenation. Removal of Trp C3β would therefore facilitate the transition to the quaternary oxy structure
26*	Malmö	β97	FG4	His	Gln	I	Erythrocytosis	Very high	His FG4 lies at the $\alpha_1\beta_2$ contact but it is not clear why its replacement by Gln should change the oxygen affinity
27, 28	Yakima	β99	G1	Asp	His	I	Erythrocytosis	Very high (1.0; normal)	Asp G1β_2 may be hydrogen bonded to NH of Glu G3β_2 in oxy. In deoxy it is hydrogen bonded to the phenolic OH of Tyr C7α_1. The mutation breaks this important bond in the $\alpha_1\beta_2$ contact of the deoxy form and destabilizes the quaternary structure
29	Kempsey	β99	G1	Asp	Asn	I	Erythrocytosis	Very high	As above
30, 31	Kansas	β103	G4	Asn	Thr	I	Cyanosis	Very low (1.3; present)	Asn G4β_2 forms a hydrogen bond with Asp G1α_1 in oxy. The mutation breaks this important bond in the $\alpha_1\beta_2$ contact of the oxy form and destabilizes the quaternary oxy structure
32	Yoshizuka	β108	G10	Asn	Asp	I	Mild anaemia	Low (normal; reduced)	Asn G10β_1 is in contact with His G10α_1, which lies in a buried position in the $\alpha_1\beta_1$ contact. The replacement of Asn by Asp may lead to the ionization of the histidine, but it is not clear how the degree of ionization would differ in the two alternative quaternary structures

* And personal communication from G. Berglund and T. Leonhardt.

The positions of the residues substituted in haemoglobins Hirose, Malmö, Yakima, Kempsey and Kansas are illustrated in Fig. 8 of ref. 38.

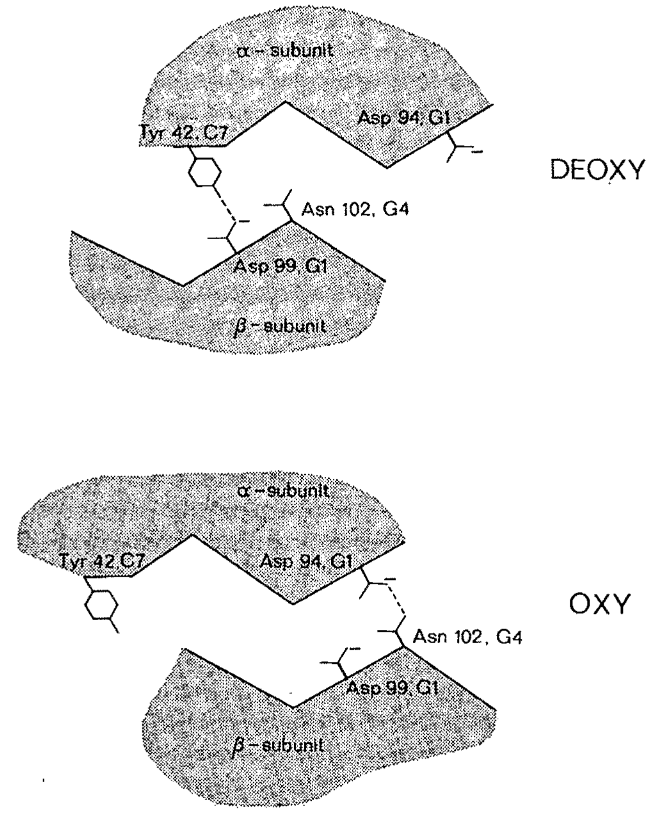


Fig. 3 Change in $\alpha_1\beta_2$ contact on oxygenation. The contact area clicks from one dovetailed position to another, involving a switch of hydrogen bonding as shown. Most of the other contacts are non-polar.

in the more inclined position which it takes up in the tertiary oxy structure. The removal of that ring in the two abnormal haemoglobins may make it easier for the haem group to turn to its more upright position in the deoxy structure.

Among the substitutions affecting the balance between the two alternative tertiary structures listed in Table 2, those of haemoglobins Shepherd's Bush and Peterborough seem to alter the relative free energies of the β subunits in the oxy and deoxy states by purely sterical effects. In Shepherd's Bush the substituted aspartic acid residue is a misfit only in the tertiary deoxy structure, which raises the oxygen affinity, while in Peterborough the substituted phenylalanine is a misfit only in the tertiary oxy structure, which lowers the oxygen affinity. In haemoglobin E the effect seems to be electrostatic, the oxygen affinity being lowered by a cluster of positively charged side chains that destabilizes the oxy structure. The substitution of lysine for glutamic acid in position F6β in haemoglobin Agenogi is interesting because the lysine seems to interact with the C-terminal residues of the β-chain which regulate its oxygen affinity. In deoxyhaemoglobin, tyrosine HC2β is tightly bound in a pocket between helices F and H, but on oxygenation that pocket narrows and the tyrosine is expelled[4]. Any forces which push the tyrosine back into its pocket, such as low *p*H, would also lower the oxygen affinity. Model building shows that a lysine in position F6 could force the tyrosine into its pocket by combining with the C-terminal carboxyl of histidine HC3β. The alanine residue in position E20 is external; its substitution by glutamic acid in haemoglobin Seattle should have no effect on the oxygen affinity. Indeed, an acid group in this position probably occurs in several mammalian species. This haemoglobin may need further study.

The substitutions listed in Table 3 seem to alter the balance of interactions between the subunits. Tyrosine HC2α_1, like the corresponding tyrosine in the β-chain, is wedged into a pocket between helices F and H in the deoxy form and gets expelled on oxygenation. This expulsion is opposed by the indol ring of tryptophan C3β_2. The two residues lie in the $\alpha_1\beta_2$ contact. Replacement of the tryptophan by serine in haemoglobin Hirose would ease the expulsion of the tyrosine and thereby raise the oxygen affinity. It would also open the α_1 and β_2 contact to water and thereby precipitate dissociation into dimers. Kansas, Yakima and Kempsey offer the most striking examples of mutations altering the allosteric equilibrium between the two alternative quaternary structures. They each affect the $\alpha_1\beta_2$ contact in the region between Cα and FGβ where the two subunits slide past each other by several Ångstroms in the course of their reaction with oxygen. In each of the two forms the contact is made up of many van der Waals interactions plus one hydrogen bond (Fig. 3 and Fig. 1 of ref. 3). In deoxyhaemoglobin A that single hydrogen bond is between aspartate G1β_2 and tyrosine C7α_1; in oxyhaemoglobin A it is replaced by another between asparagine G4β_2 and aspartate G1α_1. In Yakima and Kempsey, replacement of aspartate G1β breaks the hydrogen bond that stabilizes the deoxy form, resulting in high oxygen affinity. In Kansas replacement of asparagine G4β by threonine breaks the hydrogen bond that stabilizes the oxy form, resulting in low oxygen affinity (though there may be other factors; Greer found dislocations in the β subunits which may also affect their oxygen affinity[33]).

A diminished Bohr effect has been reported for haemoglobin Yoshizuka. The replaced asparagine residue lies in the $\alpha_1\beta_2$ contact where histidine G10α_1 is normally in contact with the amides of asparagine G10β_1 and glutamine H9β. Replacement of the asparagine by aspartic acid would tend to raise the *pK* of the neighbouring histidine, and if the rise were greater in oxyhaemoglobin than in deoxyhaemoglobin this would oppose the normal Bohr effect. But the atomic models do not suggest any reason why the degree of ionization of histidine G10α in the two forms should differ. The very high oxygen affinity of haemoglobin Malmö is also puzzling. Histidine FG4β is one of the invariant residues at the $\alpha_1\beta_2$ contact, but its part in the allosteric mechanism is still unclear. Similarly, haemoglobins Chesapeake and Capetown in which arginine FG4α is replaced by leucine and glutamine respectively, show high oxygen affinity and low haem–haem interaction which are at present unexplained[34]. Haemoglobin M Iwate has a very low oxygen affinity because it is permanently frozen in the quaternary deoxy structure[36]. Haemoglobins M Milwaukee and Boston also have low oxygen affinities; they are still under X-ray study.

These results show that the equilibrium between the oxy and deoxy forms of haemoglobin can be altered in many ways. One of the two forms may be destabilized because the mutation introduces a bigger side chain which is a misfit in one form but not in the other, or because the mutation leads to a concentration of groups of equal charge which repel each other in one but not the other of the two forms or because it leads to the loss of a hydrogen bond stabilizing one or the other of the two quaternary structures. The equilibrium between the two allosteric forms of the haemoglobin molecule is shown to be very sensitively balanced so that the loss or gain of a single pair of hydrogen bonds has a drastic effect on the oxygen dissociation curve. It is encouraging that even fine modulations of physiological properties of some proteins can now be understood, at least qualitatively, on the basis of atomic models derived from X-ray analysis.

H. M. thanks the Wellcome Trust for a fellowship.

H. MORIMOTO*
H. LEHMANN

MRC Abnormal Haemoglobin Unit,
Department of Biochemistry,
University of Cambridge

M. F. PERUTZ

MRC Laboratory of Molecular Biology, Cambridge

* Present address: Faculty of Engineering Science, Osaka University, Japan.

Received March 23, 1971.

[1] Perutz, M. F., and Lehmann, H., *Nature*, **219**, 902 (1968).
[2] Muirhead, H., and Greer, J., *Nature*, **228**, 516 (1970).
[3] Bolton, W., and Perutz, M. F., *Nature*, **228**, 551 (1970).
[4] Perutz, M. F., *Nature*, **228**, 726 (1970).
[5] Buse, G., Braig, S., and Braunitzer, G., *Hoppe Seyl. Z.*, **349**, (1969).
[6] Rossi Fanelli, A., Antonini, E., and Povoledo, D., *Symposium on Protein Structure* (edit. by Neuberger, A.), 144 (Methuen, London, 1958).
[7] Imai, K., Morimoto, H., Kotani, M., Watari, H., Hirata, W., and Kuroda, M., *Biochim. Biophys. Acta*, **200**, 189 (1970).
[8] Dacie, J. V., Shinton, N. K., Gaffney, P. J., Carrell, R. W., and Lehmann, H., *Nature*, **216**, 663 (1967).
[9] Grimes, A. J., Meisler, A., and Dacie, J. V., *Brit. J. Haematol.*, **10**, 281 (1964).
[10] Muller, C. J., and Kingma, S., *Biochim. Biophys. Acta*, **50**, 595 (1961).
[11] Winterhalter, K. H., Anderson, N. M., Amiconi, G., Antonini, E., and Brunori, M., *Europ. J. Biochem.*, **11**, 435 (1969).
[12] Hitzig, W. H., Frick, P. C., Betke, K., and Huisman, T. H. J., *Helv. Paediat. Acta*, **15**, 499 (1960).
[13] Steadman, J. H., Yates, A., and Huehns, E. R., *Brit. J. Haematol.*, **18**, 435 (1970).
[14] Cathie, I. A. B., *Great Ormond Street J.*, **2**, 43 (1952).
[15] Hollender, A., Lorkin, P. A., Lehmann, H., and Svensson, B., *Nature*, **222**, 953 (1969).
[16] Svensson, B., and Strand, L., *Scand. J. Haematol.*, **4**, 241 (1967).
[17] Hunt, J. A., and Ingram, V. M., *Biochim. Biophys. Acta*, **49**, 520 (1961).
[18] Thompson, R. B., Warrington, R. L., and Bell, W. N., *Amer. J. Physiol.*, **268**, 198 (1965).
[19] Lehmann, H., Story, P., and Thein, H., *Brit. Med. J.*, **1**, 544 (1956).
[20] White, J. M., Brain, M. C., Lorkin, P. A., Lehmann, H., and Smith, M., *Nature*, **225**, 939 (1970).
[21] Huehns, E. R., Hecht, F., Yoshida, A., Stamatoyannopoulos, G., Hartman, J., and Motulsky, A. G., *Blood*, **36**, 209 (1970).
[22] Stamatoyannopoulos, G., Parer, J. T., and Finch, C. A., *New England J. Med.*, **281**, 915 (1969).
[23] Miyaji, T., Suzuki, H., Ohba, Y., and Shibata, S., *Clin. Chim. Acta*, **14**, 624 (1966).
[24] Imai, K., Morimoto, H., Kotani, M., Shibata, S., Miyaji, T., and Matsumoto, K., *Biochim. Biophys. Acta*, **200**, 197 (1970).
[25] Yanase, T., Hanada, M., Seita, M., Ohta, Y., Imamura, T., Fujimura, T., Kawasaki, K., and Yamaoka, K., *Jap. J. Human Genet.*, **13**, 40 (1968).
[26] Lorkin, P. A., Lehmann, H., Fairbanks, V., Berglund, G., and Leonhardt, T., *Biochem. J.*, **119**, 68P (1970).
[27] Jones, R. T., Osgood, E. E., Brimhall, B., and Koler, R. D., *J. Clin. Invest.*, **46**, 1840 (1967).
[28] Novy, M. J., Edwards, M. J., and Metcalfe, J., *J. Clin. Invest.*, **46**, 1848 (1967).
[29] Reed, C. S., Hampson, R., Gordon, S., Jones, R. T., Novy, M. J., Brimhall, B., Edwards, M. J., and Koler, R. D., *Blood*, **31**, 623 (1968).
[30] Bonaventura, J., and Riggs, A., *J. Biol. Chem.*, **243**, 980 (1968).
[31] Reissman, K. R., Ruth, W. E., and Nomura, T., *J. Clin. Invest.*, **40**, 1826 (1961).
[32] Imamura, T., Fujita, S., Ohta, Y., Hanada, M., and Yanase, T., *J. Clin. Invest.*, **48**, 2341 (1969).
[33] Greer, J., *J. Mol. Biol.*, **59**, 99 (1971).
[34] Greer, J., thesis, Univ. Cambridge (1970).
[35] Perutz, M. F., Muirhead, H., Cox, J. M., and Goaman, L. C. G., *Nature*, **219**, 131 (1968).
[36] Greer, J., *J. Mol. Biol.*, **59**, 107 (1971).
[37] Bratu, V., Lorkin, P. A., Lehmann, H., and Predescu, C., *Biochim. Biophys. Acta* (in the press).
[38] King, M. A. R., Wiltshire, B. G., and Lehmann, H., *Brit. J. Pharmacol.* (in the press).
[39] Yamaoka, K., *Blood* (in the press).

Ecology of the Fossil Hominoidea from the Siwaliks of India

THE ecology of the North Indian *Ramapithecus* has been the subject of recent discussion[1–3]. In such discussion the tectonic framework and uplift of the Himalayas and Siwalik sedimentation during the Miocene must be taken into account. The Middle Miocene is usually correlated with the third major upheaval during which the Himalayas acquired their major features characterized by mountain ranges interspersed by large river valleys and swamps. The bulk of the Nagri fauna associated with *Ramapithecus* is known from Haritalyangar[4]. The ecology can be studied by correlating the extinct mammalian material including *Ramapithecus* with the flora.

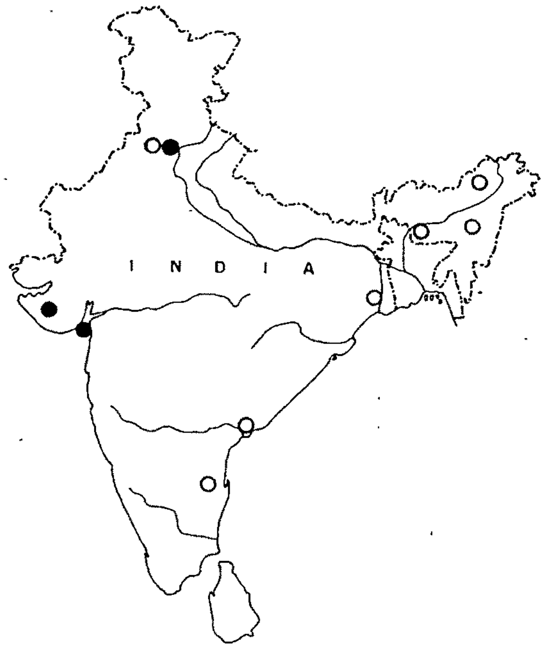


Fig. 1 Floral distribution during the Mio-Pliocene in the Indian region. ○, Miocene; ●, Pliocene.

Two species of *Hipparion* are known from the Nagri beds: the slender, widely occurring *Hipparion antilopinum* may have been a forest browser; the robust *Hipparion theobaldi* apparently preferred an open country environment where forest partially receded especially during the Dhokpathan–Pliocene. *Giraffokeryx*, which is closely allied to *Okapia*, seems to have been a forest dweller, but giraffes are known to prefer open grasslands with scattered trees. The doubtful tubulidentate (*Orycteropus*) could adapt to all types of habitats. The hystrisid and rhyzomyid rodents present in the sediments do not throw sufficient light on environment. The tragulids preferred river banks, whereas the canids and felids could adapt to varying types of habitat. Few typical gazelles are known from Nagri. Convincing evidence for a mixed type of forest vegetation accrues from the presence of dinotheres and true rhinocerotids. It is difficult therefore to draw conclusions about the ecological

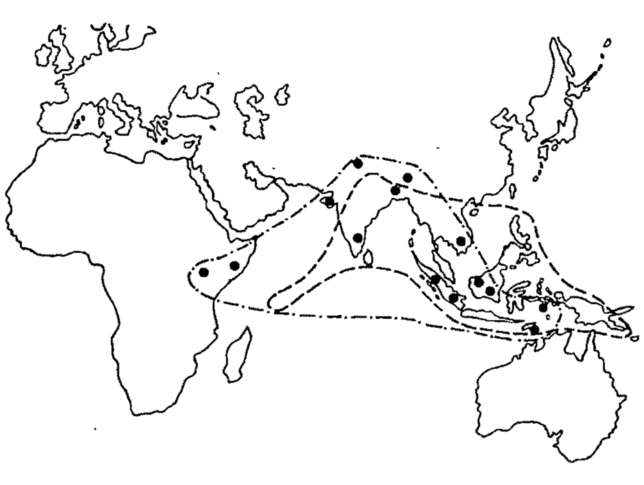


Fig. 2 Distribution of Dipterocarpaceae (modified after Chowdhury, ref. 8). – – –, Distribution today; -·-·-, distribution during the mid Tertiary.

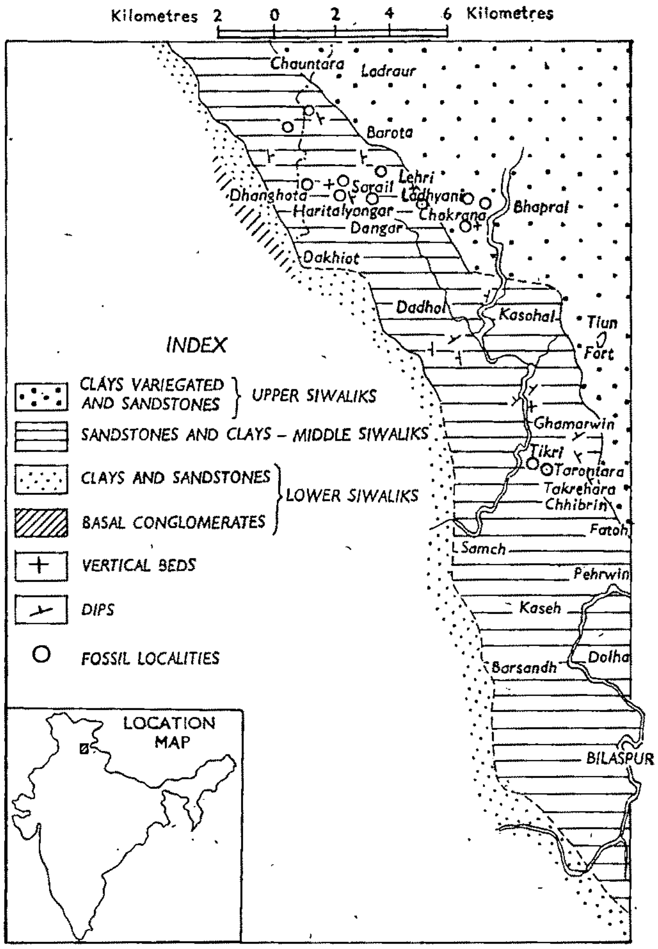


Fig. 3 Geological sketch map of the Bilaspur–Haritalyangar area.

setting of early hominids such as *Ramapithecus* without palaeobotanical evidence.

The evidence for a forest type of habitat in the Miocene lies in the presence of many dicotyledonous woods preserved in the sediments along with extinct mammalian material. Petrified leaf and wood is known from the Siwaliks[5–8]. Miocene flora of evergreen rain forest had a much wider distribution in India during the mid-Tertiary (Fig. 1). *Dipterocarpoxylon* is known from Cutch, the Siwalik Hills of Punjab and foothills region of West Bengal. Fossil woods of Dipterocarpaceae originally distributed in the Malay region are also known from East Africa[8] (see Fig. 2). During the Miocene, *Gluta* was growing in West Bengal and Assam but is now confined to the extreme south-west coast and south Burma. The presence of the moist favouring Dipterocarpaceae and several fossil woods referable to *Mesua, Gluta, Cynometron* and *Calophyllum* in south-east Asia, including the Siwaliks, and in East Africa indicates that the climate during Mio-Pliocene was tropical with plenty of rainfall and supporting heavy forest—fossil woods and other associated floral material are usually considered to be reliable indicators of past climate. The climate seems to have been similar and more or less uniform along the foothills region of the Siwaliks from Assam to Punjab.

Table 1 Distribution of Fossil Dicotyledon Woods in the Tertiary of India (Modified after Ramanujam[9])

Family	Genus and species	Age
Guttiferae	*Kayeoxylon assamicum*	Upper Miocene
	Calophylloseylon eoinophyllum	Middle Miocene
Dipterocarpaceae	*Dipterocarpoxylon garoense*	Upper Miocene
	Dipterocarpoxylon chowdhuryi	Middle Tertiary
	Shoreoxylon evidens	Middle Tertiary
	Anisopteroxylon bengalensis	Sub-Recent
	Anisopteroxylon jwalamukhi	Miocene
Anacardiaceae	*Glutoxylon burmense*	Upper Miocene
	Glutoxylon chowdhuryi	Sub-Recent
	Glutoxylon bengalensis	Pliocene
Leguminosae (Caesalpinae)	*Cynometroxylon indicum*	Upper Miocene
	Pahudioxylon bankuriensis	Upper Miocene
	Pahudioxylon sahnii	Middle Miocene
Combretaceae	*Terminalioxylon chowdhuryi*	Middle Tertiary
	Terminalioxylon tertiarum	Middle Miocene
Ebenaceae	*Ebenoxylon indicum*	Upper Miocene

Leakey[2] asks "Was the very sparse *Ramapithecus* material from the Nagri zone actually excavated from a limited area, at a single level, in association with Lorisids and Pongids?". The Nagri Stage is represented by a thickness of 1,500 m of sediments and most of the fauna have been recovered from various sites within this formation[10,11] (Fig. 3). Tattersall has suggested[1] that it is unlikely that the Nagri hominoids were subjected to heavy predation by social carnivores such as *Crocuta* as propounded by me[4]. Professor G. H. R. Koenigswald (personal communication) has suggested the possibility of predation by crocodiles in addition to hyaenids because numerous crocodilean remains are found in abundance in the Nagri sediments. I suggest therefore that the Nagri environment was one of mixed forest interspersed with streams.

I thank Dr C. G. K. Ramanujam for helpful comments on flora.

K. N. Prasad

Geological Survey of India,
Hyderabad I (AP)

Received April 20, 1971.

[1] Tattersall, I., *Nature*, **221**, 451 (1969).
[2] Leakey, L. S. B., *Nature*, **223**, 1075 (1969).
[3] Tattersall, I., *Nature*, **224**, 821 (1969).
[4] Prasad, K. N., *Palaeontology*, **7**, 124 (1964).
[5] Sahni, B., *Monograph, Sahni Inst.*, No. 1 (1964).
[6] Feistmantel, O., *Rec. Geol. Surv. Ind.*, **15**, 1 (1882).
[7] Lakhanpal, R. N., *Pub. Centr. Adv. Study, Punjab*, 5 (1968).
[8] Chowdhury, K. A., *Palaeobotanist*, 14 (1966).
[9] Ramanujam, C. G. K., *Geol. Soc. Ind.*, Mem 2 (1968).
[10] Pilgrim, G. E., *Rec. Geol. Surv. Ind.*, **63**, 4 (1913).
[11] Prasad, K. N., *Pal. Ind.*, 39 (1969).

Evaluation of Compound Probabilities in Sequential Choice

SITUATIONS frequently occur in which a successful outcome depends on an individual making a correct choice at each of several more or less independent stages. The choice of mode of transport at various stages of a journey is one example. Comparable predicaments occur in professional, administrative, political and military life, and in communication networks generally. The temporal order of the several choices is not invariably a vital factor. Furthermore, the situation as a whole may have a stochastic character in that the probability of correct choice may vary from stage to stage.

We shall describe an experiment simulating this general type of situation, which clearly requires, for a successful outcome, the multiplication of probabilities. Studies[1] of preference for locating a target in an $m \times n$ array, or in a display partitioned into concentric zones, indicate that the cells in the array or the concentric zones are not subjectively equiprobable as locations for the target. Subjects do not seem to guess, at random, the unknown location of the target. Their strategy of search rather has the character of "divining" where the experimenter has hidden it, or of locating it where they would expect it to be. Other experiments[2] suggest that many people, when faced with a situation involving compound probabilities, tend to add, instead of multiply, the chances at the different stages.

We may accordingly predict that in an $m \times n$ array, with an unknown target in each of the m rows, the subject's estimate of his chance of guessing all targets correctly will be exaggerated, as judged by the compound probability, n^{-m}, of locating the target. Our experiment is designed to test this prediction, and to elucidate the phenomena which the situation will generate.

The apparatus consisted of a board on which were set m rows each containing n receptacles, where m and n each took the values 2, 3, 4, 5 or 8. All the receptacles were empty except one in each row which contained a ticket. The number of separate stages therefore ranged from 2 to 8, with the chance of guessing correctly at each stage ranging from 2^{-1} to 2^{-3}.

The subjects were fifty grammar school boys aged 14–15 yr whose "intelligence" may be assumed to be at least as good as that of the average adult. A subject was given to understand that in order to win a prize he had to guess the correct location of the ticket in each row, only one guess per row being allowed. His task was to equate what he thought was the chance of winning the prize with one of a set of lotteries.

There were fourteen lotteries in all. Ten of them had 100 tickets each, the chance of drawing a winning ticket, based on the number of winning tickets in the particular lottery, being 0.01, 0.1, 0.2, 0.3, . . . , 0.9. The remaining four lotteries had 500, 1,000, 5,000 and 10,000 tickets respectively, with the corresponding chance of drawing a winning ticket, 0.002, 0.001, 0.0002 and 0.0001.

The range of values taken by the rows and the range of values of the receptacles allowed for twenty-five different situations. Each of the fifty subjects was assigned at random to five of these situations by means of randomized Latin squares. Ten subjects thus judged each $m \times n$ situation.

The amount of information actually required to locate the target is $m\log_2 n$ "bits", where m is the number of rows, which we will now call "stages", and n the number of receptacles, which will be designated "alternatives". The chance of drawing a winning ticket in the lottery chosen by the subject we shall treat as an indirect estimate of his psychological probability (ψ) of winning a prize.

Table 1 Psychological Probabilities (ψ) based on Choice of Lottery*

No. of alternatives per stage	No. of stages in array 2	3	4	5	8	Mean
2	0.45	0.51	0.36	0.38	0.37	0.41
3	0.41	0.21	0.21	0.21	0.17	0.24
4	0.31	0.23	0.21	0.12	0.11	0.19
5	0.30	0.22	0.17	0.18	0.08	0.19
8	0.07	0.16	0.11	0.13	0.05	0.11
Mean	0.31	0.27	0.21	0.20	0.16	

* Entries in the table represent means of ten observations.

Table 2 Ratios, ψ/p, of Psychological to Compound Probabilities

No. of alternatives per stage	No. of stages in array 2	3	4	5	8
2	1.8	4.0	5.7	12.2	95.0
3	3.8	5.9	17.8	53.2	10^3
4	4.9	14.8	54.0	118.0	7×10^3
5	7.5	27.8	106.0	563.0	3×10^4
8	4.3	82.4	465.0	4×10^3	9×10^5

The results presented in Table 1 indicate that all values of ψ are overestimates, as judged by the compound probabilities, p, thus confirming our prediction. The magnitude of the overestimation is indicated by the ratios of ψ to p in Table 2, which suggests that the realism of the estimates is confounded by an apparent tendency to take less account of the number of stages than of the number of alternatives per stage, although an analysis of variance shows that in relation to the residual variance, both the inter-stage and inter-alternative variances are significant ($P<0.01$).

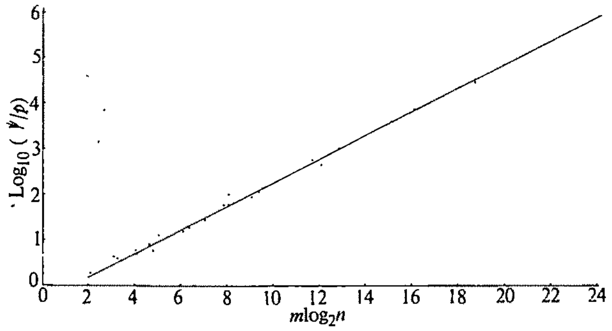


Fig. 1 Log relative overestimation plotted against the number of bits ($\log_{10}(\psi/p)$ against $m\log_2 n$). p, Compound probabilities; m, number of stages; n, number of alternatives.

This differential effect becomes clear if we plot, as in Fig. 1, the logarithm of relative overestimation, $\log_{10}\psi/p$, against the amount of information in bits, $m\log_2 n$, required to locate the target. The relation is linear, and is given by the equation:

$$\log_{10}\psi/p = 0.26\, m\log_2 n - 0.31 \tag{1}$$

This may be re-written as:

$$\psi/p = e^{(0{\cdot}86\, m\log_e n^{-0{\cdot}72})} \tag{2}$$

from which it follows that if we keep the number of stages constant, ψ/p is directly proportional to a power of the number of alternatives per stage, whereas if we keep the number of alternatives constant, ψ/p varies exponentially with the number of stages.

Given m and n, we can predict the mean value of ψ because

$$p = n^{-m} \tag{3}$$

The compound probability p is obtained by multiplying the respective probabilities of guessing correctly at each of the m stages, each of these probabilities being equal to $1/n$.

Therefore, from (2)

$$\psi = e^{-0{\cdot}72}\, n^{-0{\cdot}14\, m} \qquad (e^{-0{\cdot}72} = 0.49) \tag{4}$$

In the main, therefore, the relative overestimation of ψ/p results from a subjective attenuation of the multiplicative factor, m, the number of stages. This attenuation is of the order of six-sevenths of the number of stages, within our experimental constraints. This leads us to infer that the multiplicative element in compound probability is far from being "primitive" or intuitive, which may help to explain the special difficulties which people encounter in the study of statistics.

Our interpretation is supported by similar experiments with subjects aged 9+ and 10+ yr, in which, apart from gross relative overestimation of ψ, no trends are discernible with variations in the values of m and n. This too suggests that the multiplicative element is not primitive.

The method we have used involves an indirect evaluation of ψ. A more direct evaluation could be obtained by asking the subject to choose between different types of array. The utility of the choice, however, might then become an important factor.

This experiment elucidates the apparent tendency, in a variety of multi-stage choice situations, for the decision maker to misjudge the likelihood of his success, and therefore to adopt an inappropriate strategy which he will later regret.

Of historical interest in this connexion is the fact that the most subtle thinkers of ancient Greece, though greatly intrigued by the idea of the possible, especially in Stoic philosophy, never grasped combinatorial analysis, which had to wait until the sixteenth century for its development[3]. Aristotle himself evidently had only a small appreciation of the concept of probability. Whatever intuition of the subject he and others might have had was submerged by long established habits of thought.

The relative overestimation of compound probabilities which the experiment has revealed may be a phenomenon of considerable generality in decision and choice. If so, it merits a special designation. We propose to name it the "inertial ψ effect".

JOHN COHEN
E. I. CHESNICK
D. HARAN

Department of Psychology,
University of Manchester

Received February 3; revised April 22, 1971.

---

[1] Cohen, J., Boyle, L. E., and Chesnick, E. I., *Occup. Psychol.*, 43, 129 (1969).

[2] Cohen, J., and Hansel, C. E. M., *Acta Psychol.*, **13**, 357 (1958).

[3] Sambursky, S., *Osiris*, **12**, 35 (1956).

Similarity Measure for Mixed Attribute Types

SUPPOSE we wish to examine the relationships among n objects (units, samples) with respect to m attributes (variates, characters, tests). Such a need arises in geology and archaeology, literary studies, medicine and all the biological sciences, particularly the taxonomy of plants and animals. I define here a numerical measure of similarity between objects that can be applied when any mixture of quantitative and qualitative attributes is involved and when observations are missing.

Quantitative data are frequently presented as an $n \times m$ matrix, X. For many purposes (for example, principal component analysis) the units are regarded as points in Euclidean space, using the Euclidean distance metric

$$d_{ij} = \sum_{k=1}^{m} (x_{ik} - x_{jk})^2 \tag{1}$$

as a measure of the dissimilarity between units i and j. D can be derived from the "similarity" matrix, $W = XX'$, by the relation

$$d_{ij}^2 = \omega_{ii} + \omega_{jj} - 2\omega_{ij} \tag{2}$$

Unfortunately, in many practical situations, some or all of the attributes cannot be expressed in terms of ordered quantitative values, so that W and D cannot immediately be formed. For purely qualitative data, or presence/absence data, there are many coefficients[1] for matching which measure the similarity, s_{ij}, between units i and j. It can be shown that, for many similarity measures, S is positive semi-definite (p.s.d.) so that there exists a quantitative matrix, X_s, such that $X_s X_s' = S$. Thus, S can be regarded as the W matrix for a configuration given by X_s and therefore defines a Euclidean distance matrix by application of equation (2).

A general coefficient of similarity for mixed quantitative and qualitative attributes can be defined as follows.

If attribute k is quantitative, and scaled to have approximately unit range, let

$$s_{ijk} = x_{ik} x_{jk}$$

If attribute k is qualitative, let

$$s_{ijk} = \begin{cases} 0 \text{ when } x_{ik} \neq x_{jk} \\ 0.5 \text{ when } x_{ik} = x_{jk} \end{cases}$$

Then

$$s_{ij} = \frac{1}{m} \sum_{k=1}^{m} s_{ijk}$$

Thus, the measure is a combination of the simple matching coefficients and the W elements for individual attributes.

The resulting matrix, S_m, is certainly p.s.d., because any r-state qualitative attribute can be expressed as $(r-1)$ dummy quantitative variates to give an expanded data matrix, X_e, and $mS_m = X_e X_e'$. We may wish to scale or weight the attributes by factors, f_k, so that

$$s_{ij} = \frac{1}{m} \sum_{k=1}^{m} f_k s_{ijk}$$

This corresponds to scaling the columns of X_e and therefore the similarity matrix remains p.s.d.

The coefficient is additive over attributes or sets of attributes, which allows a recursive definition of the similarity matrix; because, if S_k is the similarity matrix for the first k attributes, and s_k is the similarity matrix for the k^{th} attribute alone, then

$$S_k = \frac{1}{k} ((k-1)S_{k-1} + s_k) \tag{3}$$

Two points should be noted. (1) In general, when quantitative data are involved, self-similarities do not equal a fixed constant, so that the units are not artificially constrained to lie on a hypersphere. There is thus no "horseshoe" effect. (2) As before, we have

$$d_{ij} = (s_{ii} + s_{jj} - 2s_{ij})^{\frac{1}{2}}$$

independent of the origin of the implicit configuration. But, when the similarity matrix itself is required (for example, for a principal component analysis), the elements of S_m must be centred; this is most easily achieved by replacing each s_{ij} by $(s_{ij} - \bar{s}_i - \bar{s}_j + \bar{s})$, where $\bar{s}_i$ is the mean of the i^{th} row of S_m, and $\bar{s}$ is the overall mean[2].

When either or both of x_{ik} and x_{jk} are unknown, s_{ijk} must be estimated. For Gower's similarity measure (first described by Smith[3]) s_{ijk} is set equal to the similarity based on available attribute comparisons between units i and j. This is unsatisfactory, because the m attributes are more or less independent; for example, in a human population, we would hardly be satisfied with an estimate of age similarity based on sex and blood group similarities. Furthermore, the resulting similarity matrix is not necessarily p.s.d., in which case the data cannot be represented in Euclidean space.

For the common attribute distributions, the best estimate of s_{iik} $(=x_{ik}^2)$ is given by the average of the available similarities for attribute k. This corresponds to estimating the original missing value by the mean of attribute k, based on known observations (using dummy values for qualitatives). Thus, S_m remains p.s.d.

A simple example may clarify this. Suppose we have three subjects whose sex, blood group and age are given in Table 1.

Table 1 Sex Characteristics of Three Subjects

Subject	Sex	Blood group	Age	Age/50
1	Male	A	15	0.3
2	Male	A	40	0.8
3	Female	B	60	1.2

Then, using a scaling divisor of 50 for age, we have

$$s_{11}=\tfrac{1}{3}(0.5+0.5+0.09)=0.3633$$

$$s_{12}=\tfrac{1}{3}(0.5+0.5+0.24)=0.4133$$

$$s_{22}=\tfrac{1}{3}(0.5+0.5+0.64)=0.5467$$

so that

$$d_{12}=(0.3633+0.5467-2\times 0.4133)^{\frac{1}{2}}=(0.0834)^{\frac{1}{2}}=0.289.$$

Similarly, $d_{13}=0.968$ and $d_{23}=0.849$.

Suppose, now, that the age of subject 1 is unknown. Estimating age similarities from sex and blood group similarities gives

$$d_{12}=0.216 \text{ and } d_{13}=1.146$$

so that $d_{12}+d_{23}=0.216+0.849=1.065<d_{13}$; in other words, the triangle inequality is not satisfied. But, when we estimate the age of subject 1 to be $\frac{1}{2}(40+60)=50$, or, alternatively, estimate s_{113} $(=x_{13}^2)$ to be $\frac{1}{4}(s_{223}+s_{333}+2\times s_{233})=\frac{1}{4}(0.64+1.44+2\times 0.96)=1.00$, we have $d_{12}=0.116$ and $d_{13}=0.825$.

A. J. B. Anderson

MRC Clinical and Population Cytogenetics Unit,
Western General Hospital,
Edinburgh

Received November 5, 1970; revised April 6, 1971.

[1] Sokal, R. R., and Sneath, P. H., *Principles of Numerical Taxonomy*, ch. 6 (Freeman, San Francisco and London, 1963).
[2] Anderson, A. J. B., *J. Ecol.* (in the press).
[3] Smith, I. W., *J. Gen. Microbiol.*, 33, 263 (1963).

Nitrogen Fixation and its Significance in Tropical Lake George, Uganda

Tropical lakes have been little studied although they often seem to be very productive, especially at those trophic levels exploitable by man. One such lake, Lake George (0°, 00′, 29° 55′ E) is a shallow lake ($\bar{z}=2.5$ m) with an area of approximately 290 km^2 including the associated channel, and is one of the smaller lakes in the western arm of the Rift Valley of Africa. An important feature of the lake is the high phytoplankton standing crop of approximately 400 mg of chlorophyll a m^{-2}. Yet, in spite of this, there is very little inorganic nitrogen in the water. There is little seasonal variation in species composition, in the numbers of the phytoplankton and in chemical and physical parameters[1,2].

Enrichment experiments have shown that the phytoplankton probably suffer a semi-permanent nitrogen deficiency[3]. An investigation of the supply of nitrogen by direct fixation of N_2 by phytoplankton is consequently of obvious importance. N_2 fixation was measured using a ^{15}N tracer and an acetylene reduction assay[4-8] during wet and dry seasons representative of a full year. A value of 0.008 atom percentage excess over a biological control not exposed to $^{15}N_2$ is significant at the $P=0.05$ level.

Table 1 Seasonal and Depth Variations of Nitrogen Fixation in Lake George

Date (1968)	Incubation depth (cm)	Organic N present (mg l.$^{-1}$)	Atom % excess in sample*	Nitrogen fixed % Fixation (l.$^{-1}$ h^{-1} × 10^3)	(μg l.$^{-1}$ h^{-1})	(mg m^{-2} h^{-1})
February 2	0	2.48	0.0557	22.3	0.55	—
February 5	15	3.18	0.1070	13.4	0.43	—
February 16	0	2.82	0.2791	50.0	1.41	1.66
	15	1.51	0.2317	41.5	0.63	
	40	3.01	0.1625	33.7	1.01	
	150	2.52	0.1479	30.6	0.77	
March 5	0	4.09	0.0618	16.0	0.65	0.44
	15	1.18	0.0457	11.4	0.13	
	40	3.96	0.0263	6.6	0.26	
	150	4.01	0.0123	4.7	0.19	
March 20	0	4.14	0.0435	7.5	0.31	—
March 26	0	4.10	0.1615	24.3	1.00	0.89
	15	3.93	0.1371	16.9	0.66	
	40	3.96	0.2353	23.5	0.93	
	150	2.34	0.0377	3.8	0.09	

* After correction for partial pressure and enrichment of $^{15}N_2$ gas used.

Table 1 shows that N_2 fixation rates of 0.44–1.66 mg N m^{-2} h^{-1} varied irregularly during wet, dry and interseason weather. This contrasts with the pattern in temperate and polar lakes where pronounced peak(s) of N_2 fixation occur during the warm water period[8-12]. The relative maximum rate of fixation, expressed as a proportion of the total amount of nitrogen present, was 0.05% h^{-1} (approximately 0.6% day^{-1}). Satisfactory reasons for these irregular and continuously high rates are not known but the following points are probably influential. First, Lake George diel variations in phytoplanktonic standing crop and productivity can be greater than seasonal changes. Second, the euphotic zone is shallow (60 cm) so that the algae receive irregular amounts of light as they are circulated in and out of the zone. Thus on successive days the physiological state, previous light history and nutrient status of the phytoplankton may often have been different even when the samples were collected at the same time and place.

It is possible that in the afternoon dissolved O_2, which may reach 250% saturation, could reduce the rate of N_2 fixation[13,14]. The frequent pronounced minima of N_2 fixation at 15 cm, which was the depth of maximum photosynthesis during much of the day, may be a direct result of high oxygen concentration, the effect of which must be quite rapid as it was found in both long and short exposure experiments.

The daily fixation against depth profiles reveals a surface maximum, a subsurface maximum at 40 cm and pronounced minima at 15 and 150 cm. This pattern, partially due to crop size, was different from the exponential decrease with depth that occurs in the English Lakes[9] but was more like the pattern found in Tjeukemeer[8] and Clear Lake[15]. Fixation at 150 cm, a region of very low light, was nevertheless appreciable, ranging from 3.8×10^{-30}% h^{-1} (0.09 μg N l.$^{-1}$ h^{-1}) to 30.6×10^{-3}% h^{-1} (0.77 μg N l^{-1} h^{-1}). This consistent fixation at 150 cm requires comment because this was a region with a light intensity well below the photosynthesis/respiration compensation point. The phenomenon of significant fixation in the dark was also found occasionally in the thermally stratified lakes of the English Lake District[9] so that it would seem to be common to a wide variety of waters. Photosynthetic bacteria are present in late summer in the anoxic hypolimnium of eutrophic Esthwaite Water, England (V. G. Collins, personal communication), and may contribute to the high rate of fixation in that lake[9]. By analogy, it is possible that photosynthetic bacteria growing at very low light intensities together with non-photosynthetic heterotrophic bacteria were contributing to fixation at 150 cm in Lake George. Results of diurnal studies of N_2 fixation in Lake George indicate that most of the fixation at 150 cm during daylight occurred in the morning when the oxygen concentration is low. Recent work showing N_2 fixation in the anoxic hypolimnia and sediments of several lakes in North Americ[16,17]a supports the idea of some bacterial contributions to low light fixation in Lake George. But no fixation was observed in illuminated, anoxic, H_2S-producing sediments of the lake and the anoxic zone immediately above the mud is subject to oxygen fluctuations which would deter strict anaerobes. The organism(s) responsible for fixation rates below the photic zone is thus not certain.

No species from the lake was isolated in axenic culture and tested for nitrogen fixation. Fixation was tentatively ascribed to planktonic species of *Anabaena* and *Anabaenopsis*, both of which are suspected to fix nitrogen, although there are few studies concerning pure cultures of truly planktonic blue-green algae. Both these algae were common in the lake[1], and possessed heterocysts, which are correlated with nitrogen fixation in lakes[9,15].

Table 2 Nitrogen Balance Sheet for Lake George—Major Features

Annual gain Source	Amount (mg)	Annual loss Source	Amount (mg)
N_2 fixation	1,280	Denitrification	?
Stream inflow *	322	Stream plankton outflow	3,180
Rainfall	277	Sedimentation	655
Hippopotamus excreta †	76-99	Commercial fish export	50–75

* Minimum values and does not include surface runoff.

† Estimated from sparse knowledge of daily herbage consumption and digestion efficiency[20], extrapolations from cattle urine excretion, and nitrogen analysis of faeces[18].

The annual nitrogen fixation is estimated to be approximately 1,300 Mg for the whole lake, or 44 kg ha^{-1} yr^{-1}. This quantity represents approximately 33% of the lake's annual nitrogen change and thus represents an important factor in the overall budget (Table 2). The details of calculations of nitrogen inflows and outflows from Lake George are given elsewhere[18]. They are based on the flow rates of the principal influents with corrections for direct rainfall and evaporation and the nitrogen content of the mean standing crop of plankton. This high percentage annual contribution is put in perspective when set against the similar value of 38% for Clear Lake, and the considerably lower value of 0.5% for the thermally stratified English lakes[9].

The percentage for Smith Lake, Alaska, was estimated to be 5–10% (Alexander, personal communication); that for Lake Mendota, Wisconsin, is 8.5% (Fitzgerald, personal communication) while that for a Russian Lake[19] was indirectly estimated to be 8%.

It would seem from our results that, in Lake George, the shortage of combined inorganic nitrogen as a nutrient for primary production puts a premium on atmospheric N_2 fixation. The daily rate at which this is carried out (0.6% of total N), although less than the highest values, is within the upper ranges reported for most other waters. More important, the relative constancy of the equatorial climate sustains this rate throughout the year, so the total N_2 fixed becomes extremely important in the nitrogen budget of the lake.

This study is part of a 5 yr International Biological Programme project sponsored by the Royal Society and was undertaken while A. J. H. was in receipt of a grant to study nitrogen fixation *in situ* from the Scientific Research Council. We thank Professors G. E. Fogg, W. D. P. Stewart and Dr J. F. Talling for their help.

A. J. HORNE

Clear Lake Algal Research Unit,
Institute of Ecology,
University of California, Davis 95616

A. B. VINER

International Biological Programme,
Royal Society African Freshwater Biological Team,
PO Box 50,
Lake Katwe, Uganda

Received January 20; revised April 20, 1971.

[1] Ganf, G. G., thesis, Univ. Lancaster (1969).
[2] Viner, A. B., *Verh. Intern. Verein. Limnol.*, **17**, 289 (1969).
[3] Viner, A. B., in *Bioenergetics and Tropical Ecosystems* (IBP/UNESCO regional symposium) (in the press).
[4] Neess, J. C., Dugdale, R. C., Dugdale, V. A., and Goering, J. J., *Limnol. Oceanog.*, **7**, 163 (1962).
[5] Fogg, G. E., and Horne, A. J., in *Chemical Environment in the Aquatic Habitat* (edit. by Golterman, H. L., and Clymo, R. C.), 115 (Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, 1967).
[6] Stewart, W. D. P., Fitzgerald, G. P., and Burris, R. H., *Proc. US Nat. Acad. Sci.*, **58**, 2081 (1967).
[7] Hardy, R. W. P., Holsten, R. D., Jackson, E. K., and Burns, R. C., *Plant Physiol.*, **43**, 1185 (1968).
[8] Horne, A. J., thesis, Univ. of Dundee (1969).
[9] Horne, A. J., and Fogg, G. E., *Proc. Roy. Soc.*, B, **175**, 351 (1970).
[10] Dugdale, R. C., and Dugdale, V. A., *Limnol. Oceanog.*, **7**, 170 (1962).
[11] Goering, J. J., and Neess, J. C., *Limnol. Oceanog.*, **9**, 530 (1964).
[12] Billaud, V. A., *J. Fish. Res. Board Canada*, **25**, 2101 (1968).
[13] Stewart, W. D. P., Haystead, A., and Pearson, H. W., *Nature*, **224**, 226 (1969).
[14] Stewart, W. D. P., and Lex, M., *Arch. Microbiol.*, **73**, 250 (1970).
[15] Horne, A. J., *Brit. Phycol. J.* (in the press).
[16] Brezonik, P. L., and Keirn, M. A., *Abst. Thirty-third Ann. Meeting Limnol. Oceanog. Soc.* (Kingston, Rhode Island, 1970).
[17] Howard, D. L., Frea, J. I., Pfister, R. M., and Dugan, P. R., *Science*, **169**, 61 (1970).
[18] Viner, A. B., thesis, Univ. London (1970).
[19] Kusnezow, S. I., *Die Rolle der Mikroorganismen im Stoff-kreislauf der Seen* (Berlin, 1959).
[20] Field, C. R., thesis, Univ. Cambridge (1968).

Bomb ^{14}C in the Human Population

IN the atmosphere ^{14}C occurs principally as $^{14}CO_2$ and is usually produced by nuclear reactions between cosmic ray neutrons and the nitrogen atoms of the air. The natural equilibrium between production and disintegration of ^{14}C determines a part of the natural background radiation to the human population. From 1955 there has been a gradual increase of ^{14}C in the atmosphere, the land biosphere and the ocean, as a result of nuclear tests. Although ^{14}C was initially not regarded as an important hazard to man[1], it was later pointed out[2–4] that ^{14}C could be a source of appreciable genetic hazard in the world's population, because of its long half life (5,700 yr).

At this laboratory we have studied the ^{14}C concentration in the human body[5]. The correspondence between ^{14}C in the atmosphere and in the human body, mediated as it is by photosynthesis, has been confirmed in 6 yr of measurements[6–8].

Since 1955, about two-thirds of the total nuclear energy liberated in nuclear tests has resulted from tests carried out in the atmosphere at high northern latitudes in 1961 and 1962 (ref. 9). The subsequent transfer of ^{14}C down to the troposphere, the biosphere and the ocean has been followed in detail[10–16]. The ^{14}C excess[16] ($\delta^{14}C$) in the northern troposphere (Fig. 1) is representative chiefly of the region between 30° N and 90° N, although the curve for the southern troposphere is representative of the region from the equator to 90° S.

The model for CO_2 exchange between the various reservoirs is shown in Fig. 2, in which the CO_2 in the troposphere is in exchange with CO_2 in the stratosphere, the land biosphere and the ocean. For CO_2 exchange between the troposphere and the biosphere we share the view of Münnich (for discussion, see ref. 17), who divided the biosphere into two parts. The first (b_1), which consists of leaves, grass, branches, and so on, is in rapid exchange with the troposphere and is combined with this reservoir, but the larger part of the vegetation (b_2) has a much slower exchange rate and is combined with the humus layer.

The ocean is divided into two reservoirs, the mixed layer and the deep ocean. The exchange of CO_2 between the troposphere and the ocean occurs chiefly in the mixed layer, but according to Craig[18] there is also the possibility of a direct exchange with the deep ocean. The ^{14}C concentration in the mixed layer of the ocean is now 10 to 15% above normal[19,20].

Using the model of Fig. 2, we have treated the decrease of $\delta^{14}C$ in the troposphere in a previous article[20]. We showed that the measured variation of $\delta^{14}C$ (x_t) in the troposphere is approximately reproduced by the following two-term exponential function:

$$x_t = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \qquad (1)$$

in which the parameters A_1, A_2, k_1, and k_2 depend on the various exchange coefficients. Because the errors on some of these coefficients are large, the extrapolation is uncertain and is given in the shaded area of Fig. 1.

The amount of bomb-produced ^{14}C in the atmosphere has increased the total natural amount of ^{14}C in nature by about 3%. According to Harkness *et al.*[8], the production of CO_2 from fossil fuel would lower the natural ^{14}C concentration in the atmosphere to about 16% below normal at the end of this century. It is thus reasonable that the ^{14}C excess caused by the atomic bomb will be more than compensated for by the dilution of inactive carbon (the Suess effect).

The transfer of ^{14}C into the human body depends on the following three factors: (1) the time between the photosynthesis in vegetational food and its consumption; (2) the diet, particularly the amount of vegetational food, and (3) the residence time of the carbon in the constituents of human tissue.

Broecker *et al.*[6] (Fig. 1) found that it took 1 and 1.8 yr before the ^{14}C concentration in blood and lung tissue, respectively, reached that in the atmosphere. They also found that the $\delta^{14}C$ value of blood had a maximum time lag of 6 months behind food.

Berger and collaborators[7,21-23] (Fig. 1) studied chiefly the metabolic turnover time of the constituents of human tissue. For this they used samples from persons who had travelled from the southern to the northern hemisphere. One result of their work was that the incorporation in these people of ^{14}C

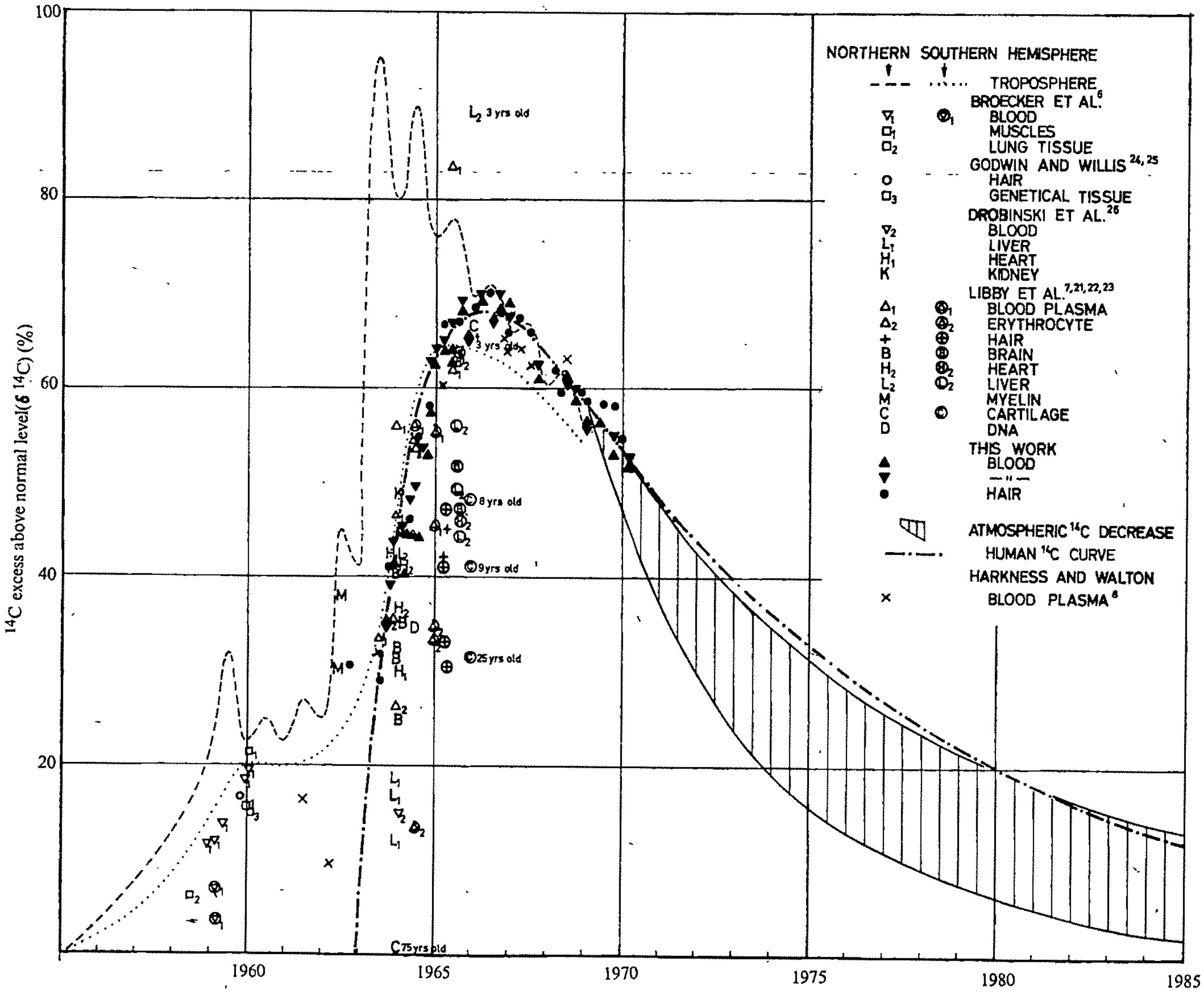


Fig. 1 Radiocarbon in the troposphere and human body.

in brain protein and lipids, liver, heart, plasma protein and erythrocyte protein was very similar to, and reflected, the atmospheric ^{14}C content present several months earlier. Collagen of cartilage was found to be metabolically inert in older persons. The concentration of ^{14}C in the human body has been studied by other workers (refs. 8, 24–26) who obtained values shown in Fig. 1.

At our laboratory the transfer of ^{14}C into the human body was studied by following the time-variation of $\delta^{14}C$ in blood and hair for three persons[5,27] (Tables 1–3). No separation between blood plasma and erythrocyte protein was performed, and the measured ^{14}C activity is thus a mean value for the total blood samples. Fig. 1 shows that there is excellent agreement between data obtained for the blood and for the hair samples. The values of the ^{14}C concentration in blood plasma obtained by Harkness and Walton[8] are slightly lower than ours.

Table 1 Carbon in Neck Hair, from a Boy (K.N.) Born in 1962

Time of collection		$\delta^{14}C$ %	$\delta^{13}C$ /‰	$\Delta^{14}C$ %
November	1962	30.7±1.3		
June	1963	29.2±0.8	−19.7	26.7
October	1963	41.0±1.0	−19.2†	39.3
March	1964	46.0±1.0	−18.9	44.2
July	1964	54.8±1.0	−19.2†	52.9
October	1964	58.0±0.8	−18.6	55.9
February	1965	66.9±1.0	−19.2†	64.9
July	1965	67.2±1.0	−18.6	64.5
February	1966	68.7±1.0	−19.2	66.7
May	1966	70.0±0.9	−19.2†	68.0
December	1966	65.9±0.9	−19.2†	63.9
February	1967	67.5±1.1	−19.2†	65.4
July	1967	65.9±0.9	−19.2†	63.9
February	1968	61.5±0.9	−19.2†	59.6
May	1968	60.2±1.1	−19.2†	58.2
August	1968	61.8±1.2	−19.2†	59.9
November	1968	59.7±1.1	−19.2†	57.7
January	1969	58.7±1.2	−19.2†	56.7
April	1969	58.2±1.2	−19.2†	56.5
May	1969	58.3±1.2	−19.2†	56.6
July	1969	58.1±1.1	−19.2†	56.4
October	1969	54.9±0.9	−19.2†	53.1
December	1969	54.7±1.1	−19.2†	52.9

† Not measured (mean value).

Table 2 Carbon in Blood Samples, from a Woman (I.H.), 26 Yr Old in 1963

Time of collection		$\delta^{14}C$ %	$\delta^{13}C$ /‰	$\Delta^{14}C$ %
September	1963	34.9±1.1	−22.0	34.0±1.1
October	1963	39.3±0.8	−22.2	38.5±0.8
November	1963	43.6±0.8	−22.7	42.9±0.9
February	1964	45.6±1.1	−22.0	44.7±1.2
April	1964	48.1±1.1	−21.7	47.1±1.1
June	1964	49.6±1.1	−21.7†	48.6±1.1
July	1964	53.7±1.0	−22.1	52.8±1.1
November	1964	62.9±0.9	−19.8	61.2±0.9
January	1965	64.2±0.9	−19.7	62.4±0.9
March	1965	65.0±0.9	−20.5	63.5±1.0
May	1965	67.1±1.0	−21.1	65.6±1.0
September	1965	69.1±0.9	−22.0	67.9±1.0
November	1965	65.6±0.9	−21.4	64.2±1.0
March	1966	69.7±0.9	−22.3	68.7±0.9
July	1966	67.6±1.1	−21.2	66.2±1.1
September	1966	69.8±1.0	−21.5	68.6±1.1
December	1966	67.4±1.0	−21.9	66.3±1.0
September	1967	62.5±0.9	−21.7†	61.4±0.9
June	1968	60.6±0.9	−21.7†	59.5±1.0
August	1968	59.9±0.9	−21.7†	58.8±1.0
January	1969	56.0±0.7	−21.7†	55.0±0.7
September	1969	55.5±1.2	−21.7†	54.5±1.2
March	1970	52.6±1.2	−21.7†	51.6±1.2

† Not measured (mean value).

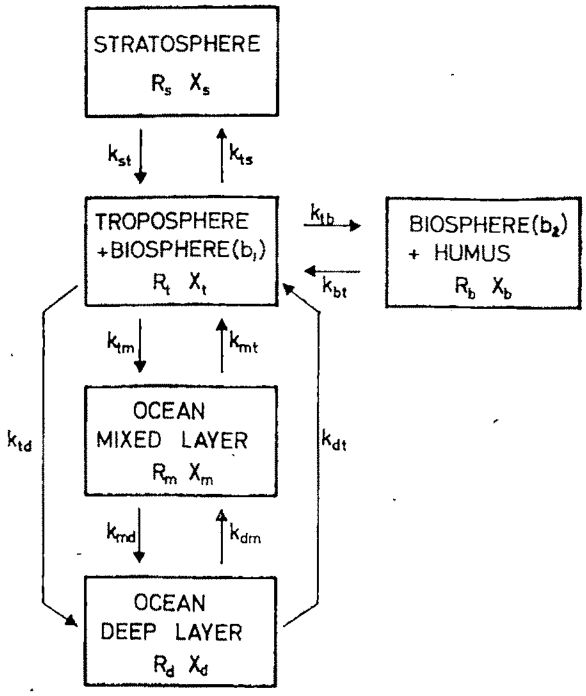


Fig. 2 Exchangeable carbon reservoirs, where: R_t, total carbon amount; X_t, ^{14}C excess; k_{ij}, exchange coefficients; i, j, a, b, m, d $(i \mp j)$.

Table 3 Carbon in Blood Samples, from a Woman (A.L.), 26 Yr Old in 1963

Time of collection		$\delta^{14}C$ %	$\delta^{13}C$ /‰	$\Delta^{14}C$ %
September	1963	35.2±0.8	−21.6	34.2±0.8
November	1963	41.5±0.6	−21.7†	40.6±0.7
February	1964	40.5±0.6	−20.9	39.3±0.7
March	1964	44.4±0.8	−22.5	43.5±0.9
April	1964	44.2±0.7	−23.2	43.6±0.7
June	1964	44.2±1.0	−22.1	43.3±1.0
August	1964	53.4±0.8	−21.9	52.4±0.9
November	1964	57.6±0.9	−20.7	56.2±0.9
January	1965	62.4±0.9	−19.1	60.5±0.9
March	1965	64.3±0.7	−20.1	62.6±0.7
May	1965	63.9±0.9	−21.0	62.5±1.0
September	1965	68.4±0.9	−22.0	67.3±1.0
November	1965	65.9±0.7	−21.4	64.4±0.8
March	1966	69.6±1.0	−22.0	68.5±1.1
July	1966	67.5±1.0	−21.2	66.2±1.1
September	1966	68.3±1.0	−20.9	66.9±1.0
December	1966	69.0±0.9	−20.8	67.6±1.0
September	1967	60.9±0.9	−21.7†	59.8±0.9
June	1968	61.4±0.9	−21.7†	60.3±0.9
August	1968	58.8±0.9	−21.7†	57.7±0.9
January	1969	56.6±0.7	−21.7†	55.6±0.7
May	1969	56.4±1.0	−21.7†	55.4±1.0
September	1969	53.0±1.0	−21.7†	52.0±1.0
March	1970	51.6±1.1	−21.7†	50.6±1.1

† Not measured (mean value).

The blood and hair data are almost representative for persons living in the northern hemisphere. Because $\delta^{14}C$ in the southern troposphere at present lags behind that of the northern troposphere by about 1 yr, there should also be a similar lag for $\delta^{14}C$ in the human populations of the respective hemispheres. After about 1970, the ^{14}C concentrations in people in the northern and southern hemispheres will be similar, and equal to those in the troposphere. Fig. 1 shows that $\delta^{14}C$ (x_H) in the human body appears as a pulse, delayed with respect to that of the atmosphere. The observed values for x_H can be fitted reasonably well by the following two-term exponential function:

$$x_H = 108\,(e^{-0.1t} - e^{-0.75t}) \qquad (2)$$

The coefficients in this function were determined by a least mean squares method, using the upper limits of the measured values for ^{14}C in the human body in the period from 1963 to 1970, and extrapolated values in the troposphere in the period from 1970 to 2000. The upper limit values were chosen because there was some excess ^{14}C before 1963 which should also be considered. There is also a tendency for the blood and hair data during the last 2 yr to correspond with the upper limit of the shaded area in Fig. 1. Function (2) was simplified by assuming that all previous nuclear tests occurred within a short time interval, and that the ^{14}C increase in the human population started in about January 1963.

The first term in the brackets of equation (2) indicates that the excess ^{14}C in the human body has a mean lifetime of about 10 yr and the second term that ^{14}C enters the human body with a mean delay time of about 1.4 yr after production in the atmosphere. The latter value is probably accurate to within 30% and agrees with previous estimates[6,7].

The hazard to the human population from artificial radiocarbon arises largely from inventory radiation of the body. Natural ^{14}C contributes with a certain dose rate, r_0, as a result of its decay rate of about fourteen disintegrations per min per gram of carbon. The average value of r_0 is about 1.06 mrad yr[9]. The dose from natural ^{14}C is distributed as follows: 1.64 mrad/yr in the bones, 1.15 mrad/yr in the cells lining bone surfaces and 0.71 mrad/yr in bone marrow and soft tissue. Applying function (2), the dose D_t absorbed in the human body during a time t can be calculated from the formula:

$$D_1 = 1.08\, r_0 \int_0^t (e^{-0.1t} - e^{-0.75t})\, dt \qquad (3)$$

For a period of about 30 yr the total radiation dose from this source will be $9r_0$. We thus obtain a total radiation dose of 16 mrad to bone, 11 mrad to cells lining bone surfaces and 7 mrad to bone marrow and soft tissue. The genetic hazard is caused by the latter. That dose constitutes about 10% of the total gonad dose from all radioactive fallout. Purdom[4] pointed out, however, that the actual gonad dose is somewhat larger because of a transmutation process in the DNA molecule, in which the decaying ^{14}C atoms are replaced by nitrogen atoms. Purdom assumed that the biological damage from the transmutation was equal to that from β-radiation.

The amount of artificial ^{14}C in the human body at about the year AD 2000 will constitute about 3% of the total amount of ^{14}C. This isotope has a half-life of 5,700 yr, and several scientists[2,3,9,28] think that it would therefore cause a most serious genetic threat. The long term radiation dose can be calculated from the formula

$$D_2 = 0.03 r_0 \int_0^t e^{-0.000125t}\, dt$$

The total radiation doses ($D_1 + D_2$) which will be received by the bone cells, the cells lining bone surfaces, and the bone marrow and soft tissue in the next 10,000 yr will be 410, 290 and 180 mrad, respectively. These doses, which are in agreement with values given in a United Nations report[9] (page 45), are more important than those from all other radioactive fallout. We question, however, the value of the long term radiation dose (D_2) because, as previously mentioned, the use of fossil fuel might reduce the ^{14}C concentration in man below normal. We are of the opinion that the only ^{14}C hazard from previous tests which should be taken into account is attributable to a total genetic dose (D) of the order of 10 mrad, received in a period of about 30 yr. This dose is, however, negligible compared with the dose received from natural sources, which constitutes about 100 mrad per yr.

We thank Norges Almenvitenskapelige Forskningsråd for financial support.

REIDAR NYDAL
KNUT LÖVSETH
ODDVEIG SYRSTAD

Radiological Dating Laboratory,
Norwegian Institute of Technology,
Trondheim, Norway

Received November 10, 1969; revised August 24, 1970.

[1] Libby, W. F., *Science*, **123**, 657 (1956).
[2] Pauling, L., *Science*, **128**, 3333 (1958).
[3] Sakharow, A. D., *Soviet Scientists on the Danger of Nuclear Tests*, 39 (Foreign Languages Publishing House, Moscow, 1960).
[4] Purdom, C. E., *New Scientist*, **298**, 255 (1962).
[5] Nydal, R., *Nature*, **200**, 212 (1963).
[6] Broecker, W. A., Schulert, A., and Olson, E. A., *Science*, **130**, 331 (1959).
[7] Libby, W. F., Berger, R., Mead, J. F., Alexander, G. V., and Ross, J. F., *Science*, **146**, 1170 (1964).
[8] Harkness, D. D., and Walton, A., *Nature*, **223**, 1216 (1969).
[9] *Rep. UN Sci. Comm. Effect of Atomic Radiation*, No. 14 (A/5814) (United Nations, New York, 1964).
[10] Münnich, K. O., and Roether, W., *Proc. Monaco Symp.*, 93 (Vienna, 1967).
[11] Bien, G., and Suess, H., *Proc. Monaco Symp.*, 105 (Vienna, 1967).
[12] Rafter, T. A., *NZ J. Sci.*, **8**, 4, 472 (1965).
[13] Rafter, T. A., *NZ J. Sci.*, **11**, 4, 551 (1968).
[14] Young, J. A., and Fairhall, A. W., *J. Geophys. Res.*, **73**, 1185 (1968).
[15] Lal, D., and Rama, *J. Geophys. Res.*, **71**, 2865 (1966).
[16] Nydal, R., *J. Geophys. Res.*, **73**, 3617 (1968).
[17] Nydal, R., *Symp. on Radioactive Dating and Methods of Low-Level Counting, UN Doc. SM 87/29* (International Atomic Energy Agency, Vienna, 1967).
[18] Craig, H., *Second UN Intern. Conf. on the Peaceful Uses of Atomic Energy*, A/CONF. 15/P/1979 (June 1958).
[19] Rafter, T. A., and O'Brien, B. J., *Proc. 12th Nobel Symp.* (Almquist and Wiksell, Uppsala, 1970).
[20] Nydal, R., and Lövseth, K., *CACR Symp.* (Heidelberg, 1969); *J. Geophys. Res.*, **75**, 2271 (1970).
[21] Berger, R., Fergusson, G. J., and Libby, W. F., *Amer. J. Sci., Radiocarbon Suppl.*, **7**, 336 (1965).
[22] Berger, R., and Libby, W. F., *Amer. J. Sci., Radiocarbon Suppl.*, **8**, 467 (1966).
[23] Berger, R., and Libby, W. F., *Amer. J. Sci., Radiocarbon Suppl.*, **9**, 477 (1967).
[24] Godwin, H., and Willis, E. H., *Amer. J. Sci., Radiocarbon Suppl.*, **2**, 62 (1960).
[25] Godwin, H., and Willis, E. H., *Amer. J. Sci., Radiocarbon Suppl.*, **3**, 77 (1961).
[26] Drobinski, jun., J. C., La Gotta, D. P., Goldin, A. S., and Terril, jun., J. G., *Health Phys.*, **11**, 385 (1965.)
[27] Nydal, R., and Lövseth, K., *Nature*, **206**, 1029 (1965).
[28] Pauling, L., *Les Prix Nobel*, 296 (1963) (Nobelstiftelsen, Stockholm, 1964).

Cosmic Ray Flashes in the Eye

THE observations by Apollo astronauts of light flashes in the eye during lunar flights have been interpreted by Fazio *et al.*[1] as Čerenkov radiation. These authors have also considered the possibility of direct stimulation of the retinal cells by the passage of cosmic rays. I wish to show that the observed effects can also be interpreted as scintillations produced in the eye lens by the passage of multiply charged cosmic rays.

It has been found in experiments here that scintillations are produced by alpha particles of 5.3 MeV in eye lenses from freshly killed bullocks. The polonium alpha source was deposited on the tip of a silver wire which was then inserted into the lens. The lens and incorporated alpha source were mounted between a pair of photomultipliers operated in coincidence and easily detectable scintillations were observed. The single electron pulse spectrum was measured by established techniques[2]; using the manufacturer's figure of 10% for the quantum efficiency of the photocathode, the measured maximum pulse height of the scintillations and the known geometry of the experimental arrangement, a figure of 1,000 photons was calculated for the approximate size of the maximum scintillations produced by 5.3 MeV alpha particles along their track length of 40 μm in the eye lens. The higher specific ionization produced by M and H group nuclei in the primary cosmic radiation should produce much larger scintillations for comparatively low particle energies of less than 1 GeV.

Light from such scintillations is of course distributed over the whole interior of the eyeball and it is necessary to postulate some focusing mechanism for some of this light to account for the point flashes reported by astronauts. The water–air interface at the front surface of the cornea will act as a partially reflecting concave mirror for light coming from within the eye. When the eyelid is not in optical contact with the cornea, the percentage of light reflected back into the eye is about 2% and scintillations in the lens will be focused to give an image on the retina. The focusing will be sharp only for certain points within the eye lens and the amount of light reflected back will be limited by the aperture of the pupil of the eye.

For a fully dilated pupil, approximately 0.15% of the light produced in a scintillation in the eye lens will be reflected to give an image on the retina and for a scintillation track of length 60 μm the light reflected will fall within an area of diameter 300 μm on the retina. Taking ten photons as the minimum detectable, a scintillation size of 7,000 photons is therefore required to give a detectable flash. In general, if a particle has sufficient energy to travel through several mm of the eye lens two points along the scintillation track will be brought to sharp focus on the retina, thus producing a double flash. If the eye is closed, reflexion will take place at the inside surface of the eyelid. The partially diffuse nature of this reflexion will mean that the flashes should appear as blobs, though this will be compensated to some extent by the improvement in the fraction of light reflected at this surface.

Scintillations large enough to be visible will be produced only by particles of high charge and relatively low energies (less than 1 GeV). Such particles are below the geomagnetic cut-off for latitudes such as have been used in manned Earth orbiting satellites and it is not to be expected that visible flashes would be reported in such flights. Measurements have not yet been made of the flux of low energy primary cosmic radiation in the regions well away from the Earth, so it is not possible to calculate an expected flash rate. However, taking a figure of 1 per min as the flash frequency observed by the astronauts leads to a flux of particles producing visible scintillations of 7 m^{-2} s^{-1} sr^{-1}, which seems to be of the right order of magnitude.

The number of visible flashes should be considerably increased if the reflexion coefficient at the front surface of the cornea is improved. This can be done by the use of contact lenses which are silvered on the front surface. As D'Arcy and Porter[3] have reported visible flashes seen by dark adapted observers, an attempt was made to see if this effect was enhanced by the wearing of a contact lens aluminized on the front surface. So far it has been possible to do this only with one observer, but flashes of two different sorts have been reported with a low total frequency of about one every 20 min. These flashes were described either as balls of light or as a cloud of light. It is suggested that attempts to confirm these observations might be made in high altitude flights, where there should be an appreciable flux of the primary radiation.

I thank Dr S. McMurry for wearing an aluminized contact lens and reporting on the observed effects, and Dr J. Fitzgerald for depositing the reflecting aluminium on the lens used.

The recent article by Charman *et al.*[4] on the effects observed when 14 MeV neutrons interact in the eye tends to support the scintillation hypothesis because knock-on protons with energies of this order could produce enough photons to be just detectable after reflexion to a dark adapted retina. The streaks reported when the neutron beam enters the eyeball laterally are to be expected since a scintillation track of finite length perpendicular to the lens axis will produce a finite line image on the retina. Knock-on protons produced by 3 MeV neutrons would not, on the basis of the figures quoted earlier, produce visible flashes. This accords with the results obtained by Charman *et al.*[4].

I. R. McAULAY

Physics Department,
Trinity College,
Dublin

Received April 30; revised May 10, 1971.

[1] Fazio, G. G., Jelley, J. V., and Charman, W. N., *Nature*, **228**, 260 (1970).
[2] Delaney, C. F. G., and Walton, P. W., *Nuclear Instruments and Methods*, **25**, 353 (1964).
[3] D'Arcy, F. J., and Porter, N. A., *Nature*, **196**, 1013 (1962).
[4] Charman, W. N., Dennis, J. A., Fazio, G. G., and Jelley, J. V., *Nature*, **230**, 522 (1971).

Structure of Acetylcholine

In a recent letter, Chothia and Pauling[1] comment on our paper dealing with the determinants of specificity at cholinergic synapses[2]. They correctly point out that we do not define the conformation of acetylcholine which is responsible for its action at nicotinic sites. Moreover, they present a calculation to show that because the acetylcholine ester grouping is not planar in the conformation shown in the figures, it represents only a negligible proportion of the molecules in solution. From this they conclude that this model of acetylcholine is not relevant to the roles of the molecule as a neurotransmitter.

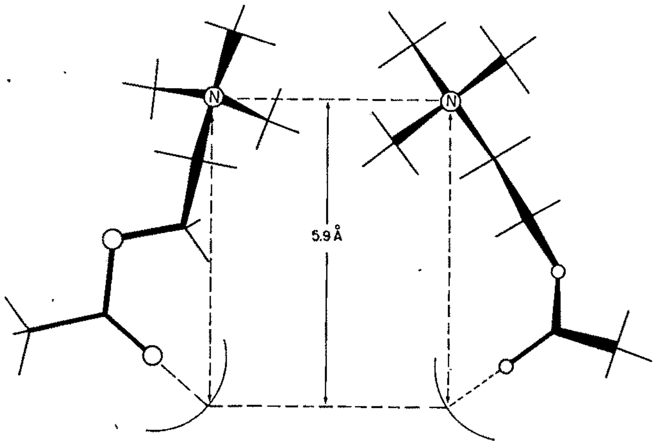


Fig. 1 The drawing used to demonstrate that acetylcholine can meet the proposed requirements for nicotinic activity in our paper is shown here on the right. In the conformation shown on the left the ester group is planar and the requirements are nonetheless fulfilled.

Our analysis is based exclusively on the spatial disposition of functional groups in cholinergic drugs which have either rigid structures or limited degrees of conformational freedom. For each of these drugs we specify and justify a specific conformation. In contrast, we suggest no specific conformation for acetylcholine, and the structure of acetylcholine represented in the diagrams was not intended to imply one. It was meant to demonstrate simply that acetylcholine can fulfil the requirements specified by the rigid drugs, which for the nicotinic receptor is a distance of 5.9 Å between the charge and H-bond. Acetylcholine can meet these restrictions in a very large number of conformations that maintain the atoms in the ester grouping coplanar (Fig. 1). The large number of conformations can be appreciated by recalling that there is essentially free rotation about the O—C bond, that rotation about the bond between the α and β carbons also occurs readily[3] and that hydrogen bonds involving oxygen as the acceptor have few, if any, geometric restrictions[4].

WILLIAM H. BEERS
EDWARD REICH

The Rockefeller University,
New York,
New York 10021

Received April 13, 1971.

[1] Chothia, C., and Pauling, P., *Nature*, **229**, 281 (1971).
[2] Beers, W. H., and Reich, E., *Nature*, **228**, 917 (1970).
[3] Burgen, A. S. V., *Ann. Rev. Pharmacol.*, **10**, 7 (1970).
[4] Pauling, L., *The Nature of the Chemical Bond*, third ed. (Cornell University Press, Ithaca, 1960).

Presence and Function of Disclinations in Surface Coats of Unicellular Organisms

IF a cell which is topologically a sphere is covered with a coat composed of identical units arranged to form a surface crystal, this crystal necessarily[1] contains screw disclinations of total strength +2 complete rotations. Harris and Scriven[2] draw attention to the micrograph of a marine bacterium, *Nitrosomonas*, published by Watson and Remsen[3], on the cover of *Science*, showing a coat of apparently spherical units in close packing. They say that intrinsic disclinations are strongly indicated. We have undertaken further analysis of the disclination structure of this coat.

The structure clearly contains a grain boundary which terminates in the visible part of the coat. Such a termination is a partial disclination. We can show that the visible part of the coat, including the two regions which have apparently been removed in preparation, contains just two disclinations each of strength +1/6. Fig. 1 reproduces the micrograph of Watson and Remsen, with the addition of lines indicating the continuous variation of a lattice direction in the crystal. To analyse the disclination structure, a point was taken that lay between the termination of the grain boundary and the margin of the adjacent missing region. The lattice direction at this point which lies roughly parallel to the long axis of the photograph was taken as the reference direction; this was also assumed to be the long axis of the organism. From this point, two circuits were described, each encircling one of the missing regions counter-clockwise. The lattice direction followed continuously round the circuit has rotated by $\pi/3$ counter-clockwise when the circuit closes. Each circuit therefore contains screw disclinations of total strength +1/6. One disclination is dissociated, and one of its partial disclinations appears as the termination of a grain boundary; the other disclination lies completely within the region which has been removed in preparation.

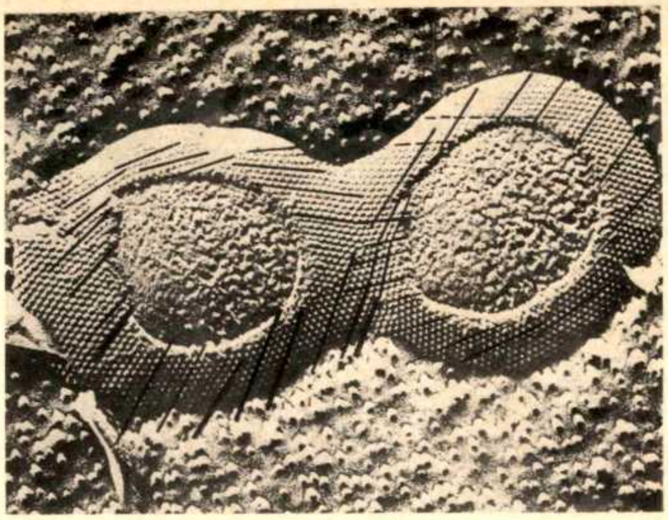


Fig. 1 Micrograph of the coat of *Nitrosomonas* with additional lines indicating the continuous variation in the lattice direction in the crystal.

A disclination of strength $(6-n)/6$ has n-fold rotational symmetry. Thus the addition of two such disclinations, one at each pole, would account for the necessary total strength +12/6 and would fit an assumed n-fold symmetry of the cell. There are exactly two values for n that imply disclinations all of the same strength: $n=5$, with a disclination of strength +1/6 at each pole, and $n=6$ with no disclinations at the poles. The case $n=7$ requires fourteen disclinations of strength +1/6 and two of strength −1/6; all other possible values of n require disclinations at the poles with strengths of magnitude greater than the minimum. Dislocation glide provides a mechanism whereby disclinations can dissociate into disclinations of lower strength and move to lower the total strain energy[2]. Therefore one expects all values of n, other than 5 and 6, to be unlikely. In the micrograph there is no evidence for flattening at the poles as is required if $n=6$. Thus one is left with the most probable result that $n=5$, that is, that there are twelve disclinations each of strength +1/6, including one at each pole.

While the presence of disclinations in coats of this kind is a geometrical necessity, they have no obvious direct biological function. They can, however, act as sources of edge dislocations[2], and edge dislocations are the natural growth points in a surface crystal[4]. This suggests the intriguing possibility of the chemotherapy of diseases associated with the growth of cells having membranes of this kind by the mechanism known to metallurgists as Cottrell locking. A suitable small molecule will be incorporated into the structure at the core of an edge dislocation in preference to an additional unit of the membrane. Further growth at this point is stopped.

F. R. N. NABARRO

Physique des Solides,
Faculté des Sciences,
Paris XI—Orsay

W. F. HARRIS

Department of Chemical Engineering,
University of the Witwatersrand,
Johannesburg

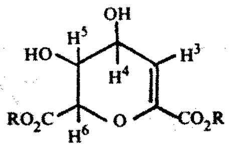
Received April 26, 1971.

[1] Harris, W. F., in *Fundamental Aspects of Dislocation Theory* (edit. by Simmons, J. A., *et al.*) (National Bureau of Standards, Washington, DC, in the press).
[2] Harris, W. F., and Scriven, L. E., *J. Appl. Phys.* (in the press).
[3] Watson, S. W., and Remsen, C. C., *Science*, **163**, 685 (1969).
[4] Harris, W. F., and Scriven, L. E., *Nature*, **228**, 827 (1970).

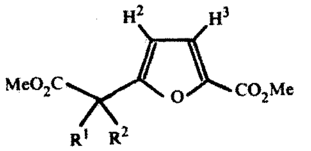
Daucic Acid and its Occurrence in Plants

DURING anion-exchange chromatography of extracts of biological materials, acidic non-nucleotide contaminants of the nucleotide fraction have often been encountered. They are usually identified by their characteristic ultraviolet absorption maxima. Thus the leaves of maize (*Zea mays*) were shown to contain chelidonic acid[1], the leaves of wheat (*Triticum sativum*) *trans*-aconitic acid (unpublished results of A. J. K., and M. J. Cornelius) and certain animal tissues ascorbic acid[2]. These compounds were present in sufficient quantities to interfere with ultraviolet analysis of the chromatographic fractions. During a study[3] on the free nucleotides of the root of carrot (*Daucus carota*) another contaminant was observed with a maximum at 240 nm. The spectrum did not change with *p*H until decomposition occurred in highly acid media. The contaminant was eluted with adenosine diphosphate which indicated a strong or multibasic acid.

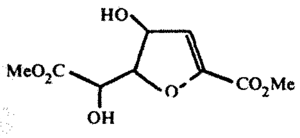
Bulk quantities of this acid, conveniently named daucic acid, were isolated from carrots (preferably mature) by extraction of the pulped roots with hot water. After the removal of solid material, the acid, together with nucleotide and polysaccharide material, was adsorbed on activated charcoal at *p*H 2. The charcoal was washed with water and the acids desorbed with 0.2 M ammonium hydroxide. The solution was concentrated and the low molecular weight ammonium salt (located by the 240 nm ultraviolet absorption), was separated from nucleotide and polysaccharide material by gel filtration on 'Sephadex G 10'. Further purification was achieved by ion exchange chromatography[4] on 'Dowex-1 X 4' (200–400 mesh). The free acid, liberated using the hydrogen form of a cation-exchange resin, was methylated with diazomethane in methanol–ether. By this procedure, 12.7 kg of carrot roots yielded 460 mg of the diester.



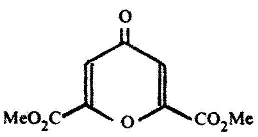
I



II



III



IV

Dimethyl daucate (I; R = Me) had a melting point (in ethyl acetate–light petroleum (boiling point 60–80° C)) of 130–131° C, an $[\alpha]_D^{24.5}$ of −102° (c, 0.9 in acetone), a mass spectrum, M^+ 232, with significant peaks at *m/e* 169, 155, 145 (100%), 139 and 127, a λ_{max} (in ethyl alcohol) at 242 nm (ε 5,600); ν_{max} (in 'Nujol') at 3,500, 1,740, 1,720 and 1,650 cm^{-1} and τ (in $CDCl_3$) 3.96 (1H, m, *H*-3), 5.34 (1H, m, *H*-6), 5.49 (1H, m, *H*-4), 5.70 (1H, m, *H*-5), 6.20 (3H, s, O*Me*), 6.23 (3H, s, O*Me*), 6.72 (2H, m, O*H*). [Found, C, 46.4; H, 5.04; $C_9H_{12}O_7$ requires C, 46.6; H, 5.2%.] INDOR decoupling experiments[5] gave $J_{3,4} = 3$ Hz, $J_{4,5}$ indeterminate, $J_{4,6} = 1$ Hz, $J_{3,5} = 1$ Hz, $J_{3,6} = O$, $J_{5,6} = 2$ Hz. These assignments were supported by a study of the diacetate[7] of the diester of $\tau(C_6D_6)$ 3.98 (1 H, m, *H*-3), 4.15 (1 H, m, *H*-5), 4.37 (1 H, m, *H*-4), 5.65 (1 H, m, *H*-6), 6.56 (6 H, s, O*Me*), 8.21 (6 H, s, $COCH_3$). INDOR experiments[4] gave $J_{3,4} = 2.6$ Hz, $J_{4,5} = 4.4$ Hz, $J_{4,6} = 0.8$ Hz, $J_{3,5} = 1.5$ Hz, $J_{3,6} = O$, $J_{5,6} = 1.8$ Hz.

The diester (I; R = Me) was treated with dry hydrogen chloride in methanol to give a 40% yield of the furan (II; $R^1 = OH$, $R^2 = H$) with a melting point (in ethyl acetate–light petroleum (boiling point 60–80°)) of 134–135°, a mass spectrum, M^+ 214, with significant peaks at m/e 183, 155 (100%) and 122, an $[\alpha]_D^{25}$ of +79° (c, 0.2, acetone), a λ_{max} (in ethyl alcohol) at 256 nm (ε 16,600), ν_{max} ($CHCl_3$) at 3,550, 1,740, 1,720 and 1,600 cm^{-1} and $\tau(CDCl_3)$ 2.93 (1 H, d, J = 4 Hz, *H*-3), 3.58 (1 H, d, J = 4 Hz, *H*-2), 4.80 (1 H, s, α-*H*), 6.16 (3 H, s, O*Me*), 6.23 (3 H, s, O*Me*), 6.74 (1 H, br. s, exchangeable with D_2O, O*H*). The furan (II; $R^1 = OH$, $R^2 = H$) was oxidized with selenium dioxide in refluxing xylene to give, in high yield, the optically inactive ketofuran (all new compounds have been analysed by micro or mass spectral analytical techniques). (II; R^1, $R^2 = O$), m.p. (ethyl acetate) 115–116°, ν_{max} ($CHCl_3$), 1,735, 1,685 $cm.^{-1}$, λ_{max} (EtOH), 293 nm (ε 13,000), mass spectrum M^+ 212, with significant peaks at m/e 181, 153 (100%) and 140, $\tau(CDCl_3)$ 2.20 (1 H, d, J = 4 Hz, *H*-2), 2.63 (1 H, d, J = 4 Hz, *H*-3), 6.45 (3 H, s, O*Me*), 6.60 (3 H, s, O*Me*). The ketofuran was further degraded by oxidation with alkaline hydrogen peroxide to furan −2,5-dicarboxylic acid. The dimethyl ester of this was identical in all respects to authentic material[6].

This correlation established the structure of the methanolysis product of the daucic ester as (II; $R^1 = OH$, $R^2 = H$). This could have arisen from either the dihydropyran structure (I, R = Me) or the dihydrofuran isomer (III). The nuclear magnetic resonance evidence favours structure I and this was confirmed chemically by oxidation of daucic acid methyl ester with chromium trioxide in pyridine to dimethylchelidonate (IV) (40% yield) which was identical in all respects with authentic material. The furanoid compounds (II; R^1, $R^2 = O$) and racemic compounds (II, $R^1 = OH$, $R^2 = H$) were synthesized by conventional methods[7] and found to be identical in all respects to the naturally derived compounds except for the optical activity of the naturally derived (II, $R^1 = OH$, $R^2 = H$). The stereochemistry of daucic acid will be discussed in another report.

Daucic acid has been detected in plants belonging to various other families, in wheat, sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus*) and tobacco (*Nicotiana tabacum*), and would seem to be widely distributed. The possible relationship to primary metabolic processes is being investigated.

We thank Mr P. N. Jenkins for conducting INDOR experiments, Mr M. J. Cornelius for help with the extraction procedures and Professor J. Edelman for his interest. B. D. B. acknowledges a CAPS award from the Science Research Council and Shell Research Ltd.

A. J. KEYS *

ARC Unit of Plant Physiology,

C. J. LEAVER †

Department of Botany,

D. H. R. BARTON
B. D. BROWN
D. A. WIDDOWSON

Department of Chemistry,
Imperial College,
London SW7

Received April 29, 1971.

* Present address: Rothamsted Research Station, Harpenden, Hertfordshire.

† Present address: Department of Botany, University of Edinburgh, Edinburgh 8.

[1] Atkinson, M. R., and Eckermann, G., *Aust. J. Biol. Sci.*, **18**, 437 (1965).

[2] Bengtsson, L. P., Deutsch, A., and Nilsson, R., *Acta Obstet. Gynec. Scand.*, **43**, 381 (1964).

[3] Leaver, C. J., thesis, Univ. London (1966).

[4] Keys, A. J., *Biochem. J.*, **108**, 1 (1968).

[5] Kowalewski, V. J., de Kowalewski, D. G., and Ferrá, E. C., *J. Mol. Spectrosc.*, **20**, 203 (1966).

[6] Yoder, P. A., and Tollens, B., *Ber.*, **34**, 3446 (1901).

[7] Gagnaire, D., and Monzeglio, P., *Bull. Soc. Chim. Fr.*, 474 (1965).

[8] Shmagina, N. N., and Moshkin, P. A., *Plasticheskie Massy*, 58 (1965) [*C. A.*, **62**, 16168e (1965)].

[9] Chakravarti, S. N., and Swaminathan, M., *J. Ind. Chem. Soc.*, **11**, 715 (1934).

BOOK REVIEWS

The Academic Profession

The British Academics. By A. H. Halsey and M. A. Trow. (Society Today and Tomorrow.) Pp. 560. (Faber: London, May 1971.) £5.

"It is virtually impossible that any professor should make the conduct and regulation of his faculty the principal occupation of his life. I myself am a practising barrister—my two immediate predecessors were beneficed clergymen." This is the evidence, before the Royal Commission on Cambridge in 1852, of Henry Main, twenty-third professor of civil law. His evidence was not anomalous: the Royal Commission on Oxford, sitting at the same time, wrote that it was desirable that a university professorship should become "a recognized profession". By 1952 there were some 11,000 professional university teachers in Britain. Today there are more than 30,000: and a graph in Halsey and Trow's book (page 140) predicts an academic profession of some 50,000 by 1980. On this prediction about one in eight hundred of the estimated working population of the United Kingdom would be a university teacher!

So the academics of Britain certainly deserve authoritative and objective study. Halsey and Trow have now provided this study, with a clarity and perception unmatched by any previous writers on the subject. It was not an easy task, for the profession is curiously ambivalent. Its members have a dual loyalty: on one hand, to the "guild" (for want of a better word) of chemists or historians or economists on whose esteem their status in the profession depends; on the other hand, to their avocation to teach in a faculty of the university. Critics of the profession commonly assume that loyalty to the guild takes precedence over loyalty to the student. But it is a profession widely misunderstood, for a simple reason. A lawyer, a doctor, a clergyman, are professionally in touch with ordinary citizens every day. A professor of (say) pure mathematics can achieve an international reputation within his guild without having communicated, professionally, with any except other pure mathematicians. This important book, if people will take the trouble to read it, will go far toward dispelling misunderstanding.

It would be unfair on the authors for a reviewer to try to summarize 560 pages of statistical data and closely argued analysis. Suffice it to say that the book, though expensive even by modern standards, is one which those concerned with decision-making in universities cannot afford to be without. For, as the authors say: "The government is the final arbiter of numbers and money but the university teachers themselves are the managers of expansion. Their assumptions and preoccupations are therefore crucial". And the assumptions and preoccupations of these teachers "shape thousands of unpublished decisions made in colleges and committees, and will continue to influence the rate and form of change in the 1970s". Halsey and Trow, whose views on the sociology of higher education have already won worldwide respect, have painstakingly analysed the motives and prejudices of the academics taking part in the great debate between elitists and expansionists.

After an introduction to "the English idea of a university" (it would be churlish, when the book is already so long, to wish that there had been more about the Scottish idea) the book anatomizes the academic profession (including an entertaining section in which ex-vice-chancellors will wryly recognize their image) into its statistical components: age-structure, pecuniary rewards, social origin and education, and the differentials in emolument and status which still, apparently, persist between Oxford and Cambridge on one hand, and civic universities on the other. The authors then turn to their main theme, which is the attitude of academics toward the expansion of universities in Britain. It is a theme of critical importance. Unless technological civilization collapses, mass higher education is inevitably coming to Britain; any government, left or right, is committed to financing it. But how the finance will be apportioned between university education, as we now understand it, and other forms of higher education (either in universities or elsewhere) is not yet decided. Through the "*crypto-dirigisme*" of the purse, the University Grants Committee, or even the Department of Education and Science, may—probably will—influence this apportionment. But in the long run it is the academics themselves who will determine the educational consequences, as surely as doctors in the health service determine diagnosis and treatment. So to ask how a university teacher's education, social origins, political persuasion, affects his views on university expansion and on the balance between teaching and research is to ask a paramount question. This is the question which Halsey and Trow ask, and answer, for Britain in the late 1960s.

To appreciate their findings in perspective, one must read the book. But in a review for a journal read chiefly by natural scientists it is desirable to offer some reassurance about the methodology and style of a book written by two social scientists. As for methodology, I yield to none in my scepticism about conclusions drawn from sociological questionnaires, and this book is full of tables compiled from replies to questionnaires. But Halsey and Trow followed up their enquiries by intensive interviews. Their experience and objectivity as interviewers are such that the natural scientist's natural suspicions can be put at rest. Another reasonable misgiving which a natural scientist has about sociological observations is that it is difficult to make them as independent of the observer as physical observations can be. What do Halsey and Trow themselves think about élitism versus expansion? And do their views bias their observations? This the reader must be left to decide for himself. Though detecting glimpses of the authors' opinion, I am satisfied that they have achieved a remarkable objectivity.

As for style, there is none of the exasperating jargon (commoner, it is true, from America than from Britain) behind which lesser sociologists conceal the ambiguity of their thoughts. The style is courageously simple and clear. It displays, for posterity, the state of the British academic profession at a critical point in its history.

Eric Ashby

Heartfelt Tale

The History of Coronary Heart Disease. (New Series: Vol. xviii.) By J. O. Leibowitz. Pp. xvii+227+15. (Wellcome Institute: London, December 1970.) £3.

Coronary heart disease is an indiscriminate killer that strikes men down in their prime. In the present century it is one of the scourges of modern life, having replaced smallpox and other infectious diseases as a major hazard to mankind. In medicine, as in other fields of human activity, knowledge of history makes the present more comprehensible even if the future remains unpredictable and, with this in mind, Dr J. O. Leibowitz has written a history of coronary heart disease.

His book provides an excellent background to understanding the present state of medical knowledge in this field. Starting with antiquity and the Middle Ages, he traces the first tentative glimmerings of knowledge of early writers who were concerned on the one hand to define the anatomy and function of the coronary circulation, and on the other to describe individual clinical problems causing sudden death. In the seventeenth century, William Harvey's discovery of the circulation of the blood provided the major physiological breakthrough on which all subsequent development was based. During the next hundred years, an increasing number of clinical reports of individual cases was published and Dr Leibowitz draws attention to a well documented account of rupture of the heart described by Harvey himself in 1649. But it was not until the eighteenth century that advances in clinical medicine and pathology began to parallel those which had taken place earlier in the basic sciences, and Heberden's classical description of angina pectoris, never bettered by any writer since, was published in 1769. Within ten years disease of the coronary arteries was recognized as causing angina pectoris and by the end of the century the work of Parry, Jenner and others had clearly established the relationship between coronary artery disease, angina pectoris and sudden death. The nineteenth century saw the development of cellular and vascular pathology, but only in the twentieth century, with the advent of electrocardiography, was coronary heart disease in its manifold forms fully recognized and Dr Leibowitz unfolds a fascinating story of the developments which have taken place during the past fifty years. Investigation at present is directed specifically to the study of aetiology and prevention, and the book concludes with an up to date account of contemporary thought.

This book is a work of scholarship drawing on the widest possible range of sources and providing an excellent bibliography. Footnotes are well written and unobtrusive, yet provide useful small print information. There is an unexpected error in the identity of a Dr Anonymous who had left his body to Dr Heberden for dissection following his death from angina pectoris in 1772. The autopsy failed to show any abnormality of the heart. Dr Leibowitz identified this public spirited patient as Dr Haygarth of Chester, a physician of outstanding repute in the field of infectious fevers. Haygarth, however, cannot have been Heberden's patient, for he lived on until 1827 when he died in Bath at the advanced age of 87. Such minor points do not detract in any way from the excellence of this scholarly account of one of the major diseases of modern times.

C. C. Booth

Catching the Light

Invertebrate Photoreceptors: a Comparative Analysis. By Jerome J. Wolken. Pp. xi+179. (Academic: New York and London, May 1971.) $9.50; £4.45.

In spite of a generalized title; J. J. Wolken's little book is an illustrative rather than an exhaustive comparative study of invertebrate photoreceptors. By way of introduction the book begins with a description of the electromagnetic spectrum and the physics of light, and this is combined with equally useful information concerning the classification and basic biochemistry of the photosensitive pigments found in plants and animals.

The comparative section begins with that group of animals which has been neglected perhaps the most by photobiologists—the Protozoa—illustrated in this case by the flagellate, *Euglena gracilis*. The fine structures of the flagellum and eyespot are described and experimental details of the animals' photobehaviour are integrated with microspectrophotometric studies of the various organs involved. This is followed by a consideration of the compound eye as found in many insects and Crustacea. Some nine genera of arthropods are described in detail, including the copepod *Copilia*, a species which has become ubiquitous in textbooks of late, presumably because of the perversely unusual visual apparatus it possesses. The invertebrate survey is concluded with a study of the structure of the photoreceptors found in the refracting eye of cephalopod molluscs, such as the squid. It was wise of the author to contrast all the invertebrate structures with the vertebrate retinal rod. The whole forms a good introduction to the study of photoreceptor structure and the subject is made all the more easy to grasp by virtue of the many fine micrographs which form the text figures.

The second part of the book is concerned with the vertebrate and invertebrate visual pigments and deals with techniques for their extraction and purification. The various absorption spectra as measured both *in vitro* and *in vivo* are then presented and discussed, as are similar results for the accessory and screening pigments found in association with the various photoreceptors.

An important generalization in the concluding section is that a functional difference exists between the "open" type of rhabdomere arrangement found in the dipterous insects and the "closed" or fused rhabdomeres of many insects and Crustacea (excepting, of course, *Copilia* which is of the open type). This difference is seen as the adaptation of the closed type to vision at low light intensities, which is fair enough except to say that many insects with closed type rhabdomeres, for example, the bees and wasps, the dragonflies and perhaps the locusts and grasshoppers, are as diurnal in habit as any of the open eyed Diptera. To correlate the structural findings with the electrophysiological evidence the author associates the open eye with the "fast" category of electroretinogram and the closed eye with the "slow" type. Once again the bees are usually considered as having the fast electrical responses, yet are shown to possess the closed rhabdomere arrangement, so that one feels the author should have presented a rather fuller argument for his interpretation of photoreceptor fine structure.

Omitting as it does almost all reference to the electrophysiological events associated with visual excitation, this book leaves the impression of a story unfinished, and this is one reason why *Invertebrate Photoreceptors* remains a good basis for the study of the subject but lacks the breadth to make it a thoroughgoing comparative account.

John Patterson

Snakes of Two Regions

A Field Guide to the Snakes of Southern Africa. By V. F. M. FitzSimons. Pp. 221+12 plates. (Collins: London, January 1971.) £2.10.

The Snakes of Europe. By J. W. Steward. Pp. 238+28 photographs. (David and Charles: Newton Abbot, March 1971.) £2.75.

Europe has thirty-three species of snakes, only five of them confined to the area. There was a general work on European reptiles nearly a hundred years ago and a book devoted to the snakes in particular nearly sixty years ago. Mr Steward brings us up to date on the basis of years of field and vivarium experience and much travel. Southern Africa has three and a half times the number of species, about forty-two of them endemic, and may well have undiscovered forms. Dr FitzSimons's handbook is based on his own pioneering studies in the museum and the field.

Both works recognize a considerable number of subspecies. Although both authors sometimes pause to express doubts about the status of some taxa neither of them discusses the nature of subspecies and makes it clear that they are subjective categories; the existence of zones of intermediacy is barely hinted. Surely the non-specialist would have been better served by an informal survey of the variant regional populations, some of which have been recognized as subspecies. I make this comment partly because I believe that, in many cases, when a larger number of characters is studied, many of them will be found to vary independently and many of the subspecies boundaries be seen to be arbitrary.

Both authors give aids to identification but neither takes the reader gently by the hand through the problems nor mentions museums as a source of information. FitzSimons groups most, but not all, of the snakes by predominant colour and pattern. If one's snake be mostly brown, however, there are fifty-nine to choose from; this can be narrowed further if it be banded, spotted, striped or plain, but a considerable choice would still remain. Steward has a dichotomous key based on simple characters but some of these, for example scale-row counts, are not explained in text or figures. So, in either case, to be reasonably sure, the novice might often finish up scanning the generous illustrations (many in colour for Southern Africa) and wading through individual accounts.

FitzSimons gives a tabular summary of distributions by regions. He gives English and Afrikaans names but only occasionally notices that the snakes have African names. A valuable feature of Steward's book is a distribution map for every species and subspecies. My only comment is that the firmness of the boundary lines can be misleading and is sometimes at variance with the text. There are outline figures of the head scales of most species, unfortunately rather small in relation to the size of the page, and some of them rather vague; several have no nostril. Another valuable feature is a list of the common names in thirteen European languages.

The great value of both of these works lies in the precise description of each form with an account of the habits, where they are known. Sometimes I pause to wonder just how many years of field experience have gone into just a couple of sentences. The books are of about the same size. FitzSimons's work understandably gives more space to snakebite. Perhaps because the European snake fauna is smaller, Steward has more room to discuss the habits of snakes, to interpret their distribution patterns and to convey his personal enthusiasm.

I wish both these works many years of useful service and further editions.

GARTH UNDERWOOD

Hodograph Equations

The Hodograph Equations: an Introduction to the Mathematical Theory of Plane Transonic Flow. By A. R. Manwell. (University Mathematical Monographs.) Pp. ix+476. (Oliver and Boyd: Edinburgh, February 1971.) £10.

ALTHOUGH this book has the title *The Hodograph Equations* it is almost exclusively concerned with the application of the hodograph method to the problem of the plane transonic flow of an inviscid gas. This approach to gas dynamics was pioneered at the beginning of this century by Chaplygin and received a tremendous impetus after the end of the Second World War as the study of transonic phenomena was intensified. The author gives a detailed account of the work up to about 1965. Although slanted to some extent towards the interests of his own considerable contributions to the subject over some twenty years, a very fair overall picture of the subject is presented and the work of the leading research workers such as Tricomi, Lighthill, Guderley, Frankl, Germain and Morawetz, is described in considerable detail. The list of references is not intended to be complete but is highly representative of the major contributions.

The book professes to be aimed at honours graduates in mathematics and engineering, and presupposes a relatively modest knowledge of fluid dynamics and a somewhat more exacting one of classical analysis, although where practicable the author attempts to provide the necessary mathematical background. This leads to lengthy sections on partial differential equations, integral equations and special functions, and accounts in part for the formidable size of the book. Indeed the whole approach is geared primarily to the mathematician, and this is reflected in the preface where it is stated that "the arrangement of the various items is as far as possible in the order of their analytical complexity". This seems a strange principle to adopt as a criterion for ordering and as a result the reader is left with an uneasy impression of having jumped about from one topic to another. With a subject of this nature—relatively narrow from the physicist's standpoint but giving scope for enormous mathematical complexity—the author has a difficult task in that he wishes to present a book which is self-contained and does not require the reader to make constant reference to research papers or other textbooks. At the same time the serious student of the subject may regret the lack of a more extensive bibliography. Professor Manwell writes with the authority of one who has read the literature widely and deeply, and would, one feels, be in a position to supply detailed references, amplified where necessary with expert comment.

Unfortunately, the general impression which the printed format of the book gives is not favourable. The intricate nature of the mathematics calls, perhaps understandably, for the introduction of a plethora of asterisks, bars, tildes, subscripts and superscripts together with a vast range of basic symbols, and there is ample evidence that at times these demands have over-extended the resources of the compositor. Moreover, there are numerous proof reading errors and a complete lack of consistency in the use of symbols, brackets and spaces. A particularly unhappy example is the way in which throughout the entire text a full stop is introduced in a random manner into the middle of mathematical expressions to indicate multiplication. (Equation (14.3) and the line above it illustrate the lack of any consistent pattern.) Other examples are the indiscriminate use of two different founts for the superscript ξ on numerous occasions from p. 59 on, and the fact that the o,O order symbols are sometimes put in italics and sometimes not. Factual errors also occur. For example, in (19.36), $\psi_{(c)}$ does not satisfy Laplace's equation as is required; its compressible analogue in (19.37) is also wrong, and is not even the correct equivalent form of the incorrect version of (19.36). In spite of the inherent interest of much of the material the book is marred by faulty proof reading, poor display of mathematical expressions and indifferent diagrams, and seems overpriced at £10.

A. G. MACKIE

Ancient Astronomy

Ancient Astronomical Observations and the Accelerations of the Earth and Moon. By Robert R. Newton. Pp. xviii +309. (Johns Hopkins: Baltimore, Maryland, and London, December 1970.) £4.75.

IN 1853 J. C. Adams pointed out that the then theoretical value of the gravitational secular acceleration of the Moon's mean motion, which agreed reasonably well with observation, was erroneous and should be reduced to little more than half. The original value stemmed from the combination of the historic discovery by Laplace in 1787 that the secular acceleration was due to the slow diminution in the eccentricity of the Earth's orbit and of a demonstrable error in the analytical derivation (the variable eccentricity had been treated as a constant during the integration of the differential equation). The resulting discordance with observation led to immense activity both in the verification and improvement of the theory, and in the critical examination of the observations.

The discordance could not be resolved since it was due to two distinct but related non-gravitational causes: the variable speed of the rotation of the Earth, giving rise to non-uniformity in the time-scale of mean solar time; the dissipation of energy, and the transfer of angular momentum within the Sun–Earth–Moon system, chiefly due to tidal friction.

In 1939 the Astronomer Royal, H. Spencer Jones, summarized all available data from modern (after 1680) observations of the Sun, Moon and planets and combined these with the results obtained by J. K. Fotheringham from ancient observations. He demonstrated conclusively the reflexion of the Earth's irregular rotation in the motions of the Sun, Moon and planets and determined the so called secular accelerations of the Sun and Moon. The results were subsequently used to define the time-scale of ephemeris time and the adopted secular acceleration of -22.44^{11} (century)$^{-2}$ in the Moon's mean longitude.

In his book R. R. Newton rediscusses the ancient observations and comes to the conclusion (in the opposite sense to Adams) that they support an average value of almost double the currently adopted value. There can be no question either of the importance and value of Newton's contribution or of the impetus that it will give (and, since preprints were widely circulated in September 1968, has given) to research in this field, particularly the analysis of both ancient and modern observations.

The basic unresolved problem is geophysical in respect of the medium term behaviour of the Sun–Moon–Earth system: can the dissipative effects have changed by a factor of 2 in the past 2,000 years, or are either the ancient or modern observations (or analyses, or both) suspect? The question throws doubt on the efficacy of the determination of ephemeris time from observations of the Moon in indicating possible cosmological differences from atomic time. Newton only touches this matter lightly in his final chapter, being otherwise content to present the evidence.

The main part of the book is devoted to a detailed investigation into the records of ancient eclipses and their interpretation. This is a specialist field, much aided now by precise predictions, but involving scholarship of high order and judgment; Newton is very critical of the methods of his predecessors who, in his view, tended to introduce bias by forcing identification. Not all will agree with his interpretations, however; and his methods of analysis are not always as precise as might be desired. But he provides very full information of his sources, his arguments and his treatment; he reviews critically most modern investigations, and he gives a valuable digest of the many confusing notations that have been used. It is a quite invaluable contribution and one that no research worker in either the astronomical or geophysical fields concerned can afford not to have.

D. H. SADLER

Peculiar Stars

Stellar Spectroscopy : Peculiar Stars. By Margherita Hack and Otto Struve. Pp. 317. (Observatorio Astronomico di Trieste: Trieste, 1970.)

THIS book is the second part of the updated revision of a text originally written about ten years ago but not published at the time because of Otto Struve's death in 1963, and it fills a substantial gap by providing a detailed survey of the results of stellar spectroscopy from a chiefly observational point of view. Normal stars were dealt with in the first part of the series.

The concept of peculiar stars is relative: some unusual stars are merely extreme cases of processes in stellar evolution or stellar atmospheres that are believed to be fairly well understood, while other groups of quite frequent occurrence are more or less baffling in the present state of knowledge. Furthermore, it is convenient to group together stars of widely differing evolutionary status having common characteristics such as emission lines. This book accordingly concentrates on three broad classes: hot stars with emission lines; novae and other explosive variables (which also have emission lines); and magnetic, metallic-line and related stars. Other groups like T Tauri and symbiotic stars are mentioned only in passing. The text is partly expository and partly composed of skilfully blended quotations from review articles.

The task of providing a coherent survey of these diverse and only partially understood types of stellar spectrum is difficult, and Mrs Hack has done remarkably well in making the material coherent and reasonably up to date. There are gaps: for example, the possibility of collisional excitation in nova spectra has been ignored and there is no reference to the important work done on non-dipolar fields in magnetic stars. Also some of the arguments used do not have a clear physical basis, particularly on the rather fiendish problems involved in interpreting the behaviour of magnetic spectrum variables. However, the book is a good summary of the observational data in its chosen fields and will provide an excellent starting point for students embarking on an investigation of peculiar stars.

BERNARD PAGEL

Chemical Texts

Physical Methods of Chemistry. Edited by Arnold Weissberger and Bryant W. Rossiter. Part 1A: *Components of Scientific Instruments.* Pp. xi+433. £9.50. Part 1B: *Automatic Recording and Control, Computers in Chemical Research.* Pp. 330. £8. (Techniques of Chemistry, Vol. 1.) (Wiley-Interscience: New York and London, May 1971.)

"TECHNIQUES of Chemistry" is the replacement for the well-known series "Techniques of Organic Chemistry" and the first volume, *Physical Methods of Chemistry,* succeeds *Physical Methods of Organic Chemistry.* Part 1A, *Components of Scientific Instruments,* contains chapters on mechanical and electronic components, the latter being preceded by a chapter on basic electrical principles. Finally, there is a chapter on the generation and modulation of waves (both electromagnetic and acoustic).

The standard of presentation is variable, and in some cases the rate of progress from an elementary start to more sophisticated matter is alarming. Thus, much of the material must appear to be either far too elementary or too difficult for any particular reader. This volume is therefore unlikely to be attractive to individuals and will only find a ready sale to libraries. Even here it would seem that, for a good proportion of the material, likely users will find other specialized texts (not necessarily more advanced) more valuable. It is particularly irritating to find, in a volume as expensive as this, much wasted space. While this is clearly a personal view, I find it difficult to believe that pages of photographs devoted to "view of typical printed circuit card, card rack, hand-wired circuit board, typical multi contact connectors", and such like, can be justified.

Part 1B, *Automatic Recording and Control, Computers in Chemical Research,* contains four chapters. The first, on detection, is principally concerned with various transducers for converting in most instances a change in a particular physical property into an electrical signal, and related matters. I found this chapter interesting and believe that it could be helpful to many. The next very short chapter on automatic recording reads like a collection of technical specifications for various recorders. Such information is freely available, indeed it is difficult to avoid receiving much of it, from manufacturers.

The third chapter, on automatic control, discusses linear feedback control networks, the ways of obtaining data and applications of basic principles to specific systems and the use of feedback for some typical controllers used in chemical apparatus. For those concerned with designing apparatus for chemical research this chapter could be valuable and should enable them to talk sensibly with their senior electronics technicians. The title of the final and longest chapter, "Computers in Chemical Research", is misleading: nearly sixty pages are devoted to Fortran. Surely nobody wishing to learn this language will turn to a book of this type. Similarly, although they make quite easy reading, the history, basic principles and a long and elementary discussion of binary arithmetic and Boolean algebra are out of place in a book about techniques of chemistry. Part of the relatively small fraction of this chapter on digital computers has some real value in the context of this volume. I can imagine very few individuals buying this volume, for more than half of the material is readily available elsewhere at considerably lower cost.

H. M. FREY

CORRESPONDENCE

Science and Development

SIR,—I wish that your discussion of the relevance of science in developing countries (*Nature*, **232**, 73; 1971) could have started some 25 years ago when, after centuries of foreign rule, most of the countries under the old British Empire were beginning to emerge as free nations. It could probably then have saved some of the new governments of those countries from taking decisions which have led to serious economic and social problems. The crying need of millions of people in these countries is for food and shelter and if science and technology has any relevance to humanity then it should be the foremost aim to provide these basic necessities as soon as possible. Development of agriculture and the related industry must therefore be the first task. In order to develop agriculture the research must be oriented to develop better strains of wheat, rice, etc., on one hand and "intermediate" tools of agriculture on the other hand. (What I mean by "intermediate" tools is the sort of tools which are in between the primitive plough drawn by the horse or bullock and the complex tractor.) The intermediate tools would have the advantage that they could be more efficient than the primitive tools used at present and be mechanically simple to be looked after by the simple farmer himself.

Communication to the men in the villages of these countries (and about 80% of the population in a country like India lives in villages) is another equally, if not the most, important aspect to which very little attention has been paid with the result that whatever developments were made in these fields remained confined to reports in the files of government officials.

Without the communication of these new developments to the farmers in the villages, no real progress on the farms can be achieved since it is the farmers in the villages and not the officials who work on the farm. I believe that it is here that the medium of television could provide the means to communicate to the farmer, in his own language, the new developments in tilling the soil, sowing, fertilizers, maintenance of his tools, etc., since any written material, such as books, is no good to him because he cannot read. Even illiteracy could be effectively tackled by the programmes on the television specially designed for this purpose. Italy is known to have achieved, during the last decade, a great success in its agriculture and literacy programme by use of television. I am sure the same can be done in the case of developing nations. Here is a place for the advanced technology in the economics and social advancement of slowly advancing countries.

Education of the illiterate masses through television, I am sure, will make a great impact on the quality of life to millions of people and bring about an awareness of the world around them. As such, at present they are in complete darkness about the better way of life existing a few miles away in their own land, not to mention foreign lands. Television could be used to teach them simple ways and means to improve their own lot and their children's future in the circumstances in which they find themselves. I can visualize the application of television to convince the people of the need to control the population explosion, to improve their sanitary conditions, how to avoid malnutrition with available food. Without the use of this medium, I am afraid, the progress in developing countries will take too long, which would have a disastrous effect the world over. The cost of providing a network of television stations throughout these countries would be well worth the improvements in the lives of millions of people and would be repaid by the increase in production on the farms.

Organized research on an international scale, as you mention, of problems such as health, cheap housing, etc., can easily contribute towards the development of the developing countries. But what is foremost is the need for the realization by the leaders of these nations to orient, in the interest of the people they serve, their policies so as to attract capital from inside and outside the countries in order to enable them to exploit the technology which already exists or is developed by their own efforts for the solution of these problems. This would not only generate the growth of their economies but at the same time enable them gainfully to employ their trained manpower, since unemployment and under-employment of the vast number of the school leavers and graduates lead to serious social problems and no democracy can survive and grow with the existing social conditions of extreme poverty and misery.

Yours faithfully,

K. C. TRIPATHI

16 *St John's Way,*
Hempton,
Deddington,
Oxford OX5 4QR

Obituary

Sir W. Le Gros Clark

WHEN Wilfrid Le Gros Clark went to Oxford in 1934 (after holding chairs at St Bartholomew's and St Thomas's Hospitals) to become Dr Lee's professor of anatomy at the age of 39, he was already the acknowledged master and leader in three exciting and expanding fields. Largely through the example of his own meticulous and imaginative investigations of the visual and olfactory pathways and of the connexions of the thalamus, descriptive neuroanatomy had begun to make way for much more significant enquiries into cerebral functional organization. The basis of Le Gros Clark's success in this field was laid by his researches into the comparative neurology of lower primates, particularly of tarsier and tree shrews, specimens of which he obtained during his time as principal medical officer in Sarawak (1920–23). This in turn led him deeply into primate and human evolution; his pre-eminence in this field was marked by the publication in 1934 of his influential *Early Forerunners of Man*, later revised in several editions as *The Antecedents of Man* (1959).

His creative and original approaches to neurology, morphology and phylogeny led him on to his reforming activities in both teaching and research in human anatomy and physical anthropology. In anatomy he insisted on experimentation to reveal the functional significance of structure as the way forward. His *Tissues of the Body*, now in its fifth edition, must have reconciled many a medical student to the load placed on him by an over-traditional subject. And he strove for the introduction of shorter textbooks, more in keeping with a modern curriculum, bringing out his own *Practical Anatomy* (1948) and later contributing to the *Text Book of Human*

Anatomy (edited by W. J. Hamilton). Likewise, in physical anthropology, an old-established subject in Oxford, Le Gros Clark firmly opposed the traditional fare of craniometry and typology, advocating instead a genuine study of human variation in relation to both environmental and genetic factors.

All these issues were fostered by him in Oxford over the next 25 years. His vision and integrity, his positive outlook, his critical and creative power, his erudition, his unfailing kindliness, as well as the example of his own sustained productivity, made him an impressive and inspiring head of a large and many sided department. A stream of visitors and postgraduate students came to the department to work with, under, or just near 'Le Gros'.

In the war years a team under his lead demonstrated a new facet of functional anatomy by designing equipment to meet the visual, auditory, manipulative and postural requirements of Service personnel such as gunners performing complex tests of sighting and tracking. This experience of "human engineering" or ergonomics convinced Le Gros Clark of its value for everyday activities, particularly in industry. An MRC unit, attached to his department and of which he was honorary director (1948–1962), pursued a programme devoted largely to practical applications of functional anatomy, human physiology and physical anthropology.

In the last decade of his tenure at Oxford, Le Gros Clark continued his neurological work but became more and more immersed in his palaeontological interests. On a visit to South Africa he was able to study at first hand the australopithecine material, accumulated by Raymond Dart and Robert Broom. He became entirely convinced of the hominid status of these creatures, though he remained sceptical of the proved existence of a form as advanced as *Homo habilis*. He also carried out the definitive descriptions of the pre-hominid material (notably Proconsul) discovered by Louis Leakey. In all this he was never a mere taxonomist, but strove always to define the phylogenetic processes at work. His conclusions on the course of primate and human evolution are set out succinctly in three notable books, *History of the Primates* (8th edition, 1967), *The Fossil Evidence for Human Evolution* (1955), and *Man Apes or Ape Men?* (1967). Le Gros Clark also contributed significantly to the solution of the long standing Piltdown problem (1953).

Somewhat to his surprise Le Gros Clark encountered not inconsiderable opposition both to his reforming activities and to his views on fossil man. Polemics he found distasteful and distressing, but he was a formidable protagonist in controversy, deriving his strength from the painstaking and concentrated consideration and reconsideration he gave to everything he studied.

In some ways Le Gros Clark was a man of paradox. While he went to considerable trouble to discourage the study of anatomical minutiae, he himself retained an awesome knowledge of the detailed macro and micro-anatomy of the human frame and indeed delighted in the art of topographical disquisition. He heartily disliked administration, the role of "organization man" or "scientist-politician" was not to his taste; yet he directed his large department unobtrusively and with efficiency, paying careful attention to its rebuilding, expansion and modernization. He remained a firm believer in simple apparatus and simple experimentation, and often expressed scepticism of the need for expensive equipment, even of the electron microscope; nevertheless when fully convinced, he would spare no trouble to see that the necessary equipment was obtained. For a generation Le Gros Clark was the outstanding British anatomist and its leader, yet he strove always to keep aloof from scientific politics, though in a few crises when he felt impelled to intervene his influence was decisive.

Le Gros Clark was elected to the Royal Society in 1935 and awarded a Royal Medal in 1961. He was president of the International Anatomical Congress at Oxford in 1950, president of the Anatomical Society in 1952 and 1953 and president of the British Association in 1961. He also served on the Medical Research Council. He was a member and master (1954) of the Salters Company and fellow of Hertford College, Oxford. He was knighted in 1955 and retired in 1962. He published a volume of memoirs, *Chant of Pleasant Exploration*, in 1968.

In 1923 he married Freda Constance Giddey, by whom he had two daughters. Freda Le Gros Clark died in 1963. In 1964 he married Violet, widow of Dr Leonard Browne.

The combination of Wilfrid Le Gros Clark's gifts and the diversity of his interests has without doubt made a long-lasting impression on the content and style of British anatomy and allied disciplines.

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:
12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature+ Nature Physical Science	**£24**	**$83**	**$90**
Nature+ Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, August 6 1971

NATURE Volume 232 AUGUST 13 1971

Have We Been Here Before?

ON the face of things at least, the expedition to the Moon two weeks ago was a dramatic vindication of those who have managed, in the past eight years, to keep their faith in the Apollo programme. Nobody will grudge the crew of Apollo 15 the congratulations they deserve for their resourcefulness and persistence. Nobody will pretend that the work that they have done is unimportant—the chances are that after a few months of analysis, the samples now returned from the Moon will help to account for many features of the solar system which are at present annoying puzzles. At the same time, however, it is only proper to insist that what appears now to be the crowning achievement of the Apollo programme is neither a justification of the programme of space development on which the National Aeronautics and Space Administration has embarked nor an argument for thinking that still further rockets should be added to the Apollo series. On the contrary, the way in which the programme appears to have reached a plateaux of achievement is in many ways an incentive for a closer re-examination of what should happen next. Nobody should be surprised if the problem of how to develop a strategy for NASA, sharpened as it has been by the most recent successful expedition to the Moon, seems an echo of the arguments which marked the early sixties.

So far, the direct outcome of the Apollo programme has been four successful landings on the Moon. In simple money terms, each expedition has cost the best part of $4,500 million, comparable with the whole expenditure of the National Science Foundation since the Apollo programme was conceived. Even though there is every reason to applaud the recovery of substantial amounts of rock from the surface of the Moon and although it is beyond dispute that the same samples have added in unique ways to the understanding of the astronomy of this part of the Galaxy, much greater benefits have sprung from the less spectacular sponsorship of basic research in the United States which the National Science Foundation, for all its faults, has undertaken. To say this, is not to pretend that the crew of Apollo 15 risked their necks for nothing or even to deny that there are adventitious benefits—spin-offs as the saying goes—from mounting gigantic enterprises like the Apollo programme. The truth is simply that in circumstances in which scientists must share with all other kinds of mortals a sense that few benefits are obtainable without cost, the undoubted benefits of the Apollo programme are not worth what has been spent on them. Moreover, even now that the system is a going concern, the marginal cost of extra expeditions to the Moon is quite substantial. The Apollo programme is reckoned to cost about $600 million in the financial year that has now begun and will not yield more than two expeditions for such a sum of money. The Glomar Challenger could be operated for the best part of a century on such a budget, and with more valuable results. To acknowledge as much is not in any sense to decry the value of what has been accomplished. It is merely that at a time when all kinds of enterprises for the public good, not simply scientific enterprises, are starved of funds, there is a case for asking whether extravagance is justifiable.

If, however, there remains a case against the continuation of the Apollo programme after the two landings scheduled for the coming year, there is a very much stronger case against the more ambitious scheme to develop a re-usable shuttle rocket aircraft for carrying people and instruments into orbits about the Earth. In retrospect, it is hard to think that even NASA can be happy with the commitment to this enterprise. The most serious objection to the shuttle project is to know what useful purpose it will accomplish. Two years ago, it is quite clear, the intended function of the shuttle project was to keep NASA's image burning bright. With the impending end of the Apollo programme and with a danger that no amount of success would persuade Congress to continue the exploration of the Moon, NASA considered it would be prudent to have a large and indivisible programme with which to make a claim on public funds. Although there is something in the argument that a single coordinated programme of development is likely to provide a more identifiable focus for the activities of great teams of technical people, the overriding weakness of the concept of the shuttle is that it is almost certain to turn out to be a project which it is more fun to work on than to complete. Briefly, although in theory a re-usable shuttle would be a powerful means of reducing the average cost of a space launching, these benefits are most likely to be apparent for comparatively low orbits about the Earth and are in any case unattainable if there is not a sufficient volume of launching work to do. And even if Congress should allow NASA to go the whole hog and to make a functioning shuttle by the late seventies, it is exceedingly improbable that there will also be money to launch such a large number of orbiting Earth stations that one or two of the shuttle machines could be economically employed on servicing them. Far from being the workhorse of space exploration, the shuttle is well on the way to becoming the white elephant.

Luckily, it now appears that the dogmatic line of the past year is being modified. At the beginning of June, the new administrator of NASA, Dr James C. Fletcher, let it be known that NASA was seriously considering the use of conventional rocket boosters to launch upper stages capable of attaining orbits about the Earth but of returning under their own wings. On this view, it would be possible to keep the cost of the shuttle programme within more modest limits. With luck, the annual cost might be a mere $1,300 million and not the maximum of somewhere between $1,600 and $1,800 million originally expected in 1976–77. This modification, apparently devised by the Grumman Company, is obviously a departure from the simple concept of an entirely re-usable rocket—indeed, not merely would the booster be the lowest stage of a Saturn rocket of the kind used in the Apollo programme but the fuel tanks for the upper stage would also be jettisoned as they were emptied.

By making the shuttle half a shuttle, NASA has also made it possible for Congress to say that it will pay for one half and not the other. That is what NASA should now admit. And given the possibility that hidden snags may further delay what is, after all, an exceedingly complicated programme, it is entirely possible that the full-blown shuttle, re-usable booster and all, will be a plaything for the eighties, not the seventies. Two years ago, when the project was first launched, such a course was advocated by NASA's critics as the prudent course to take. If events have forced NASA to take this path, so much the better. And it is only fair to say that a comparatively modest approach to the design of the shuttle and in particular the use of the upper stage as a means of testing the recovery of gliding aircraft from a height of 100 miles or so would be technically quite valuable. Whether the same machinery is needed to service the orbiting houses called Skylab is another matter. After all, conventional rockets such as Saturn can do just that. At this point in the development of these advanced programmes, would it not be much more prudent for NASA to aim at a degree of flexibility in its planning? In short, is there not a case for deciding now that the Saturn rocket will remain the workhorse of the large booster programme in the United States, at least until 1975, when prototypes of the shuttle engine will have been flown? That will be soon enough to define the parameters of the next generation of large space rockets. By then, NASA may have a clearer idea of what it wishes to achieve.

What should that be? The first thing to say is that there has never in the past ten years been a scheme for the use of satellites and rockets that compared in attractiveness with the oldest proposal of all for putting satellites to work—the scheme for communications satellites first suggested by Mr Arthur Clarke immediately after the Second World War. By comparison, even weather satellites are less obvious pieces of machinery, whereas the scheme for using satellites to identify the constituents of the Earth's crust and even to spot corn blight in the Middle West has an air of desperate unreality. The best use so far suggested for Skylab is in X-ray astronomy and, although the scientific community is unlikely to look such a gift horse in the mouth, who would seriously claim that such an expenditure of money will bring a commensurate return? Although in the past two months it must have seemed to NASA that its constituency was being undermined by economic pressures and the scarcity of public money, by technical setbacks (of which there have been precious few) and by the wayward disaffection of American taxpayers, the truth is that its most serious weakness is the lack of an obvious goal at which to aim. In a funny way, NASA is ahead of its time, perhaps even a decade ahead. In the circumstances, it should concentrate on two quite separate objectives. First, there is the development of more sophisticated kinds of rockets for the exploration of the solar system—it is a great misfortune that enterprises such as the Grand Tour of the planets should in present circumstances have to wait until the astrologers say that the planets are properly placed. Second, there is a great deal of useful exploration to be done with existing hardware, near the Earth, on the surface of the Moon and in the directions of the nearer planets. If NASA had the strength to wash its hands of the shuttle except in the most truncated sense, it might yet win back its admired place in the scheme of things.

Throwing in the Sponge

ALL managers of research and development are well aware that the most difficult part of their job is to know when to abandon projects to stop throwing good money after bad. Ever since the managers of research began to acknowledge, in the late fifties and early sixties, that it is possible to set about objectively to understand the process by means of which a bright idea becomes a marketable product, they and those who act as their critics have been on the look-out for ways of codifying the structure of research and development. In the early sixties, the Gibbs–Zuckerman report, originally written as a means of instructing the British Government in the management of its own research facilities, put forward the then fresh discovery that the process of research and development necessarily involves the attrition of bright ideas. A company may set off enthusiastically after a dozen different hares, and may spend quite large sums of money on each of them, but if it is to be reckoned a serious business enterprise, the time will come when it will have to abandon the majority of its research enterprises so as to concentrate on the technical and commercial development of one or two of them. More recently, in the United States, the Charpie report put the same point differently—the successful small companies are those with self-restraint enough to concentrate their energies at the points at which they are likely to be profitable. In Britain, the issue has most recently been taken up by an organization called the Centre for the Study of Industrial Innovation, and in particular Dr W. Duncan Reekie of the University of Edinburgh has set out to look at a third aspect of the problem—the abandoned projects and the reasons why they were abandoned (Centre for the Study of Industrial Innovation, 162 Regent Street, London W1R 6DD, *On the shelf: a Survey of Industrial R and D Projects Abandoned for Non-Technical Reasons*, £0.50). Unhappily, the report is rich on detail and poor on analysis and generalization, with the result that those who read it will be well supplied with anecdotes that other people have made but perhaps even misled by what the survey has to say about the principles to be adopted in the management of research and development.

The centre was lucky enough to have a good response to a series of enquiries among companies in the United Kingdom—twenty of them were visited and talked to. To read between the lines, the companies concerned seem to have been rather glad of an opportunity of bringing their skeletons out of the cupboard. In a laconic summary of these confessions, the report explains how a company that set out to make an acid-resistant paint found that the research was successful but the price too high, that the successful development of an axial feed pump for feeding water into boilers under pressure had to be abandoned because the only customer changed his mind, a scaled down version of the huge machine that levelled land on highway projects was abandoned because it turned out for one thing to be too expensive for the potential customer and because it was either too heavy or too light for the applications in which its use had been suggested, and a sophisticated system of design for a voltmeter came to nothing, partly because the sophistication turned out to cost more money than the designers had hoped and partly because the manufacturer, in any case, made quite satisfactory voltmeters in the first place. None of these case studies will surprise those who work in industrial research and development in Britain, the United States or elsewhere. None of them, indeed, will seem anything but a part of the unavoidable ups and downs of commercial life.

In circumstances like these, what morals can be drawn from the abandoned projects described by Dr Reekie? His own conclusions are that the reasons for abandoning a project were usually extrinsic to the company concerned. Sometimes the market would turn out to be too small—and there is some evidence that British companies often find it hard to make a market bigger by promotion. Sometimes companies seem to have embarked on technical developments which were frustrated not merely by changes of mind by monopoly buyers but also without adequate assurance that a potential customer was in a position to buy. Other projects seem to have been abandoned because, when all the figures were available, it turned out that potential competitors were already selling competitively. And then there were problems about uncertainties over the supply of materials or the durability of the product of a successful development campaign. One way and another, Dr Reekie seems to have been given a comprehensive tour of the field in which industrial companies are required by the wish to stay in business to make decisions about the deployment of their resources on research and development.

The flaw in Dr Reekie's paper is its innocence. For decades now, in Britain, it has been customary to wring hands whenever a research project is abandoned, shedding tears not merely for the money that might have been spent elsewhere but for the manpower that has been wasted. In part, the explanation is that technical resources of all kinds were exceedingly scarce in the days immediately after the Second World War, so that there came about a tendency to regard them as a non-renewable resource to be spread as thinly as possible. The result is that even successful research and development projects in Britain are understaffed. Dr Reekie has picked out a few cases on his list where structural weaknesses in the organization of a company account for the failure of a research project, but it is earnestly to be hoped that the appearance of his report will not discourage companies elsewhere from taking the view that abandoned research projects are not merely inescapable, given the uncertainties of commercial life, but that a lack of them probably implies either that too many projects are being carried through to development, with the risk that financial resources will be overstrained, or that nothing worthwhile is happening.

Howzat!

THE grievous condition of cricket, and the agonies of the Marylebone Cricket Club, should not go unremarked simply because British athletes, youths and schoolboys have begun kicking leather balls between upright sticks of wood. In the past year, the pretence has been abandoned that the game of cricket is an open-ended business—teams will open hostilities against each other on a Thursday morning and not be finished until the following Tuesday. Instead there are now games of cricket in which only a few hours are set aside for play and often all this happens, historians will record with fascination, on Sundays. The whole objective seems to be to make the game more fun to watch without robbing it of that contemplative air which in the past has been calculated to keep the spectators away.

If the managers of the game are serious, they should take more radical steps to keep cricket alive. It is several years now since the Marylebone Cricket Club turned down a proposal for a cricket ball with a moment of inertia much greater than is at present possible, with the result that spin bowlers would be more effective or at least more puzzling to batsmen. There is no reason why it should not in the 1970s experiment with cricket pitches that are shorter, wicket to wicket, than the standard 22 yards (which is, after all, a non-metric measure—who now knows what a chain is ?). Presumably batsmen would score less for lack of time to play elegant shots but would on the other hand be able to scamper more quickly between the wickets and be less in danger of being run out. Other innovations that come readily to mind are larger cricket stumps, thinner (or thicker) bats and shorter boundaries. The MCC should give particular attention to the last of these, for it is well known that the spectators are especially delighted whenever a player strikes a cricket ball over the boundary. On the principle of rendering to Caesar what he paid to come to see, is it not time that even bad batsmen were enabled to hit a six ?

100 Years Ago

THE GUN-COTTON EXPLOSION AT STOWMARKET

THE disastrous explosion of gun-cotton, which occurred on Friday last on the premises of the Patent Safety Gun-cotton Company, is a calamity of unusual significance. Besides the large number of killed, amounting, we believe, to five-and-twenty persons in all, there were as many as seventy maimed and injured, many of them too, in such a manner as only violent explosions are known to torture and lacerate their victims ; and when it is taken into consideration that in all probability a dozen tons of the material actually exploded, the grave nature of the accident is in truth not surprising. The whole group of factory-buildings and out-houses were levelled to the earth at one fell swoop, and for miles away the effect of the catastrophe was acutely felt.

But it is not only from a social point of view that the affair is to be deplored. As a result seriously affecting the science of explosives, the occurrence is peculiarly unfortunate ; for the belief in the safety of gun-cotton as an industrial and military agent will now be gravely shaken. It is all very well for scientific men to adduce a plausible reason for the occurrence, and to prove conclusively that with due care and precaution a disaster of this nature could not possibly have happened ; but the public unfortunately will not be satisfied with a theoretical assurance of this kind ; and indeed measures should certainly be taken, not only to guard against such wholesale death and destruction, but to render the same absolutely impossible.

From Nature, **4**, 309, *August* 17, 1871

OLD WORLD

NUCLEAR WEAPONS

Aldermaston Decision

THE decision that the Atomic Weapons Research Establishment at Aldermaston should be transferred from the Atomic Energy Authority to the Ministry of Defence, anticipated as long ago as April by the Rayner report, was announced on August 5 by Mr John Davies, the Minister for Trade and Industry. This brings to an end a period of great uncertainty at Aldermaston which goes back to the early sixties and the abandonment of the advanced strategic weapons systems then being developed in the United Kingdom—the Blue Streak missile and the warheads that were to have been fitted to the Skybolt air-to-ground missile. For the past few years, it has been increasingly apparent that if Aldermaston has a place anywhere in the British Government's arrangements for research and development, it is with the Ministry of Defence and not the Atomic Energy Authority.

According to the statements last week in the House of Commons and the House of Lords, the government expects that the necessary legislation to transfer the Aldermaston establishment to the Ministry of Defence will have been dealt with in time for the transfer to be complete by the summer of 1972. The government has promised to consult the staff and trade union representatives about how the transfer will affect people's jobs, and Lord Carrington, the Minister of Defence, said that "the general aim will be that the terms and conditions taken as a whole shall be no less favourable than those provided for in existing contracts". At present, something like 19 per cent of the expenditure at Aldermaston concerns projects which are not part of the military programme, and 10 per cent of this work is nuclear in character. The government has been at pains to emphasize that the transfer to the Ministry of Defence will not necessarily reduce the amount of non-military work carried out at Aldermaston, but in future, projects such as those undertaken for the development of the fast reactor programme, will be covered by contracts placed with Aldermaston by the Atomic Energy Authority or one of its dependants.

Mr Anthony Wedgwood Benn, previously responsible as Minister of Technology for the Atomic Energy Authority, complained that it would be dangerous to go against the example shown by most other countries in the West by putting the nuclear weapons research directly under the military, and that the transfer would in any case spoil opportunities for further diversifying the work of the establishment.

So far, there is very little to suggest how the remainder of the Atomic Energy Authority will be affected by the impending reorganization of government research establishments, although a statement last week did say that the government is considering "how best the electricity supply industry and the nuclear industry could be more closely involved in reactor research and development". This formula can, of course, mean anything from the transfer of the reactor development station at Winfrith to the Central Electricity Generating Board, to the amalgamation of the design group of the Atomic Energy Authority with the Central Electricity Generating Board. Now that the fuel manufacturing capacity of the authority has been embodied in British Nuclear Fuels Limited; the way would seem to be open for a daring administrator to bring the Atomic Energy Authority entirely to an end.

SCIENTISTS' PAY

Mighty McCall

MORE than 3,000 disgruntled government scientists gathered in Central Hall, Westminster, last Tuesday in the final meeting organized by the Institution of Professional Civil Servants to protest against the government's pay offer. The purpose of the meeting was to give point to the outrage that scientists in the Civil Service have been expressing about the recent pay award.

Mr William McCall, the general secretary of the IPCS, said that the meeting was the biggest in the history of the IPCS and that there were larger issues behind this dispute. Mr McCall complained that the government was taking advantage of the economic situation and of the extensive unemployment among scientists to depress the pay of scientists. The implications of the government's offer, added Mr McCall, spell disaster for the national economy.

Indirectly, 27,000 scientists are affected by the offer and of these 17,000 are being offered no pay increase (Table 1). Mr McCall claimed that the government's treatment of the pay claim fits into a determined policy to reduce the size of pay increases. The offer of the Civil Service Department, said Mr McCall, was a declaration of war—an attack on the living standards of scientists. No other group in Britain has this year been offered no increase at all, he said; the offer means a cut of 10 per cent in the living standards of scientists. Mr McCall went on to say that as the government employed 27 per cent of all scientists in Britain, its attitude will make the pay of scientists everywhere go down and the ability of the country to make science an attractive profession will be impaired.

Mr McCall also complained that the government has never turned its back so completely on arguments advanced by the IPCS—which does not augur well for the status of the scientist in Britain. The institution's interpretation of the report of the Pay Research Unit differs profoundly from that of the Civil Service Department. Mr McCall pointed out that after investigation by his office it was revealed that the average age of the "equivalent" scientist employed in industry was nine years younger than in the Civil Service. This younger age reflected the better chances of promotion of a man in industry and if nine incre-

Table 1 The IPCS Claim and the Government's Offer

Numbers involved	Present scale	IPCS claim	Government offer
Principal Scientific Officer			
1,700	3,902 max	4,208 (7.8%)	nil
	2,820 min	3,174 (12.6%)	
Senior Scientific Officer			
1,200	2,703	3,398 (25.6%)	nil
	2,193	2,483 (13.2%)	
Scientific Officer			
600	1,982	2,077 (4.8%)	2.2%
	1,162	1,486 (27.9%)	9.7%
Chief Experimental Officer			
300	3,873	4,208 (8.6%)	nil
	3,258	3,714 (14.0%)	
Senior Experimental Officer			
2,100	3,099	3,396 (9.6%)	nil
	2,529	2,849 (12.7%)	
Experimental Officer			
3,900	2,177	2,583 (18.6%)	nil
	1,725	1,973 (14.4%)	
Assistant Experimental Officer			
1,500	1,578	2,077 (31.6%)	nil
	741	1,196 (26.4%)	9.9%
Senior Scientific Assistant			
1,200	1,777	2,077 (16.9%)	nil
	1,367	1,579 (15.5%)	2.4%
Scientific Assistant			
4,500	1,253	1,450 (15.7%)	5.7%
	490	550 (12.2%)	7.1%

ments were added to the salary of the government scientist it would then be a fair comparison.

One of the implications of this offer that worries the IPCS is that it will not be practical to implement the Fulton report on the restructuring of the Civil Service if the scientists' salaries fall much below those of the equivalent grades in the administration and engineering classes. If the Principal Scientific Officer gets no increased offer then his salary will be £500 less than the other grades within the service and an amalgamation of the grades will thus prove impossible.

Mr McCall was given a tumultuous acclamation by the scientists that packed the hall. The meeting, however, could only be regarded as a show of strength as no better offer was received by the IPCS before the official tribunal sat yesterday, August 12, to arbitrate the dispute Mr M. J. Mustill, QC, the chairman, is expected to announce the tribunal's decision early next week and Mr William McCall will certainly be a very disappointed man if no better offer than that already made by the government is recommended.

ENZYME RESEARCH

More Chemists Needed

The Science Research Council has come out with a declaration that more should be done to interest undergraduates in enzyme chemistry and technology and that practising chemists should be encouraged to devote their special skills to the subject. These points emerge from a review carried out by the Enzyme Chemistry and Technology Committee of the Science Research Council (*Support of Enzyme Chemistry and Technology 1969–71*, Science Research Council, 1971). The committee, under the chairmanship of Professor Sir Ewart Jones, was set up in 1969 following recommendations of a specialist panel of the Science Board.

The continuing importance which the SRC attaches to interdisciplinary research is indicated by the extension of the original two year life of the committee to four years. Since the beginning, the committee has recommended the award of forty-four research grants, of which the largest was £371,000 for the establishment of the Enzyme Group at Oxford. The committee makes no apology for its decision to award five of the grants over periods as long as five years, because it considers that long term funding in suitable circumstances increases the possibility of recruiting really competent staff and encourages the full and unhindered development of new ideas. But several applications have been turned down because they did not involve sufficiently close collaboration between disciplines.

Although fourteen research studentships a year have been made available to the committee for distribution to its grantees, the review makes it clear that the chance of obtaining more training grants specifically for enzyme chemistry and technology is slim. But as industry begins to play a useful role (and the interest of Whatman Biochemicals in enzyme purification is a case in point) it should be possible to make more use of the SRC's Cooperative Awards in Pure Science. And workers in enzyme chemistry can still apply for the research studentships meted out by the Chemistry, Chemical Technology and Biology Committees of the SRC. Not surprisingly the committee is trying to encourage the teaching of enzyme mechanisms in university courses and it suggests that enzyme isolation, purification and reactions should form part of some undergraduate practical courses.

On research grants, the committee says that many aspects of the subject are still failing to attract sufficient grant applications. Enzyme isolation and the production of pure enzymes and cell abstracts are two examples. The SRC is already partially supporting the extension of facilities at the Microbiological Research Establishment, Porton, so that the availability of partially purified enzymes can be increased, but the committee is particularly keen to encourage more proposals for work on enzyme separation procedures, especially those involving chromatographic methods.

The structural study of enzymes was singled out in the original SRC panel report as an important feature of the continued development of enzyme chemistry and there seems to be no lack of suitable applications. Funds have been provided for a high field NMR spectrometer for the Oxford group and some instrument time will eventually be made available to others working on biological problems. The committee has also been consistently of the opinion that studies of peptide synthesis are essential to the understanding of enzyme mechanisms and the review calls for more fundamental work to be done; it cites the problems of the unambiguous formation of disulphide cross-linkages, the protection of histidine and the incorporation of tryptophan as worthy of further investigation.

The application of enzymes in industrial processes is still apparently a completely open field and it is impossible to say in what ways the unique reaction-mediating properties of enzymes will be used. The SRC has awarded grants to three groups working on the attachment of enzymes to solid supports (for example, a chromatographic column or a glass tube) and this kind of research could lead to the production of viable systems which use enzymes to catalyse chemical processes that are difficult to perform by conventional means.

METRICATION

Waiting for a Policy

The announcement (see page 439) that the United States Department of Commerce is recommending metrication to Congress may make some reluctant British companies finally decide to change to the metric system. Several companies which have been exporting their goods to the United States have so far resisted the British Metrication Board's entreaties to go metric, and this has been a major stumbling block to the process that was started in 1965. Very few countries have not so far either changed to the metric system or made a firm commitment to do so. The United States is, of course, the largest country that has not yet decided to change, and it keeps company with such bastions of the imperial system of measurement as Barbados, Burma, Gambia, Liberia, Muscat and Oman, Nauru, Sierra Leone, Southern Yemen, Tonga and Trinidad.

What progress has been achieved in Britain during the past six years? The aim was to convert British industry to the metric system by the end of 1975. The British Standards Institution, taking the construction industry as an example, reports that nearly all the companies involved with public contracts have already changed and that two thirds of the others have either changed or are scheduled to change. Elsewhere, most larger companies have made the change and the smaller and middle sized companies are thinking of following suit.

In Britain, there is as yet no legislation to compel change by 1975 or any other date. Moreover, the Metrication Board is required to work chiefly by exhortation. The original aim, decided by the British Standards Institution in collaboration with industry, was that 75 per cent of all companies in Britain should convert by 1975, and the institution reports that this will be achieved at the present rate; it even hopes that the final figure will be more like 85 per cent.

The advantages of metrication to industries involved in overseas trade are self-apparent, but what are not very clear are the advantages to industries that do not export. The British Standards Institution is finding that companies that have converted have saved a great deal of money in the metric rationalization process. The philosophy that the costs of metrication "lie where they fall" has not encouraged such companies to go metric, but the many examples of cost saving that are now becoming obvious, together with the realization that in the near future components made to imperial standards will cost more, are making many companies rethink their policy.

If the industrial sector is well on the way to its original aim, there is a serious delay in the public and educational sectors. The present government has not been over-enthusiastic about metrication but has not, on the other hand, made any decisions to alter course. A White Paper on metrication that will spell out the government's policy has been expected for some time, but it will not appear at least until Parliament returns after the summer recess. The original intention was to introduce metric road signs in 1973, but Mr John Peyton, Minister for Transport, announced in late 1970 that this will not now be the case and that the government has no alternative date in mind.

There is no doubt that a definite statement of government policy is needed to reassure educationists who are already heavily committed to metrication. After 1972, all school examinations will be set in metric units and the SI system of units will be universally accepted. Textbooks have also been rewritten to conform with these standards. If a change of government policy towards metrication in education is expressed in the White Paper it will, as a spokesman of the National Union of Teachers said this week, cause consternation.

BUILDING RESEARCH

Constructive Policies

The Building Research Station, now under entirely new management—at least in name—looks likely to spend almost £2½ million in the financial year 1970–71, according to the annual report (*Building Research Station 1970*, HMSO, 85p). The station, which is at Garston, Hertfordshire, and its Scottish Laboratory, now fully established in new quarters at East Kilbride, is the construction research arm of what used to be the Ministry of Public Building and Works. In the reorganization of central government last autumn, however, the BRS, in company with the Road Research Laboratory, came under the wing of the new Department of the Environment.

The BRS is chiefly concerned with research into environmental design—for example, sound insulation techniques, the design of safe buildings and ways of mechanizing their construction and the performance and improvement of building materials. All of these interests are well illustrated by the Finchampstead project where the construction of 172 houses was planned and analysed along BRS lines. One measure of the success of this project is the estimated 900 man-hours required for each dwelling. A recent survey has shown that 1,200 man-hours per house is a more usual figure. The BRS provides an advisory unit, established in May 1970, and of course the station is the technical agent for the Agrément Board which takes responsibility for the testing and approval of new building materials.

On the international front, the station has recently played an important part in the sale of a 5,000-tonne hydraulic press to the Yugoslavian Government. The press, worth about half a million pounds, was designed and built at Garston by a consortium of interests including the BRS called Go-Con Concrete Ltd and is used for the rapid production of large concrete panels. Finance for the construction of the press was arranged with the help of the National Research and Development Corporation.

ELECTRICITY SUPPLY

Computing the Future

Planning the development of an electricity supply system so that costs are kept to a minimum can no longer be carried on without a computer. Both the Central Electricity Generating Board in Britain and the Commission of the European Communities now make extensive use of computing methods to decide the optimum mix of the types of power plant at present available or expected to prove themselves before the end of the century.

When only coal-fired plant was available, planning entailed the solution of a classic problem in operational research—how to place a number of coal-users in such a way that the cost of transporting coal from the coalfield was minimized. But the evolution of oil-fired and nuclear plant and the increasing use of gas turbines for meeting peak demand have considerably complicated the problem. Planning now involves not only the estimation of the distribution of demand throughout the year but also the relative capital and running costs of different types of plant and some prediction of the way in which nuclear technology is likely to develop.

The approaches to the problem which have been adopted by the CEGB and the research group at the Joint Research Centre at Ispra (where the European research is being carried out) are somewhat different. Both aim to evolve a plan for the next thirty years or so which can be continually updated, but the work at Ispra takes more detailed account of the possible development of nuclear technology.

The whole problem is a system analyst's dream. The CEGB programme, for example, has been evolved over a period of about ten years and contains an expression for the total cost of electricity during a given year. The objective is to minimize this expression under the applied constraints such as government regulations about the use of capital and the cost and availability of fossil fuels.

Several factors have to be taken into account in setting up the expression for the cost. The total amount of generating capacity which must be available is, of course, determined by the peak demand even though this may last for only a few days and at first sight there is an obvious merit order for power plants which is bound to affect the distribution between the different types. Nuclear plants are the cheapest to run, followed by oil, coal and gas turbine. On the other hand, the order of capital costs is reversed; gas turbine plants are the cheapest and the capital cost of a nuclear station can be about fifty per cent higher than that of an oil-fired station of comparable capacity. The philosophy behind the programme is that the type of generator with the cheapest running cost should supply electricity for as much of the year as possible and that the more expensive forms of power generation should only be used to meet the higher power demands that occur for short periods of the year. As more and more of the plant with the cheaper running costs is introduced into the system, the more expensive and older plant can be run for shorter periods and eventually phased out. The CEGB programme has predicted the future power plant mix shown in the Table.

Generator Plant Mix predicted by the CEGB Programme

Year	Nuclear	Conventional
1975	13%	87%
1980	>20%	<80%
2000	60%	40%

Although the computer work at Ispra is along similar lines, it is in many ways more detailed. Among its predictions for the European electricity supply industry are that the amount of electricity generated by non-nuclear means will level off in 1985 but will increase again in 1995 when more capacity will be required to supply electricity in the peak periods. The use of fast reactors will increase at the maximum rate soon after their introduction (estimated to be in 1985) but after a further fifteen years or so, the growth rate will be determined chiefly by the rate of increase of the power demand. The progress made by the high temperature re-

SRC Members

The Science Research Council has announced the appointment of six new members to its research boards so as to make up for the regular retirement of previous members. On the **Astronomy Space and Radio Board,** Professor J. Ring (Imperial College, London) and Mr M. O. Robins (Ministry of Aviation Supply) replace Mr R. Lees (Ministry of Aviation Supply) and Professor P. A. Sheppard (Imperial College, London). On the **Engineering Board,** Professor G. D. S. MacLellan (University of Leicester), Professor R. J. A. Paul (University College of North Wales) and Professor R. W. H. Sargent (Imperial College, London) replace Professor J. G. Ball (Imperial College, London), Mr H. R. Galleymore (Procter & Gamble Limited) and Dr M. R. Gavin (Chelsea College of Science and Technology). On the **Nuclear Physics Board,** Professor R. G. Chambers (University of Bristol), Professor L. L. Green (University of Liverpool) and Dr K. F. Riley (University of Cambridge) replace Professor W. E. Burcham (University of Birmingham), Sir Derman Christopherson (University of Durham) and Dr K. Mather (University of Southampton). Dr T. Emmerson (Guest, Keen and Nettlefolds Limited) and Professor Sir Ronald Nyholm (University College, London) have retired from the **Science Board** but no new appointments have been made.

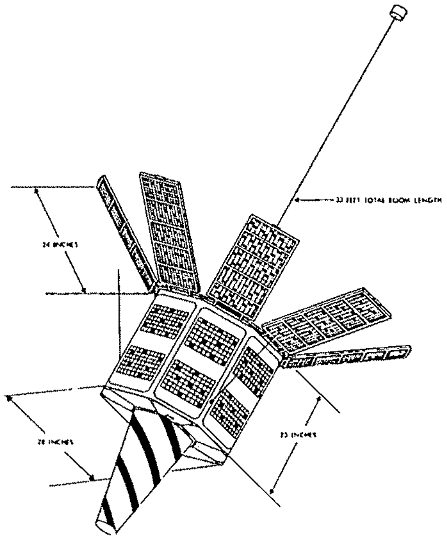
actors is expected to be determined almost entirely by comparisons with the fast breeder reactor during development and the installed capacity will not increase at all once the fast breeder reactor becomes established.

METEOROLOGY

Come in Please, No. 24

A weather satellite that obtains its data not from cameras but by the interrogation of automatic sensors carried into the atmosphere by balloons is to be launched next week. The balloons will take the sensors into a region which, because of its remoteness, is *terra incognita* on weather maps—the oceans of the southern hemisphere.

Although the satellite is experimental in the sense that the electronic system to be used for collecting data has not been applied before, the experiment is on a sufficiently large scale to make a significant contribution to the World Weather Programme. Five hundred sensors and balloons are available for launch from three locations in Argentina at latitudes of 33°, 39°, and 55° S by the French Centre Nationale d'Etudes Spatiales, which also developed the satellite to be launched on or after August 16 by NASA.



The French Eole weather satellite. The long boom is the gravity gradient stabilization system, so that the conical spiral antenna for balloon interrogation and data collection points towards the Earth.

In previous trials, balloons launched from southern hemisphere stations have lasted for several months, circumnavigating the Earth several times until lost because of icing. The French satellite carries a 4 Watt, 464 MHz transmitter for the interrogation of the balloon-borne sensors, a receiver for collecting the data transmitted by the sensors, and a 136 MHz transmitter for relaying the data to ground stations.

The philosophy of the experiment seems to be the recognition that in spite of the impact that pictures of cloud cover from conventional weather satellites have had on weather analysis and forecasting, there is still an urgent need for more measurements of temperature, pressure and wind speed which ground stations provide. In the absence of a technique that allows pressures and wind speeds in the lower atmosphere to be measured directly from sensors on satellites, the approach being tried by CNES and NASA is the most promising way of obtaining data from remote areas that are of meteorological interest.

Although the techniques of communications satellites have been used before for the collection of data from automatic sensors, the French system is an important advance on earlier American experiments because of its large capacity, allowing the tracking of as many as 500 balloons. It will be possible to determine the horizontal location of the balloons to the nearest 3 km (their vertical position will be governed by their inflation pressure so that they float at 11.85 km), a considerable improvement on a similar data collection system that has been tested on the Nimbus satellites and better by far than tracking from the ground.

EASTERN SCIENCE

Comecon Looks Ahead

from our Soviet Correspondent

The new "complex programme" of economic integration of the COMECON countries, promulgated by the 25th session of COMECON, raises some interesting questions about the future of science and technology in eastern Europe. This programme, which will be implemented during the next 15–20 years, is aimed at the "further deepening and improvement of economic co-operation and the development of the socialist economic integration of the member countries of COMECON" by measures including a "collective currency policy", the sharing of research and development experience between the member countries, a common policy of external trade, and a coordinated plan for basic research and industry. This, however, is to be accomplished on a basis of "respect for national sovereignty, independence and national interests, non-interference in the internal affairs of member countries, complete equality of rights, mutual advantage and comradely cooperation". The programme will be based on "multilateral and bilateral mutual consultations", and will not be accompanied by the creation of new "supranational bodies".

This stress on national autonomy within COMECON seems to have been largely at the insistence of the Romanian delegation, which has been active in resisting what would appear to be the logical outcome of COMECON — virtual integration of the eastern bloc with the Soviet Union. But it seems unlikely that this view will stand up against the implicit supranational nature of the projects proposed by the programme. These include, in particular, the "planned expansion of international specialization and cooperation in industry, science and technology", the end result of which, whether decided by a supranational body, or by "direct links between the ministries and other state organs concerned", will be, to some extent, a curtailment of national autonomy as far as the choice of project is concerned.

A large section of the programme deals specifically with cooperation in science and technology. The emphasis here is on matters of policy, such as the planning and solution of important scientific and technical problems, cooperation in research and the interchange of information on research and development and mutual training programmes. The list of basic problems to be tackled using the "most effective forms of mutual cooperation" is long and somewhat vague in its terms, but seems to concentrate mainly on biology and biophysics, with special reference to agriculture and food production, mechanization, automation and computers, more efficient use of natural resources, including marine resources (the Pacific Ocean is especially mentioned, in a manner almost suggesting that it is a private preserve of COMECON), semi-conductors, and the demands of metallurgy and industry in general.

Much attention is given to the "organizational forms and legal principles" of the proposed cooperation, but no concrete projects are indicated. Information exchange is greatly emphasized, and, on the basis of the existing COMECON International Centre of Scientific and Technical Information in Moscow, a system of information exchange will be set up during 1972–73. The production of scientific instruments and apparatus will be one of the first fields to be reorganized and, during the next year, proposals are to be worked out by the member countries on the establishment of international societies for the production of high-precision instruments (including apparatus for nuclear research) and the establishment of a coordinating centre for cooperation on biomedical and clinical research equipment. Production of research materials is also to be integrated, including materials for optical spectroscopy, experimental physiology and high vacuum work.

Although the programme speaks throughout of the "member countries" and the "interested countries" involved in the various cooperative projects, it is inevitable that the Soviet Union will play the leading part in the planning. Although a COMECON scientific centre has sometimes been established outside the Soviet Union (Weed and Pest Control at Poznań, Timber Research at Bratislava), the location of the Information Centre in Moscow will tend to orientate all major planning towards Moscow.

EUROPEAN RESEARCH

Aigrain Projects Persist

Plans have now been laid for a Pan-European conference on scientific and technical research in Brussels in November. The initiative comes from the Commission of the European Communities, and in particular from the technical working groups under Mr Pierre Aigrain which have in the past two years sought to define the fields in which European countries might collaborate on scientific and technical projects. At a meeting in Brussels at the end of July, representatives of nineteen countries in Europe picked out a number of particular problems from the shopping list which has been accumulating since 1969. The intention is that there should be a conference in the second half of September to settle questions such as the organization and financing of joint research projects, and that the conference of ministers in November should then set up a series of specific collaborative projects on which work could begin immediately.

According to an announcement in Brussels, the original list of proposals has been narrowed down—a term which must be understood to include selection as well as refinement—to a number of specific issues. It has, for example, been decided that the most tangible way of making progress in collaborative data processing is to set up a European Data Transmission Network and then a program library. This, as it happens, is a project developed by the staff of the European Communities on the instructions of the Hague Conference on technical collaboration in September 1969. It is now proposed that the collaboration of European states should be invited in long-term studies of passenger transport, what are called "various means of combating nuisances", a term now understood to include not merely the properties of sulphur in the atmosphere but the treatment of mud and other solid waste. A newish departure is a series of projects in metallurgical research. There is talk of collaboration on radio wave propagation.

One striking property of the shopping list now current is the disappearance of some of the more ambitious projects such as the scheme for the development of a large computer. This, as it has turned out, is at once such a risky venture and one that promises such large commercial gain that the companies able to make a contribution to the project have preferred to get together among themselves and—prudently—to enlist American interest as well. Similarly, the original proposal for collaborative research in telecommunications is conspicuous by its absence—nobody pretends that Europe has too much good work of this kind, but it is clear that there are too many companies in Europe who think of themselves, perhaps with justice, as potential competitors of AT&T for collaboration to be easy at this stage. And, of course, from the beginning of these discussions about the future of European collaboration on technical developments, it has been assumed that, having burned its fingers with Euratom, the European Community would not for the time being be able to turn its attention to fostering collaboration in the development of nuclear reactors. That is a stable door that nobody has the courage to shut even if the horse has not yet bolted.

HOVERCRAFT

How Fast for Hovertrain?

With the delivery this week from Vickers's Swindon factory of the first full-scale British tracked hovercraft, a new phase in the British development of this novel type of high speed surface travel is initiated. So far, the building programme of government-financed Tracked Hovercraft Limited (THL) at Cambridge has taken rather longer than scheduled for contractors to build a preliminary three miles of elevated box-beam track along the bank of a disused seventeenth century canal on the nearby Fens. In parallel, at the Cambridge laboratory, a variety of scale trials and studies of fundamental design features of the proposed vehicle have taken place, particularly concentrating on the specially developed single-sided linear electric motor for propulsion and the characteristics of the table-top sized hoverpads that will provide both lift and guidance.

The newly delivered 75 foot car is being equipped at a trackside workshop at the Erith terminal of the test monorail. All the interior equipment, the hoverpads and the linear motor installation are being handled by THL staff. The fitting out is expected to take some two months. The craft will then weigh 51 tons. Trials on track will then start. In view of the short length so far completed, the RTV3L vehicle is not intended to exceed 150 m.p.h. this year though rated for 300 m.p.h. (450 k.p.h.). The hope is to extend the track to five or eight miles next year when speeds up to 250 m.p.h. can be attempted. The craft will travel without a driver or passengers during the trials period. It is more efficient to control it electronically from the trackside and to use the interior space for every conceivable instrumentation so that no feature of "ride", vibration, acceleration, braking and so forth goes unrecorded.

There is one question to be answered: having received delivery of a 300 m.p.h. vehicle, is THL going to be able to exploit it? It seems to depend on a further subvention from government for extending the track to at least eight miles: eighteen were originally in view. The £3½ million initial investment is not yet exhausted, but by the end of 1971 the need of further investment will be becoming urgent. So far the government has studiously avoided committing itself in any way.

NEW WORLD

Metric Standards Round the Corner

The National Bureau of Standards has predictably recommended that the United States should switch over to a metric system of measurement in the course of the next decade. In the report of the US Metric Study, which has occupied the bureau since the passing of the Metric Study Act by Congress in August 1968, the principal theme is that the metric system is for practical purposes unavoidable. "Eventually the United States will join the rest of the world in the use of the metric system as the predominant common language of measurement." But even the adoption of externally designed units of measurement may become a patriotic challenge. "Americans working cooperatively can resolve this question once and for all."

The report of the Metric Study has the inelegant title *A Metric America: A Decision whose Time has Come* (US Government Printing Office, $2.50). The supporting evidence presented at the hearings of the study group has been published separately in twelve volumes. Responsibility for the study, originally placed with the Department of Commerce by Congress, has been delegated to the National Bureau of Standards but the Secretary of Commerce has also received advice from a large representative committee. Most of the evidence on which the report is based was collected during the summer of 1970 at public meetings which lasted altogether twenty days.

It remains to be seen what Congress will make of the report on metrication and of the argument that metrication is historically necessary. Congress failed to respond to Jefferson's commendation of the metric system as long ago as 1790, even before the definition of the metre. Since then, Congress has built up a long record of flirtation with bits and pieces of the metric system, especially at the turn of the century. Most recently, the United States seems to have been prodded into action partly by the National Bureau of Standards itself and partly by the spectacular example of countries such as Britain which have astonished not merely the tourists but themselves in their conversion to the metric cause.

The report of the study group says that the deliberate encouragement of the tendency towards metric could quite quickly create a situation in which the metric system was as widely used as the conventional system in the United States. Schools, it says, should emphasize metric measurements. Industries could be persuaded to conversion before they were forced by circumstances to measure metrically, and public authorities responsible for traffic signs and the like should seize their opportunities to make a captive audience think in kilometres.

In the same spirit of gradualism, and with some regrettable signs of falling for the confusion between units of measurement and standardization of sizes, the committee seeks to comfort anxious Americans by pointing out that it will never be necessary to change the size of an American football field or to alter the gauge of the great railroads (which may in any case be out of business before metric ways are adopted in the United States).

But what will the United States gain from such a development? The study group points out that agreements such as that between the United Kingdom, Germany and France on metric electronics arts, themselves the prototypes of more ambitious agreements yet to be negotiated, could exclude many American products from important markets. The study group has even calculated that American exports likely to be at risk in this sense in 1969 accounted for $14,000 million and that an American failure to move towards the metric system will mean that something like $600 million worth of exports will be unsold in 1975.

The Metric Study report points out that the compatibility of United States standards with those of other countries, particularly in Europe, may be particularly important at a time when the United States share of world trade is diminishing steadily—it fell by more than ten per cent during the sixties—and when arrangements such as the European Economic Community will provide further deterrents to American exports. Multi-national corporations are yet another source of pressure towards metrication.

The study group's enquiries among industrial companies seem fully to support its conclusions. In a sample of 4,000 companies chosen to represent American industry as a whole, those for the metric system represent a majority of the labour force, with larger companies more anxious to switch over. As a whole, 70 per cent of the labour force seems to be employed by manufacturers anxious to make the change. Among the general public, the study group's chief discovery seems to be that ignorance is the most common condition—only 40 per cent of the respondents to a social survey were able to name a single metric unit. In industry and among the public as a whole, however, it seems to be accepted that if there is to be a change, it should be planned as a coordinated operation and spread over the next ten years.

With this starting point, the study group urges that there should be a coordinating organization to superintend the changeover. The study group has also singled out two points at which an early start could be made on adaptation to the future—closer participation in the making of international standards and the incorporation of metric measurements into school curricula. In the long run, there will have to be special studies of particular problems such as that already being undertaken by the Industrial Fasteners Institute on the definition of metric thread sizes. Eventually, there will also have to be some decisions about the extent to which collaboration between manufacturers on the development of metric standards can be freed from the threat of anti-trust prosecution. The study group has also committed itself to a complicated cost-benefit analysis of the transition to metric. It assumes, conservatively perhaps, that the benefit of an extra $600 million a year on the balance of trade is more than $1,000 million for the economy as a whole. Moreover, the study group points out that there would be a number of important economies arising from the way in which the transition to a metric system would provide opportunities for simplifying present practice and, for example, for reducing the numbers of standard items of equipment such as screw thread sizes. The cost of the transition, for manufacturing industry, is estimated to be between $6,000 million and $40,000 million spread over ten years. In general, therefore, the committee suggests that the costs are unlikely to be much greater than the benefits.

Whether the report now published will finally tip the scales in the United States in favour of conversion to metric may be an open question, but there is no doubt that the report will do a good deal for the moral of the Metrication Board in the United Kingdom, recently much cast down by the apparent indifference of the Conservative Government. Indeed, the working group declares that the British metrication programme should be regarded as a pilot for the conversion in the United States. Lord Ritchie-Calder, chairman of the Metrication Board, will no doubt be reminded once more that prophets are not highly regarded in their own countries.

NEWS AND VIEWS

The Universe Considered as Hole

DURING the past few years, astronomers have gained an insight into black holes (or collapsars) which has made the study of these phenomena an increasingly important part of astronomy. This field of research was once largely the preserve of mathematicians concerned with the nature of space-time in an abstract way; observational developments now suggest, however, that collapsars play a vital part in the evolution of stars, galaxies, and the Universe as a whole. The latest calculations pointing to the possible importance of collapsars in binary systems appear in this issue of *Nature* (page 465). G. W. Gibbons and S. W. Hawking have followed up the line of investigation pointed to by Trimble and Thorne (*Astrophys. J.*, **156**, 1013; 1969) and conclude, from a study of the properties of seven systems shown to be binary stars by the Doppler motion of lines in their spectra, that there is suggestive evidence for the presence of black holes in some binaries. This must also lend weight to the contention made by Cameron and supported by Stothers that there is a collapsar in the system ε Aurigae (*Nature*, **229**, 178 and 180; 1971).

But what exactly is a collapsar? The general scientific imagination may well be captured by this concept of a "hole in space", but all too often it is felt that, because the prediction of this effect stems from Einstein's general theory of relativity, an understanding of it requires specialized mathematical knowledge. This is far from the truth. Like many of the fruits of this elegant theory the prediction, once made, can easily be understood in physical terms. Any star with more than 1.4 solar masses left when it has exhausted its nuclear fuel cannot support itself against gravitational collapse. The pressure at the centre of the cold star becomes so great that the fundamental nuclear forces are overcome, and matter is compressed beyond the density of atomic nuclei. This process is self sustaining, because such compression results in a higher density, a stronger gravitational field, and thus still more rapid collapse. Eventually, the collapsar has the properties of a point source of infinitely dense material. Curious as this is, it is the effect of the gravitational field produced on the space outside the collapsar which is responsible for the current wave of interest in black holes.

Within a critical radius (the particle horizon), the field is so intense that no matter, nor any radiation, can escape from the collapsar, although material can continue to be absorbed from the Universe. Hence the name black hole —the only way in which these objects can be detected is by their gravitational fields. This has great appeal to cosmologists, many of whom believe that there should be more matter in the Universe than can be seen in the form of bright galaxies. It also opens the way to intriguing mathematical games concerned with the possibility of extracting energy from these sources.

The most extravagant suggestion is that matter swallowed up by black holes re-emerges into the Universe from white holes, which lie at the centres of active galaxies and quasars. This could ensure the overall conservation of mass/energy while offering some explanation of the extremely violent events observed in some astronomical objects. At a more sophisticated level, it seems that energy could be extracted from the immediate vicinity of a collapsar by the break up of another object passing nearby. During a conference held from July 19 to 21 at the Institute of Theoretical Astronomy in Cambridge, which will shortly be reported in *Nature*, there was discussion of the properties of the ergosphere, a region just outside the particle horizon. A mass passing through this region can break up under certain conditions in such a way that one fragment falls into the black hole while the other is accelerated, gaining energy in the same way that the proposed grand tour space probes will gain energy from the rotational energy of the giant planets—a slingshot effect. But the escaping fragment can gain so much kinetic energy that it leaves the region of the collapsar with more mass/energy than that of the entire incident mass.

This mechanism must unfortunately remain a toy for the mathematicians, because the collapsars required to explain the activity of many observed galactic nuclei in terms of this effect would be implausibly large. For the present, however, practical astronomers and physicists would prefer to seek some other energizing process for these active galaxies and quasars. Black holes need not, however, be quite so extremely violent as this conventional picture conjures up. To be sure, objects like stars can only disappear inside their own particle horizons when compressed to very great densities. But while the mass of a sphere of matter at some uniform density varies as the cube of the sphere's radius, the gravitational force falls off only as r^2. For any density chosen there is some critical mass above which the sphere is within its particle horizon, or, alternatively, for any mass there is some critical density for which this occurs, and the greater the mass chosen the smaller is the required critical density. So although gravity is a weak force, its action over very great distances implies that a reasonably dense galaxy —that is, one with a density comparable to the density of some stellar clusters in our galaxy—could contain enough mass to prevent the escape of matter or radiation from its immediate vicinity.

Once again, the cosmologists who wish for more matter in the Universe are delighted—now, it seems, whole galaxies can be hidden in black holes. But why do the cosmologists want yet more matter in the visible galaxies? In short, it is to provide enough mass for the Universe to be closed. This would solve many problems regarding the nature and origin of the Universe, and would fit many of the observations of distant sources made at radio, optical and other wavelengths. But if the Universe is closed—the finite, yet unbounded three-dimensional analogue of the surface of a sphere—then the world is in essentially a rather large black hole. The corollary to this is that the expansion of the Universe must ultimately be reversed, eventually producing a compression to densities where present understanding of physics breaks down, with, perhaps, a further expansion from the primaeval matter in a cycle of pulsations which had no beginning and will have no end.

Significance of Sex Linked Antigens

THE observation that individuals of the same species may differ from each other antigenically is now so well established as to hardly bear remarking. Antigen hunting, the identification of more and more antigens, has rightly fallen into disrepute as a worthwhile activity, yet it cannot be claimed that there is any clear idea of the biological significance of the remarkable antigenic variation observed in most mammalian species.

Two extreme views for this variation can be put forward. The first is that it arises as a result of a random event, mutation, but that one form of the antigenic material confers some kind of biological advantage, and the alternative form confers another kind of advantage. Antigenic variation, as pointed out by E. B. Ford, would thus form an example of a balanced polymorphism, and takes its place with such phenomena as variation in rings on snail shells and spots on insect wings as part of a more or less homogeneous group of biological variations. On this hypothesis there is a need to look only for a cause for the maintenance of the antigenic variation, and it may be fortuitous that the variant materials are antigenic. They have a role in the cell membrane, and one antigenic form may be more advantageous in one environment than in another.

The alternative hypothesis is that it is the antigenicity of the materials which is important. There have been many hypotheses that have utilized this idea—one of the most recent is the suggestion of Jerne (*Europ. J. Immunol.*, **1**, 1; 1971) that histocompatibility antigens, such as H-2 antigens in the mouse, may play a part in the generation of immunoglobulin diversity. It has also been frequently suggested that histocompatibility antigens play a part in distinguishing neoplastic cells from normal cells, although the way in which this is done is far from clear.

These two kinds of hypothesis can, of course, be merged into one, because all variation must originate in mutation, and it can hardly be the case, for example, that the need to distinguish neoplastic and normal cells gave rise to antigenic variation—the origin of any variation must precede its utilization.

Even if this view of the origin of variation is accepted, it is still necessary to look for the reason for its survival, that is, for its "function". The direct demonstration of the mouse H-Y antigen, controlled by a gene on the *Y* chromosome, on mouse spermatozoa by Goldberg, Boyse, Bennett, Scheid and Carswell (see page 478 of this issue of *Nature*) puts this antigen firmly on the map as an antigenic system the biological role of which ought to be investigated. The use of skin grafts to investigate the H-Y antigen has hitherto clearly limited what could be done.

If it is acknowledged that antigen systems like H-Y in the mouse, and the human Xg^a blood group antigen (controlled by an *X* chromosome gene), may have biological roles, why are the genes that control them on the *X* or *Y* chromosomes? The same question can, of course, be asked of other genes present in moderately high frequency in the population, such as those on the human *X* chromosome which affect colour vision. One should not, perhaps, make too much of this—the *X* and *Y* chromosomes represent a considerable proportion of the animal's genetic material; that it can all be concerned with sexual differentiation seems unlikely.

Apart from these rather vague speculations, it is much easier to comment on the usefulness of these antigens to investigators. Race and Sanger, in their book *Blood Groups in Man* (Blackwell, Oxford and Edinburgh, 1968), point out how the investigation of the Xg^a blood group has revolutionized knowledge of the human *X* chromosome linkage group, and indeed of human genetics as a whole. The development of a direct cytotoxic test for the mouse H-Y antigen by Goldberg *et al.* could do the same for the mouse. Although many of the investigational problems which beset human genetics do not exist in the mouse, the development of a serological test will be a great spur to the investigation of the mouse *Y* linkage group, and it might one day lead to an answer to the question posed earlier—whether or not the H-Y gene is present on the *Y* chromosome fortuitously.

It is worthwhile drawing attention to another specific feature of Goldberg's work, that at least some, and therefore possibly all, *X* bearing spermatozoa carry the Y antigen and thus reflect the antigenic constitution of their precursor cells rather than the capabilities of the genetic material contained in the sperm; similar observations have now been made on several antigenic systems in spermatozoa. This therefore precludes the use of an immunological method, with any antigen system so far discovered, of either distinguishing or separating *X* or *Y* bearing spermatozoa, and is unlikely to form the basis of a technique for deliberate selection of male or female offspring.

DNA REPLICATION

Selective Inhibition

from our Cell Biology Correspondent

THE substituted uracil derivative, 6(p-hydroxyphenylazo)-uracil (HPUra) is today unlikely to be found outside the laboratory of N. C. Brown in Baltimore, who since 1966 has diligently been investigating its effects on DNA synthesis and DNA replication in a variety of bacteria. But to judge from what he has to say in the *Journal of Molecular Biology* (**59**, 1; 1971), the manufacturers of this compound, Imperial Chemical Industries Limited, can anticipate a spate of begging letters. For, according to Brown, HPUra inhibits the semiconservative replication of DNA without affecting DNA repair.

During the past five years Brown has shown that although the compound selectively and reversibly inhibits DNA replication in *Bacillus subtilis* and a range of Gram positive bacteria it does not bind with great affinity to DNA, it does not stop the replication of phage in *B. subtilis*, neither does it impair transcription, translation or the synthesis of bacterial cell wall materials. Attempts to characterize the way in which the drug acts by adding it to a range of membrane fractions, preparations of *B. subtilis* exposed to lysozyme and apparently even cells made permeable by exposure to toluene, have all failed. The DNA synthesis which these systems support *in vitro*, like the replication of phage (Brown, *Proc. US Nat. Acad. Sci.*, **67**, 1454; 1971), is refractory to HPUra.

Because of this Brown was forced to re-examine the effects of this compound on intact bacteria because they alone support the DNA replication process sensitive to HPUra. He selected the strain of *B. subtilis* 168 *thy*$^-$ *ind*$^-$ for this work, for as Pettijohn and Hanawalt have shown, cells of this strain, after exposure to ultraviolet irradiation, support extensive DNA repair synthesis and they therefore offer an opportunity of comparing the effects of HPUra on repair and replication. In an elegant series of experiments Brown has shown that although the semiconservative replication of the chromosome of this strain is blocked by HPUra, the repair of DNA damage caused by the ultraviolet irradiation continues unabated. For example, he labelled the DNA made after irradiation and in the presence of the drug with radioactive bromo-deoxyuridine and then analysed it on caesium chloride and alkaline gradients. The bulk of the labelled DNA turned up in short chains covalently linked to the parental DNA. Moreover the synthesis of this DNA was greatest in cells given the highest doses of irradiation, and it was inhibited by inhibitors of excision repair synthesis such as actinomycin D.

Because Brown found last year that the replication of virulent phages in *B. subtilis* is not blocked by HPUra he was at pains to eliminate the possibility that the synthesis of DNA in irradiated cells, which is insensitive to HPUra, is nothing more than the replication of a defective prophage induced by the irradiation. The properties of defective *B. subtilis* prophages make this idea implausible and furthermore he has shown, first, that synthesis of DNA in irradiated cells does not immediately depend on protein synthesis and, second, that mitomycin C treatment, which like irradiation induces defective prophages of *B. subtilis*, does not induce an HPUra-refractory DNA synthesis. In short, there seems little doubt that it is the repair of irradiation damage that continues in the presence of HPUra.

If HPUra is as selective an inhibitor of DNA semiconservative replication as Brown's work indicates it should prove an extremely useful tool for identifying the real DNA replicase. But Brown's claim that DNA synthesis in toluenized cells continues in the presence of this drug (and unfortunately he has not yet published the detailed evidence on which he rests this assertion) must come as a blow to cell biologists who have pinned their hopes on toluenized cells as a system for investigating DNA replication *in vitro*. The experiments reported by Sueoka's group in *Nature New Biology* (**232**, 111; 1971), for example, certainly suggest that toluenized *B. subtilis* cells support DNA replication rather than repair synthesis, but Brown's findings raise new doubts about that. If Brown is right, toluenized cells may have to be added to the scrap heap of bacterial preparations which, although they make DNA *in vitro*, do not support chromosomal replication identical to that occurring in intact cells.

ANTIQUITIES

Fooling the Experts

from our Archaeology Correspondent

NEW thermoluminescence work on supposedly ancient ceramic objects, all recently acquired by museums and collectors, shows how successfully forgers and fakers are taking in their clients. Two studies undertaken at the Research Laboratory for Archaeology and the History of Art at the University of Oxford indicate not only the effectiveness of the thermoluminescence method, but the great difficulty that experts have in distinguishing genuine objects from false ones simply by their appearance.

The first study, by M. J. Aitken, P. R. S. Moorey and P. J. Ucko (*Archaeometry*, **13**, 89; 1971), exposes as fakes no fewer than forty-eight out of sixty-six bowls, figurines and anthropomorphic vessels, which have supposedly come from illicit excavations at the prehistoric site of Hacilar in Turkey. Since the successful excavations there by James Mellaart in 1957 and subsequent years (*Excavations at Hacilar*, I and II: Edinburgh Univ. Press; 1970), the attractively painted pottery and baked clay figurines discovered have rightly had an important place in the early history of art. Similar objects from illicit excavations have fetched up to £3,000 on the antiquities market.

Thermoluminescence glow curves obtained by Aitken show that most of the specimens examined emitted very little light on heating. The thermoluminescence observed is proportional to the radiation which the clay has undergone since it was fired—the radiation originates chiefly from radioactive isotopes in the clay or in the soil in which the object was buried—and hence upon its age since firing. Forty-eight out of sixty-six objects were evidently recently fired. The remote possibility that genuine ancient pieces had undergone heating in recent times to consolidate them, thereby removing the accumulated thermoluminescence, was checked by S. J. Fleming and D. Stone-

Streptovaricins Suppress Oncogenicity

LAST March Brockman and his colleagues reported in *Nature* (**230**, 249; 1971) that the streptovaricins—a family of antibiotics, the structure of which is similar to that of the rifamycins—inhibit the activity of reverse transcriptase in mouse leukaemia virus particles. At concentrations of 40 μg/ml. the enzyme's activity was inhibited by 75 per cent. In next Wednesday's *Nature New Biology* Brockman's group describe their latest experiments with these drugs which show that streptovaricins not only inhibit the transformation by mouse sarcoma virus of mouse 3T3 cells growing in culture, but also selectively inhibit the progress of erythroblastic leukaemia and spleen enlargement induced in mice by Rauscher leukaemia virus. Their work thus suggests that streptovaricins might become useful chemotherapeutic agents for the treatment of cancer.

At concentrations which do not slow down the rate of cell division, those streptovaricins which inhibit reverse transcriptase also block by from 25 to 50 per cent the induction of foci of transformed cells by mouse sarcoma virus. This fact, together with the finding that the antibiotics are ineffective 24 h after infection, suggests that the inhibition of transformation results from the inhibition of reverse transcriptase activity. Moreover the inhibition of transformation by streptovaricins was enhanced if the cells were exposed simultaneously to interferon, which Brockman *et al.* suggest specifically inhibits the translation of viral messenger RNAs.

But inhibiting transformation *in vitro* is one thing; inhibiting the induction of a cancer in an intact animal is another. Can streptovaricin alter the course of events after mice are infected with a virus such as Rauscher leukaemia virus? According to Brockman and his colleagues it can. They find that mice fed streptovaricin in their food such that the concentration of the drug in their serum is from 1 to 3 μg/ml. are protected to a significant extent against splenomegaly induced by Rauscher murine leukaemia virus. The spleens of mice fed the drug before and throughout the infection are only about 50 per cent as large as those of mice given the virus but not the drug. Moreover when the drug is not given until 8 days after infection, when spleen enlargement is well under way, it can still significantly reduce further enlargement.

These findings, together with the observation that streptovaricin does not inhibit transformation *in vitro* if it is given 24 h after infection, lead Brockman's group to conclude that *in vivo* the drug is blocking secondary focus formation in the spleen. The drug does not, however, reduce the proliferation of normal haematopoietic cells, as judged by spleen focus counts, neither does it impair spleen enlargement in mice inoculated with the L1210 transplantable lymphoma cells.

In short, in mice the streptovaricins seem to block a virus-induced oncogenetic event rather than affecting the proliferation of malignant cells. In the intact animal it may therefore be acting as an inhibitor of tumour virus reverse transcriptase.

ham. They used the "pre-dose" technique, which depends on the enhanced susceptibility, after heating, to artificial irradiation shown by specimens which have previously had a radiation dose. Most of the samples with negligible initial thermoluminescence showed a small increase in susceptibility to irradiation after simulated re-firing, which suggests that they never had experienced the appreciable dose of radiation received over 7,000 years, in the normal course of things, by the genuine antiquities.

The second investigation, by S. J. Fleming, H. Jucker and J. Reiderer (*ibid.*, **13**, 143; 1971), reveals that most or all of the Etruscan wall paintings on terracotta recently on the Swiss and American art markets are very palpable fakes. The museums owning these painted slabs will have paid anything up to £10,000 each for them. Fleming *et al.* used both the fine grain and the inclusion thermoluminescence methods—the inclusion method utilizes the large quartz and other mineral grains present as inclusions in the clay—and a comparison of the results shows that the painted plaques were made some time during the past twelve years.

The layman, of course, always derives a little innocent pleasure from seeing the experts fooled—a pleasure which will not be shared by the museums in Bern, Basel and Munich owning "Etruscan" terracottas, or by the custodians of the Hacilar material around the world. A more serious consequence of such faking activities is brought out in Ucko's discussion. The fakes include a number of variants and ostensibly new types accepted, until now, as genuine by most authorities. Indeed the abundance of new "finds" on the market led Mellaart to propose that looters had discovered the hitherto unknown cemetery at Hacilar, unearthing vases which "probably were used only in ritual functions in shrines and graves". Ucko now suggests that the few genuine pieces could all have come from illicit digging in the settlement itself, so that the whole interpretation of the meaning and function of such genuine pieces as remain becomes very different.

It is a sobering thought too that, for most materials other than baked clay, no method as reliable as thermoluminescence exists for detecting fakes. Luristan bronzes and Cycladic marble figurines are but two more of the fields where collectors and museums have been consistently and profitably defrauded. There are far more of them in the museums of the world than ever were excavated. The obvious moral is that museums should be less eager to encourage illicit excavation by paying large sums for unprovenanced antiquities. By doing so they are supporting, often very generously, looters and fakers alike.

BEHAVIOUR

Displays Analysed

from our Animal Behaviour Correspondent

THE most striking aspects of an animal's behavioural repertoire are often the "displays" it gives in sexual or aggressive encounters. Much is now known about the behaviours which birds and fish show during courtship and aggression, and there has been much speculation as to the underlying physiological mechanisms which give rise to these conspicuous displays. Elements of displays of animals, and sometimes whole behavioural sequences, can now be produced by electrical stimulation of the brain or by injection of hormones. The idea that such displays might be a particularly fruitful meeting ground for ethology and physiology prompted the meeting of the Association for the Study of Animal Behaviour at the University of Sussex from July 26 to 29. As Professor R. J. Andrew (University of Sussex) pointed out, displays have been selected during evolution to provide an accurate "read out" of an animal's internal state to another animal, and so may well be able to do so for the human observer.

The meeting provided an excellent opportunity for the exchange of ideas, but it soon became clear that there is still a long way to go before the physiology of even the most commonly observed displays is elucidated. There were several reports of researches into the role of various brain structures in controlling behaviour, but the precise function of these areas and their interrelations with other parts of the brain are still obscure, possibly because the

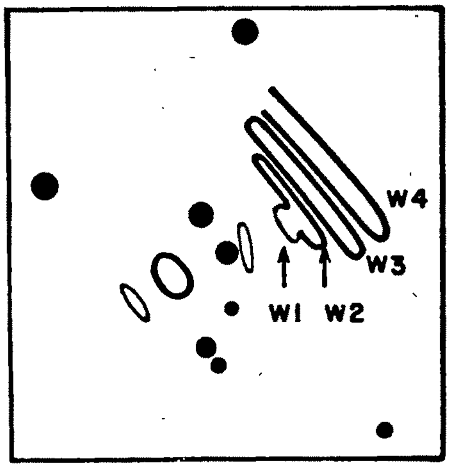
Exploding Magnetosphere May Cause Crab Glitches

BEFORE detailed timings of the Crab pulsar became available, astronomers were concerned to explain the amazing regularity of pulsar pulses. But now it is the small irregularities that have been revealed in the periodicity of the Crab pulsar for which explanations are being sought, in terms of the view that pulsars are rapidly spinning neutron stars which provides a satisfactory explanation of the gross features of pulsars. A review of the considerable data now available on the irregularities of the Crab pulsar—the largest and most sudden of which are called glitches, although an entire spectrum of events of different magnitudes and time scales can occur—is given by Jeffrey Scargle of Lick Observatory and Franco Pacini of the Laboratorio di Astrofisica at Frascati (Italy) in next Monday's *Nature Physical Science*.

The popular view that the irregularities are caused by what are called "starquakes" in a solid outer crust surrounding the neutron superfluid core of a neutron star does not find favour with Scargle and Pacini. Although changes in the internal structure of a neutron star, such as a starquake, would lead to an alteration in the moment of inertia of the spinning star that would be reflected in a change in the periodicity of the pulses, Scargle and Pacini point out that this notion is unsatisfactory on energy considerations.

The reason is that some filamentary concentrations of gas adjacent to the Crab pulsar seem to move in tune with the glitches, requiring more energy than can be provided by the starquake explanation. As an alternative explanation Scargle and Pacini suggest that the gas is excited from time to time by the sudden release of plasma from the magnetosphere of the pulsar, which would also lead to changes in the dynamics of the spinning pulsar that would be reflected in the pulse periodicity.

These occasional outbursts supplement the continuous injection of energy into the Crab Nebula by the escape of particles from the pulsar along open field lines. Much work needs to be done to investigate the kinds of plasma instabilities that can occur in the magnetospheres of neutron stars, but on the face of it this model seems to be a neat way of accounting for several features of the Crab pulsar.



The Crab pulsar, at the centre of this diagram, seems to be associated with a series of wisps of gas, W1, W2, W3 and W4. According to the review by Scargle and Pacini, Wisps 4, 3 and 2 seem to have been generated from Wisp 1, possibly as a result of the activation of Wisp 1 by the explosive release of plasma from the magnetosphere of the pulsar. The impact of this plasma pushes Wisp 1 out from the pulsar until the pressure is balanced by the pressure of the plasma and magnetic field of the Crab Nebula. Wisps 2, 3 and 4 have been generated by the magnetosonic waves which this motion of Wisp 1 produces.

brain, like a computer, can be "programmed" to do different jobs at different times. Indeed, the animal-computer analogy was heard more than once, from Dr J. D. Delius (University of Durham) and also from Dr D. J. McFarland (University of Oxford) who argued that animals allocate their time between different behaviours by "time sharing".

Physiological interpretation of a behaviour pattern is facilitated by an accurate description of the phenomenon to be explained, so that methods of recording and analysing behaviour are extremely important. Dr U. Weidmann (University of Leicester) illustrated his technique of frame-by-frame film analysis of the displays of mallard ducks as a method of discovering spatial and temporal relations between the birds that would be impossible by more conventional means. Dr P. Slater (University of Sussex) talked of the difficulties of describing and making sense of complex sequences of behaviour, and Drs R. Dawkins and M. Dawkins (University of Oxford) presented some methods of describing and predicting moment to moment changes in the fine structure of a behavioural act.

Better methods of description and the application of statistical techniques to uncover underlying patterns may well prove as useful a method for gaining an understanding of what goes on inside an animal as the already fruitful physiological approaches.

PROTEINS

Oxidation to Order

from our Molecular Biology Correspondent

A GROUP at the University of Padua, Jori, Scoffone and their colleagues, have cornered the market in photo-oxidation of proteins. By a series of chemical subterfuges they have shown how the scope and precision of the method can be extended to make it potentially one of the most useful resources of the protein chemist. In their latest article (on papain) they present a striking demonstration of the delicate molecular surgery that is now feasible (Jori *et al.*, *J. Mol. Biol.*, **59**, 151; 1971).

Papain contains a single thiol group, which is a part of the active site, and is readily modified. A suitable chromophoric group attached to it can then be used as a sensitizer for the photo-oxidation of reactive side chains in the immediate vicinity. Jori and his colleagues examined two such sensitizers—the dinitrophenyl and the fluorescein thiocarbamyl groups. Their insertion caused no disturbance in the structure of the protein, as reflected in its optical properties. The dinitrophenyl group gave rise to its own Cotton effect in circular dichroism, indicating that it was rather rigidly set in its place in the protein. The fluorescein chromophore on the other hand showed no such effect, and from its low fluorescence polarization it could be inferred that there was little restraint on its rotation.

It might consequently be supposed that with its less restricted orientation it might function as a less discriminating photosensitizer. This expectation is borne out: the dinitrophenyl chromophore permits the destruction of only two residues, which were identified by separation of tryptic peptides as a histidine and a tryptophan. The fluorescein chromophore sensitizes the oxidation of both of these and also of a further tryptophan in the early stages of irradiation, and then increasingly indiscriminate destruction of residues in all parts of the chain. If the protein is denatured before irradiation, the side chains in the dinitrophenyl derivative are also extensively oxidized on irradiation.

From the papain structure, and indeed from what is known of its chemistry, the two residues destroyed in the native dinitrophenyl derivative are known to be in the active site, and no more than about 5 Å from the substituted cysteine. The protein so modified retains its native conformation, with unimpaired thermal stability. The second tryptophan initially modified in the fluorescein derivative is not in the active site, but is tucked into a tight-packed internal region close by. When this residue is oxidized, previously inaccessible groups become exposed, the optical properties change, and all the indications are that the tertiary structure has broken down. The conclusion is that the tryptophan in question is indispensable for maintaining the integrity of the conformation of the native enzyme.

A further refinement to the photo-oxidative approach has been evolved in the same laboratory (Jori *et al.*, *Biochim. Biophys. Acta*, **236**, 746; 1971). It is well known that paramagnetic ions are efficient quenchers of electronically excited states, and presumably for this reason are able to afford protection against photo-oxidative damage. The possibility then arises of making use of specific binding of such ions by proteins to achieve selective protection of some of the side chains which would otherwise be destroyed in a sensitized photo-oxidation process. Lysozyme is known to have a single strong binding site for zinc or cobalt. The site is known from protein magnetic resonance studies to consist of the carboxylate groups of glu–35 and asp–52. When the zinc complex is irradiated in the presence of proflavine as photosensitizer, tryptophan and methionine side chains are annihilated and the enzymatic activity vanishes. If on the other hand the paramagnetic cobalt ion is substituted for zinc the activity after irradiation does not fall below 85 per cent of native, and two indole rings are found to have been degraded. These are external side chains (trp–63 and 123), and do not perceptibly affect the conformation of the protein.

Experiments with ribonuclease gave similar results: the free enzyme or its zinc complex were extensively damaged but the cupric complex was modified only at two residues, met–79 and his–105, with substantial retention of activity. This result answers finally the question of whether met–79 is implicated in the enzyme mechanism. The protective effect of the paramagnetic ion is clearly a function of distance and orientation, and the possibility exists of exerting a finer control on the reaction by the choice of metal ions with different orientations of ligand orbitals.

Accurate Radio Source Positions from Cambridge

As the relationship between radio, optical and high energy emission from sources both inside our galaxy and from extragalactic sources is becoming increasingly important to an understanding of the processes operating in these objects, so it is of increasing importance that radio sources should be pinned down by accurate measurements of their positions. Without the painstaking work which this implies, no correlation can be made between radio sources and optical objects. Equally important is the establishment of an absolute reference scale, so that positions determined using different antennae can be adequately compared In next Monday's *Nature Physical Science* J. W. Smith reports the latest progress made in this field by the team operating the Cambridge One Mile radiotelescope.

By carrying out simultaneous observations at 2.7 and 5 GHz, corrections allowing for the curvature of the atmosphere, and seasonal variations in the electrical lengths of the delay cables in the equipment are included—these terms are as small as 0.01 arc s. The overall accuracy of the positions given by Smith for twenty-eight sources is not, of course, as accurate as 0.01 arc s. But the better than 0.05 are s accuracy quoted for right ascension, and better than 1 arc s for declination, is already pressing the optical astronomers, who will now need to improve their own measurements before a deeper insight into these sources can be achieved.

THE EARTH

Chondritic After All?

from our Geomagnetism Correspondent

THE chemical nature of the Earth's outer core has been a source of speculation ever since seismological evidence proved unequivocally that this part of the Earth is fluid. It is now completely accepted that the principal element in the core is iron although Earth scientists are rather less inclined to speculate upon to what extent such iron may resemble conventional molten iron under the conditions of temperature and pressure which obtain in the core. But this problem apart, the principal area of disagreement remains the content of the core's minor element. For various reasons the prime contenders here are nickel and silicon; but Murthy and Hall (*Phys. Earth Planet. Interiors*, **2**, 276; 1970) have suggested that the most important minor element may be sulphur.

It has long been known that where hydrated minerals (for example, hydrous silicates) are present, the low-temperature accretion of an Earth which is roughly chondritic requires the retention of sulphur as FeS under all conditions of temperature and pressure and thus requires the relative solar proportion of sulphur in the Earth as a whole. Various considerations thus led Murthy and Hall to argue that the first melt to form during the early history of the Earth was an FeS–Fe eutectic which would have effectively transferred chalcophilic elements from the lithosphere to the core, that a core with 15 per cent sulphur would have a density lower than that of any Fe–Ni alloy and would thus be more in line with many seismological estimates, that thermal models of the Earth which take account of the presence of FeS indicate earlier core formation than other models, and that to have had early core formation and differentiation of the Earth would have required neither high accretion temperatures nor a basic difference in chemistry between chondrites and the proto-Earth. There are, nevertheless, serious objections to a chondritic Earth in the first place, one of the most important of which is that the alkali metals, potassium, rubidium and caesium, are several times less abundant in the lithosphere than differentiation of a chondritic Earth would indicate.

Taking up the matter where Murthy and Hall left off, Lewis (*Earth Planet. Sci. Lett.*, **11**, 130; 1971) now argues that the presence of sulphur in the core removes many of the apparent discrepancies between the real Earth and the chondritic Earth. His basic thesis is that, contrary to popular supposition, the three "missing" elements, among others, have "appreciable chalcophilic tendencies" and that as a result their depletion in the lithosphere is balanced by their enrichment in a sulphur-bearing core. In other words, the relative lack of abundance of these elements in the lithosphere should not be extrapolated downwards and thus does not necessarily imply that the Earth cannot be chondritic as a whole.

Needless to say, there is little direct evidence for this, but Lewis can nevertheless point to certain factors which support his case. Thus although the oxide–sulphide equilibrium is not relevant, as such, to any process occurring in the interior of the Earth, equilibrium constants show clearly that the alkali and alkali metal sulphides are extremely stable with respect to the corresponding oxides—a fact which Lewis believes to be indicative if not definitive. Second, in ordinary chondrites the alkali metals are almost always present in the silicate minerals and not in oxides, sulphides or oxysalts. A consideration of the reactions of Mg_2SiO_4 and $CaSiO_3$ with FeS in relation to olivine at a temperature of 240° C above the FeS–Fe eutectic then indicates that small amounts of CaS and MgS are stable relative to FeS and thus are likely to enter an FeS–Fe melt even with silicates in the same state of oxidation as in the present mantle. Moreover, CaS and MgS have been found in many enstatite chondrites and achondritic meteorites; and some of such enstatite chondrites also contain sulphide phases containing Na, K and Ti. In Lewis's view there seems no reason to suppose that where K goes Rb and Cs should not also follow, especially because the equilibrium constants for these elements in the oxide–sulphide reaction are very similar.

The difficulty in proving Lewis's point lies largely in the lack of thermodynamic data on the behaviour of the relevant sulphides in FeS-rich melts; and matters are not helped by the almost complete absence of alkali metal sulphides in terrestrial rocks known to geologists. But in spite of the latter deficiency and the admitted difficulty of forming solid sulphides of Mg, Ca, K, Ti and Na, the impossibility of producing an FeS-rich melt with, say, a one per cent content of such sulphides is by no means clear. Indeed, the circumstantial evidence for such a mixture in the core is quite strong.

Lewis rightly points out that if the Earth's fluid core really is enriched in

---

Growth Regulation in Normal and Transformed Cells

IT is classic observation that mouse fibroblasts (3T3) cells stop growing when they reach confluence or when they are deprived of serum, whereas the same cells transformed by the oncogenic virus SV40 continue to divide. This system would seem ideal for gaining insight into the normal regulation of cell growth and the genesis of cancer, and consequently much information has accumulated about biochemical changes in the cells after transformation or during growth limitation. In next Wednesday's *Nature New Biology*, Hershko *et al.* extend these observations, and, grasping the whole nettle boldly by the hand, propose a unifying theory of growth regulation in animal cells and its disruption by tumour viruses.

They show that depriving untransformed, but not SV40 transformed, 3T3 cells of serum produces a marked inhibition of protein and RNA (particularly ribosomal RNA) synthesis, a reduction in the uptake of certain nucleic acid precursors and a stimulation of protein degradation. They argue that each of these diverse metabolic reactions is, independently, part of a coordinated programme, or "pleiotypic response", of normal cells to changes in growth conditions. The response may be "negative" or "positive" depending on whether growth is limited or stimulated. Considering the possible mechanism of the pleiotypic response, Hershko *et al.* show that all the changes elicited by serum deprivation are reversed by insulin, even when bound to 'Sepharose' and unable to enter the cell. This suggests that the pleiotypic response may be coordinated by an intracellular "mediator" generated at the cell membrane, much in the same way that cAMP brings about the diverse responses of tissues to activators of adenylate cyclase.

In support of their idea that several aspects of cell function are coordinated by a single, underlying mechanism, Hershko *et al.* draw a striking analogy between the negative pleiotypic response in animal cells and the "stringent" response in bacteria starved of essential amino-acids. The stringent response, controlled by a single *Rel* gene, was originally thought to affect only ribosomal and tRNA synthesis, but it now seems that other processes are involved, including the uptake and phosphorylation of certain nucleic acid precursors and glucose, the formation of polyribosomes and the degradation of protein, which are all elements of the pleiotypic response. For the present, however, the case for the defence must rest there, for the nucleotide ppGpp, implicated in the mechanism of the stringent response, does not seem to be present in normal, growth-limited, animal cells, and is therefore unlikely to be the postulated negative pleiotypic mediator.

K, Rb and Cs the consequences are at once numerous and far reaching. First, the relative depletion of the three elements in the lithosphere becomes immediately explicable; and the terrestrial abundances of the major elements become compatible with a chondritic source. Second, it is no longer necessary to invoke volatilization during high temperature accretion to account for the apparent disappearance of terrestrial potassium and sulphur. Third, it may no longer be necessarily true that the chondritic Earth model, which explains the observed terrestrial heat flow so well, is not valid simply because the crust and mantle do not contain enough K, U and Th. Fourth, as much as seven eighths of the ^{40}K in the Earth may be in the core, thereby forming a major heat source of about 10^{20} ergs s^{-1}. Even if the efficiency of conversion is only 10^{-4} there would be enough energy here to maintain the convection to produce the geomagnetic field.

The fifth point is that the rough equality of oceanic and continental heat flow, notwithstanding the greater concentration of radioactive heat-producing minerals in the continental crust, may be explained by convective upwelling of deep ^{40}K beneath the oceans. Sixth, the low abundance of ^{40}Ar in the atmosphere compared with the abundance to be expected from the decay of ^{40}K in a completely degassed chondritic Earth could be related to the impossibility of degassing radiogenic ^{40}Ar from the core. And, finally, the low abundance of ^{87}Sr in the crust may be the result of the removal to the core of much of the Rb early in the Earth's evolution.

But can so many consequences, covering, as they do, such a wide range of geophysical and geochemical problems, really stem from such a simple premise? It is possible, of course, that Lewis has underestimated the complexity of some of the problems his basic thesis purports to solve. Even so, his wide-ranging conclusions must surely force a re-evaluation of the problems which have turned most scientists away from the simple chondritic Earth.

PESTICIDES

Leaching from Soil

from a Correspondent

IT is well known that fish and other aquatic organisms may concentrate organochlorine pesticides from very low levels in the water in which they swim until the amounts in their bodies are either lethal or too high for the fish to be eaten by man, so that this form of environmental contamination has received considerable study. The 1970 report of the Agricultural Research Council's Research Committee on Toxic Chemicals showed that some organochlorine pesticides were likely to come from rainfall, but that factory effluents and accidents such as spills of sheep dip were more important. Measurable amounts of pesticides are washed off the surface of treated fields especially by flash floods, but "underground water, which has percolated through soil, has not been found to contain measurable quantities of organochlorines".

This conclusion is based on many observations, mostly in temperate countries. The British Ministry of Agriculture suffered some criticism for suggesting that the best way of disposing of surplus and unwanted DDT, shortly to be banned for use in gardens in Britain following the recommendations of the Advisory Committee on Pesticides and Other Toxic Chemicals (the Wilson Committee), was to rotovate it into the soil, though this is, in Britain, a method which is likely to localize the insecticide's effects, and is far safer than such methods as disposal down the drain, or bulk burial.

A disquieting report from Pakistan is contained in an article by Aslam I. Nasim, M. M. H. Baig and K. A. Lord, of the Toxicology and Pesticide Laboratory, Karachi (*Envir. Poll.*, **2**, 1; 1971). These authors have found that organochlorine insecticides are widely distributed in soils in East Pakistan and are extracted by water from the soil. Nasim *et al.* examined six soil samples from three different localities near Dacca by silica-loaded paper and gas chromatography and found that all were contaminated with organochlorine insecticides and contained 1 to 3 p.p.m. of DDT. Furthermore, three of the samples contained 0.1 to 0.5 p.p.m. gamma BHC and three others about 0.5 p.p.m. dieldrin. BHC has been used in the past in Pakistan for the control of rice pests and dieldrin is used in the treatment of bamboo, but there seems to be no easy explanation for the DDT contamination.

Because insecticides are leached from soil by water, the presence of 1 to 3 p.p.m. of DDT and other insecticides in the soils examined by Nasim *et al.* could pose a threat to the fish life inhabiting paddy fields as well as the more direct contamination of water sources. Nasim *et al.* consider it essential therefore that further monitoring continues—freshwater fish are important sources of protein for the population of East Pakistan, and any deleterious effects of the insecticides on the fish populations could be serious.

Hormone-responsive Tumours

A FAVOURITE pastime of steroid endocrinologists in recent months has been hunt-the-receptor-protein, and attention is now turning towards the determination of the physiological relevance of these proteins. One aspect of this concerns their role in steroid-controlled growth of certain tumours. In general, hormone responsive tumours show a progression from a responsive to an unresponsive state, and this is associated with a loss or diminution of specific steroid-binding capacity (King, Smith and Steggles, *Steroidologia*, **1**, 73; 1970).

In next Wednesday's *Nature New Biology*, Kirkpatrick, Milholland and Rosen report a decreased amount of corticosteroid receptor in unresponsive P1798 lymphosarcoma as compared with the corticosteroid-responsive line. This confirms the earlier work of Hollander and Chiu (*Biochem. Biophys. Res. Commun.*, **25**, 291; 1966), but Kirkpatrick and his colleagues have taken advantage of a factor not usually available in this type of work, that a parallel biochemical response (DNA synthesis and membrane permeability) can be measured as an indication of the relevance of the steroid protein interaction. They also show that, contrary to one of the current vogues of cancer research, changes in the cell membrane cannot explain their results.

An unanswered question is what or where is the primary lesion causing this loss? Recent reports indicate that steroid specificity is controlled at the nuclear level as well as by the presence of a cytoplasmic protein (Steggles, Spelsberg, Glasser and O'Malley, *Proc. US Nat. Acad. Sci.*, **68**, 1479; 1971) so it is possible that a change in binding may also occur at the nuclear level. Kirkpatrick *et al.* found that nuclear binding was low in the responsive cells, but do not mention the unresponsive ones.

A study such as this has several aspects that are relevant to tumour biology. First, it indicates that receptors are involved in steroid mediated inhibitory as well as activation processes. Second, it suggests that receptors may be translatable into clinical tests for the hormone responsiveness of human tumours; it is of considerable practical importance to decide which breast cancer patients will benefit from ablative endocrine surgery and oestrogen-binding experiments look hopeful in this respect. Third, the presence of a regulatory protein in a responsive cell and its loss or diminished binding potential in unresponsive cells (none of the experiments distinguishes between these two possibilities) have obvious analogies with enzyme induction in microorganisms.

AIRCRAFT DYNAMICS

Scheme of Notation

from a Correspondent

THE basic equations of aircraft dynamics are those of classical mechanics, with the elaboration that the forces and moments of aerodynamic origin are themselves influenced by the motions which occur. Aeronautical engineers are greatly addicted to dimensionless expressions because, when used with discernment, they often render generalities more apparent, increase the clarity of the work and yield succinct results. The equations of motion of an aircraft, together with appropriate systems of symbols, have therefore been the subject of much dimensionless invention for more than half a century.

The most familiar system in the United Kingdom has been that of Bryant and Gates (Aeronautical Research Council Reports and Memoranda (R&M) No. 1801 ; 1937) which separately defined dimensionless aerodynamic derivatives and inertial parameters. In the United States, a somewhat different system with broadly the same intentions was adopted. There have also been sundry variations on these themes and—to borrow a felicitous phrase from the latest number in the R&M series—the "clash of symbols" has been considerable. Moreover, the pre-war systems were devised largely in the context of small perturbations from a simple undisturbed condition, using only first-order derivatives. The behaviour of modern aeroplanes and the increasing importance of cross-coupling effects, the demands of aeronautical engineers concerned with missiles and the use of computers has rendered such systems inadequate.

R&M 3562 (H. R. Hopkin, *A Scheme of Notation and Nomenclature for Aircraft Dynamics and Associated Aerodynamics*, Parts 1 to 5, HMSO, 1970, £6.75) proposes a unified system of notation and nomenclature for the study of aircraft dynamics which seeks to avoid the limitations of the earlier systems, to be more self-consistent and to be capable of further expansion. It is the product of some years of discussion at the Royal Aircraft Establishment in cooperation with the National Physical Laboratory and is a most comprehensive and closely argued work. In the course of explaining the basis of the new system, it presents a useful survey of the whole of the fundamental concepts and definitions used in the field. The geometry associated with aircraft attitude, incidence and axis systems is presented in detail, although the quality of the diagrams is no more than adequate—rather better ones are provided in the Engineering Sciences Data Unit (ESDU) Data Sheets published by the Royal Aeronautical Society. The basis of the system is, inevitably, somewhat similar to that of R&M 1801 save that it has been recognized that it is preferable, in defining "normalized" or non-dimensional quantities, to treat the aerodynamic data and the equations of motion in slightly different ways. The dynamic-normalized system is intended for investigations of the dynamic behaviour of an aircraft of certain mass acted on by aerodynamic and other forces when travelling at a certain speed. The aero-normalized system is convenient for expressing functions of the aerodynamic forces acting on an object of certain dimensions when it is in a stream of air moving at a certain speed. An important change, compared with the previous British system, is that the unit of force is now the datum dynamic head multiplied by an area, as opposed to twice this quantity. The characteristic lengths are now the mean aerodynamic chord for longitudinal quantities and the span for lateral quantities. The new aero-normalized derivatives are therefore similar to the "old" derivatives (and are denoted by similar symbols) but differ, in some cases, by factors which may involve powers of two and ratios such as the mean aerodynamic chord of the tail moment arm. "Concise" derivatives are introduced, obtained by dividing a derivative by the appropriate inertial quantity and incorporating a sign change so that most derivatives become positive (for example, $m_q \doteqdot -M_q / I_y$). The system is then arranged so that the equations of motion have the same form whether they are dimensional or non-dimensional. The precise significance of a symbol can, if necessary, be made explicit by a suitable "dressing", but in most cases the context will be such that the dressings can be omitted, as has mostly been done in the ESDU Data Sheets. As Hopkin observes, however, "the undressing of symbols should be discreet". In the absence of dressings, the symbols will look very similar to those of the older system although their numerical values, signs and non-dimensionalizing factors will usually be different. Until the new system has gained general acceptance, authors should be careful to explain exactly how they are using it.

The Engineering Sciences Data Unit has already issued several Data Sheets on Aeronautical Dynamics using this system, and it seems that it will have the blessing of the International Organization for Standardization.

Understanding the Origin of Petroleum

FATTY acids have long been regarded as possible predecessors of n-paraffins in petroleum. Some years ago Jurg and Eisma (*Science*, **144**, 1451; 1966) heated behenic acid ($C_{21}H_{43}COOH$) with kaolinite and with kaolinite and water for varying lengths of time and produced small amounts of n-paraffins—principally $C_{21}H_{44}$—and unsaturated hydrocarbons and fatty acids. In next Monday's *Nature Physical Science* Shimoyama and Johns describe an experiment in which they heated the same fatty acid in the presence of Ca-montmorillonite.

The chief product was insoluble material deposited on the clay which they describe as kerogen-like. As in Jurg and Eisma's experiments the chief n-paraffin product was $C_{21}H_{44}$, formed by decarboxylation of the fatty acid. Smaller quantities of n-paraffins down to C_{16} were determined, and generally smaller amounts of n-paraffins in the C_{22} to C_{28} range. No fatty acids were detected in the products in contrast to Jurg and Eisma's findings and also to expectations based on their proposed reaction scheme. Failure to find new fatty acids may result from the use of Ca-montmorillonite instead of kaolinite or such acids, although formed, may have lost their identities by firm attachment to the kerogen-like product.

The production of $C_{21}H_{44}$ seems to have been halted by poisoning of the clay catalyst by the kerogen-like material. Shimoyama and Johns conclude that the C_{16} and C_{17} n-paraffins are produced by breakdown of the C_{21} paraffin, whereas C_{18}, C_{19} and C_{20} develop in the early stages directly from the fatty acid and later by disruption of $C_{21}H_{44}$. Composite plots of amounts of individual n-paraffins and behenic acid against time show the braided form suggested by Hobson (*Earth Sci. Rev.*, **2**, 257; 1966) for a breakdown sequence $A \rightarrow B \rightarrow C$ . . . . The dual origin of C_{18}, C_{19} and C_{20} yields hybrid curves.

With increasing age and maturity the larger n-paraffins in sedimentary rocks show a decrease in their carbon-preference indices, a measure of odd-carbon number dominance in the distribution of the molecules. Shimoyama and Johns's data for the C_{16} to C_{20} range show a comparable decrease with increase in time of heating and increase in temperature. But limited data on the C_{22} to C_{28} range show even-carbon number dominance, an unusual condition. Kvenvolden and Weiser (*Geochim. Cosmochim. Acta*, **31**, 1281; 1967) have noted a crude oil in which there is even-carbon number dominance in the C_{20} to C_{26} range and odd-carbon number dominance in the C_{27} to C_{33} range.

Tentative calculations based on the experimental data suggest the attainment of "mature" n-paraffin distributions under conditions approaching geological requirements of time and temperature.

SEAFLOOR SOILS

Engineering Properties

from a Correspondent

MOST of the surface of the Earth is covered by a film of marine soil, the properties of which determine the cost, hence the feasibility, of constructing on the seafloor all things from observatories to drilling installations. Do marine soils which have been recently deposited have a finite strength? A symposium to consider how best to cope with the fragility and other peculiarities of soils which have never dried was held from July 26 to 29 at the University of Washington, under the auspices of UNESCO and the National Science Foundation.

Charges on the sides and ends of settling particles cause marine soils to have an open, card-house fabric which is subject to collapse. Their sensitivity to stress is consequently extreme and disturbance may cause their strength to drop to a substantial degree. Dr D. G. True (US Navy Civil Engineering Laboratory, Port Hueneme, California) considered how best to measure the sensitivity of soils *in situ*, because it is impossible to transfer samples to the laboratory without changing them. A shear tester forces into the soil a cone or vane to about 3 m, then rotates it at one revolution per hour to measure "undisturbed" strength; this is re-observed after remoulding the sample by rotation at ten revolutions per minute.

An outline of current information about seafloor spreading by Dr R. S. Dietz (National Oceanic and Atmosperic Administration, Miami, Florida) made plain how profoundly tectonic processes affect everyday affairs; the areas adjacent to the continents are those which would be most useful for buildings, yet these are the areas of greatest instability. In some areas, for example the foreset slopes of major deltas and the flank of oceanic trenches, it may always be impossible to install permanent structures, because any disturbance could induce the downhill slide of sediments which have accumulated for millennia.

A theme present throughout the meeting was reminiscent of the exploration of space; is reward maximized by investing resources in a small amount of manned exploration, or in a comparatively large amount of purely instrumental exploration? Although submarine vessels have penetrated the deepest areas of the oceans, it is apparent that locomotion and manipulation in great depths remain awkward and expensive. Dr I. E. Mikhaltsev (Institute of Oceanology, USSR Academy of Sciences) made plain the advantage of operating on the seafloor rather than at the phase and storm interface occupied by surface vessels. This and other contributions brought into relief the fundamental importance of television as a means of controlling both manned and unmanned devices.

The strength of marine soils is difficult to assess because there is only a limited time in which to apply tests. Geophysical techniques, usually acoustic, encounter this difficulty in extreme form, because the stress used in tests lasts for only a fraction of a second. Dr D. Taylor Smith (University of North Wales) showed that such factors as acoustic attenuation bear a useful relationship to geotechnical properties including porosity, itself descriptive of long-term strength. Drs R. C. Bostrom and M. A. Sherif (University of Washington) pointed out that in sediment piles slopes diminish until stability is reached, so that seafloor topography is a description of long-term strength and can be used to quantify it. The stress is that of infinite period applied by gravity. The most urgent need, perhaps, is the development of a technique capable of observing long-term strength from a ship under way.

A special topics session discussed, among other things, the use of marine soils to cover waste material. It transpires (Dr B. Lehman, Lockheed Building and Construction Corporation) that it would cost $5 per ton to transport compressed waste from New York to the nearest tectonic trench, as against $10 to $20 to incinerate it. In an area penetrated by channels the bottom soil acts as a natural seismic recorder (Dr R. E. Sylwester and others, University of Washington) so that it is possible to map active fault traces invisible on land.

MICROEARTHQUAKES

Geothermal Fault

from our Geomagnetism Correspondent

THE discovery that fluid injection may produce seismic activity along previously inactive faults could prove to be the most socially significant geophysical advance of the century should it prove possible to apply it to the gradual release of strain in active, and potentially dangerous, faults. At present, however, the successful modification and control of earthquakes are far from reality, and a greater understanding of the fundamental processes involved will be required before such hopes can progress much beyond the theoretical stage. Work proceeds in the meantime on several fronts which range from controlled experiments away from human habitation to the observation of natural events and seismic situations created inadvertently by man himself.

Perhaps the most obvious situation in which nature provides its own fluids is the geothermal field; and it seems likely that such fluids will have some effect on faults which cross the field. It is possible, of course, that faults in geothermal regions might be active even in the absence of fluids; but what is quite certain is that microearthquakes are observed in many major geothermal areas such as those in Japan, California and Iceland. And as Ward and Jacob (*Science*, **173**, 328; 1971) now show from a study of the Achuachapan Geothermal Field at El Salvador (Central America), these microearthquakes may be used to map active faults. Indeed, where large earthquakes are either rare or completely absent microearthquakes may provide the only means of fault mapping.

As it happens, between 1961 and 1970 the US Coast and Geodetic Survey reported thirty-two events with magnitudes greater than 3.5 and having epicentres within 60 km of Achuachapan; but depths of focus ranged from 16 to 276 km, so that it seems unlikely that these events were related directly to near-surface geothermal activity. By contrast, between December 7, 1969, and August 10, 1970, Ward and Jacob detected about 500 microearthquakes with magnitudes ranging down to 0. From the distribution of S-wave minus P-wave arrival times it is clear that two groups of events were present. Some 350 of the events had S–P times of more than 8 and were probably associated with a seismic zone which dips north-eastwards under El Salvador. But almost all of the remaining 150 or so had S–P times within the range 0–2; and it is these which were apparently related to surficial geothermal activity. These local events frequently occurred in swarms of ten to twenty events separated in time by up to several weeks. Unfortunately, because of operational problems the foci of only seventeen of the local microearthquakes could be located with any degree of precision, but these were sufficient to define a fault plane intersecting most of the foci and passing through a point where fault breccia was observed in a drill hole. This plane was roughly parallel to normal faults previously mapped to the north-west.

This is not the first time that microearthquakes have been used to map an active fault; but it is a significant case because of the association with geothermal fluids. Thus Ward and Jacob interpret the detected fault as the one which allows the hot geothermal water to reach the surface. If this is a valid interpretation the technique should prove to be of economic importance in that it would be of obvious advantage to site geothermal production wells close to the chief source of upwelling hot water.

Statistical Mechanics and Quantum Mechanics

G. E. UHLENBECK

Rockefeller University, New York

Part of the acceptance speech on the occasion of the award of the Lorentz Medal by the Royal Netherlands Academy of Sciences on June 27, 1970.

ARE the problems of statistical mechanics truly fundamental? I have often changed my opinion and now I would like to elaborate on it. When I was a young student, kinetic theory of matter seemed to me an example of a theory which truly explains something. With much care and effort I worked my way through the *Lectures on Gas Theory* by Boltzmann and the *Elementary Principles of Statistical Mechanics* by Gibbs. Much escaped me and became clear only after I read the famous encyclopaedia article by the Ehrenfests. It was a revelation, not only because of its great clarity but also because it contained a careful summing up of the series of more-than-twelve *lacunae* in the work of the masters. These were like frontier posts and a young student could thereby learn where the real problems lay. How difficult it is to find this out nowadays. The present pollution of the scientific literature makes the finding of clear water, the fundamental concepts, an extremely time consuming occupation. This is true not only for the new student but for anyone who tries to learn something outside his own speciality, as I well know by experience.

Though I passed my examinations successfully, I knew very little of quantum theory and even less of the theory of spectra. I learned that, with Goudsmit's help, when I returned from Rome in 1925 and became Ehrenfest's assistant. I shall not elaborate on the collaboration with Goudsmit, which I shall never forget and which led to the discovery of electron spin. Both Goudsmit and I have often related our memories of this unforgettable period and I mention it only because at that time my conception of statistical mechanics changed completely. I considered it to be clearly on a secondary level. Quantum mechanics on the other hand provided a foundation from which everything should follow, including the behaviour of gases, liquids and solids. This seemed to be confirmed by the success of the electron theory of metals. Pauli and Sommerfeld (both recipients of the Lorentz Medal) showed how all difficulties disappeared as soon as one applied the true quantum statistics. A few riddles remained, such as superconductivity, but in our optimism we felt sure that all would be straightened out eventually. My dissertation in 1927 about statistical methods in quantum theory was therefore a kind of optimistic synthesis of the encyclopaedia article of the Ehrenfests and the new quantum mechanical ideas. The number of unproved assumptions was reduced to three.

Later in Ann Arbor, the beautiful experiments of N. H. Williams on thermal noise and shot noise aroused my interest in the theory of Brownian motion, one of the nicest applications of statistical mechanics. I thought it very interesting but of course it was not fundamental. I remember very well that when I told Pauli about it he called it "desperation physics". I didn't like this but I really agreed with him. For a physicist of the quantum mechanics generation to which I also belonged, the fundamental problems of the 1930s were the theory of the positron, quantum electrodynamics and the developing theories of nuclear structure and beta radioactivity; these were the things on which to work.

Let me say at this juncture that the image of progress in science as a kind of conquest of an unknown domain with a definite "frontier" and successive "breakthroughs" seems to me more and more to be only a romantic illusion. This picture was clearly inspired by the great breakthrough of quantum mechanics and it has influenced my judgment for a long time. I might even say that I was afflicted by it.

My opinion about the fundamental character of statistical mechanics began to change in 1937 to 1938 when I, together with Boris Kahn, became involved in the so called condensation theory. The question why a gas condenses below a sharply determined critical temperature at a sharply determined density has never been called to attention since Van der Waals and the proper understanding of it seemed difficult to us. During the lively discussion about this question at the Van der Waals Congress in 1938 it was even doubted whether the basic assumptions of statistical mechanics contained the answer even in principle. This doubt was not justified, but it made a deep impression on me. As long as such common phenomena as the equilibrium between liquid and vapour and the existence of a critical temperature were not truly understood, the field was not yet conquered, not everything had been explained in principle and thus there existed fundamental aspects of statistical mechanics which I had not appreciated.

(When I showed Pauli the article by Kahn and myself on condensation theory he looked at it and said, "Yes, one should read this". He did so and ridiculed somewhat the quasi-mathematical rigour of our work but I believe that he appreciated the fundamental character of the problem.)

After the war, I continued to follow the new developments in quantum electrodynamics and the theory of beta radioactivity, and from time to time I contributed a little. But my interest moved more and more towards the fundamental questions of statistical mechanics. In the 1950s I began to write a book on statistical mechanics with my pupil and friend, the late T. H. Berlin. I hoped that it would become a modernized and expanded version of the encyclopaedia article which had made such an impression on me. I hoped that we could determine the foundations of the theory in the same critical way as the Ehrenfests, and that we could make clear the nature of the still unsolved basic problems. In short, I hoped that we could discover the "structure" of statistical mechanics.

We worked hard on it but did not get very far and the sudden death of Ted Berlin in 1962 makes it doubtful that our plan will ever be realized. I have learned a lot from it, however, and I have the feeling that I more or less understand the structure of classical statistical mechanics at least. I believe that one always has to keep in mind that the task of statistical mechanics is to study the relationship between the macroscopic description of

Che istry Librar

physical phenomena and the microscopic molecular description. These two pictures are in a certain sense independent; moreover, they are, so to speak, on a different level and are therefore, even qualitatively, totally different. If one sticks to this idea, one can see that Boltzmann, Gibbs, Einstein, Ehrenfest and Smoluchowski have formulated the true basis on which one has to build further. One sees also that there are still many unsolved problems, such as condensation and other so called phase transitions, on which much work is being done. All these problems are very difficult but they are, as a mathematician would say, *bien posés* and therefore one can work on them.

In my opinion the situation is somewhat different for quantum theory. The relationship between the classical and quantum mechanical descriptions of molecular phenomena is rather clear, but this is not so for quantum mechanics and the macroscopic theory. I am aware that this opinion is not shared by most of my colleagues. I believe that the most widespread opinion is that quantum mechanics can be "grafted" onto the classical statistical mechanics of Boltzmann and Gibbs and that therefore quantum mechanics does not require anything essentially new. I had thought so earlier too, but I have slowly retreated from that conviction. The recent discoveries of so called macroscopic quantization and interference phenomena in liquid helium and superconductors have had a great influence on my ideas. They seem to me to show that the existing theories of superconductivity and superfluidity do not provide a complete explanation and that the true macroscopic description of the superfluids has not yet been found. It would be no surprise to me that this is so because the foundations of quantum statistical mechanics have not yet been sufficiently clarified. One only needs to think of the persistent currents to feel doubt about the general validity of the ergodic theorems in quantum theory. Questions like these make low temperature physics so fascinating for me; they have had a rejuvenating effect on me and I am convinced that there is still much to be done on this "frontier" of physics and that profound surprises are possible.

Because I use the word "frontier" let me finally return to the romantic image of advances in science which I sketched earlier. I do not believe in it any longer. There are many frontiers and it comes down to the fact that in science one can only sometimes talk of progress. Whenever there is an advance there is a frontier, not the other way around. As to the direction of the advance, every investigator follows his own nose and does what he can. In my opinion this applies equally to space travel and to high energy physics and radio astronomy. These pursuits exist because they are possible and as long as the expense does not become too exorbitant one must, of course, continue them. But I think that one must oppose all fashion and prestige arguments. There is no natural hierarchy of problems and moreover, as Poincaré remarked long ago, a problem is never completely but always only more or less solved.

It seems better to me to view progress in science as the expansion of different circles of investigation, each autonomous and often apparently entirely independent of the others. The deep problems are to determine how these areas hang together, and how one can arrive at a larger unity. Biology and physical–chemical research, for example, form two such large circles. Their interrelationship seems to me to represent one of the deepest questions man can ask, a real *mysterium tremendum*. On a much smaller scale macroscopic and molecular physics form two such circles and statistical mechanics attempts to fathom their relationship.

Research Environments and Productivity

S. P. GUPTA & K. D. SHARMA

Research Survey and Planning Organization, CSIR, New Delhi-1

The productivity of Indian scientists has been investigated with a view to identifying the environmental factors that influence it. These findings may help policy makers and science managers to create the necessary environmental conditions.

OUR study of productivity has been based on data collected for a report called *Opinion Survey of Scientists and Technologists*[1]. About 9,000 questionnaires were sent to the heads of all the national laboratories, other government and autonomous research institutions, private/industrial research organizations and departments of science, engineering and medicine in universities. The heads of institutions were requested to distribute the questionnaire among an approximately equal number of researchers not below the rank of

Table 1 Characteristics of the Sample

Characteristics: age group	Percentage	Characteristics: organizational settings	Percentage
21–25+	1.9	National laboratories	18.1
26–30+	17.9	Govt. res. institutes	22.2
31–35+	26.7	Private/industrial res. institutes	6.2
36–40+	26.3	Univs./teaching institutes	53.5
41–45+	12.1		——
46–50+	8.2		100.0
51–55+	4.5	Years since PhD	
56–60+	1.0	0–5+	45.6
Above 60	1.4	6–10+	30.6
	——	11–15+	13.5
Total	100.0	16–20+	5.8
Median age	(36.6)	Above 20	4.5
			——
		Total	100.0

Table 2 Average Publication per Year with Standard Deviation for PhD Scientists according to Age Groups

Age groups	No. of respondents	Mean* publications per year	Standard deviations
21–25+	10	1.3	1.6
26–30+	92	1.8	2.5
31–35+	137	2.4	3.5
36–40+	135	2.6	3.4
41–45+	62	3.2	4.5
46–50+	42	2.7	3.0
51–55+	23	4.6	3.9
56–60+	5	3.4	2.1
Above 60	7	2.8	2.2
Total	513	2.6	3.4

* Statistically significant at 0.05 level.

laboratory assistants. No information was available as to how the distribution in each institution was effected, but the sample seems fairly representative of various ranks and disciplines.

An important factor influencing the size and composition of the sample was the willingness or unwillingness of heads of institutions to cooperate. In the absence of a roster of Indian scientists and of the ability to collect the data personally, the only course was to approach the respondent through heads of institutions to ensure a maximum, rapid return. But in many cases cooperation could not be obtained and, as a result, some institutions returned no questionnaires. In all, about 2,300 replies were received in five months—roughly a 25% return. After several rejections due to incomplete information, the total sample analysed stands at 2,143.

We have considered PhD scientists only because scientific productivity is assessed in relation to "professional age"[2] (the number of years since obtaining the PhD). Table 1 summarizes the important characteristics of the sample considered in this article.

We have measured scientific productivity by objective criteria, such as the number of papers, books and patents published. In constructing the index of productivity, a book was considered equivalent to four scientific papers in terms of contribution and efforts[2].

Individual Variables and Productivity

In the first stage of analysing our findings, we tried to discuss some major individual variables, such as age and place of work, with relation to productivity. Table 2 shows the mean number of publications per year and standard deviations in different age groups and also for the combined groups. The age of the respondent varied from 21 to 65, with a median age of 36.6 years, and the mean output of the combined groups during the period under study was 2.6 publications per year. The consistent upward trend in publications with age is indicated up to the age of 55, after which it decreases. The average number of publications per year is highest in the age group 51–55. When group means within each category are compared, the findings are statistically significant at the 0.05 level.

Table 3 Average Number of Publications per Year with Standard Deviation according to Four Organizational Settings

Organizational setting	No. of respondents	Mean* publications	Standard deviation
National laboratories	93	2.2	2.4
Government research institutes	114	2.9	3.6
Private/industrial research institutes	32	2.6	4.3
University/teaching institutes	275	2.6	3.4
Total	514	2.6	3.3

* Not statistically significant.

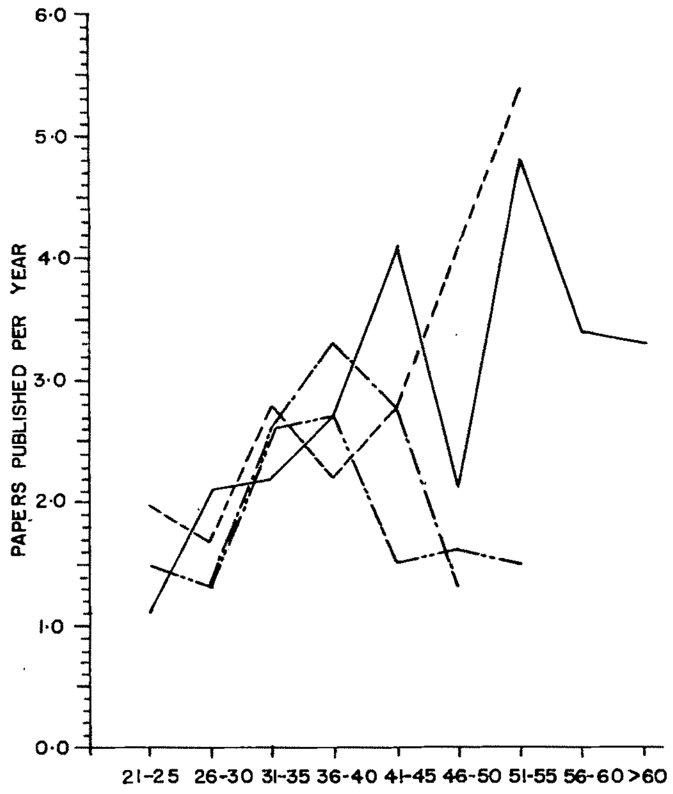


Fig. 1 Productivity of scientists in different age groups and types of organization. —··—, National laboratories; ----, government research institutions; — · — · —, private industrial research institutions; ———, universities and teaching institutions.

Agewise Productivity

Table 3 shows that organizational setting has no influence on the mean publication rate. The insignificant difference between the mean number of publications in different organizations could be due to large variance within each category. Hagstrom[3] finds considerable differences between the contributions of industrial and academic scientists to the growth of science. This is because the managers of industry are anxious to see the findings of their research scientists fully covered by patents before publication in scientific journals. The results of our study do not support Hagstrom's hypothesis. In India, it is quite likely that industrial management puts no constraint on the publication of papers in scientific journals.

Fig. 1 shows the productivity of scientists for different age groups in various organizations. In national laboratories the productivity rate seems to be highest when scientists are in their late 30s and the curve shows a sharp decline thereafter. A similar trend is evident for the scientists in private and industrial research institutes. The findings for these two types of organization are consistent with those of Lehmann[4] and Meltzer[5], who found the greatest productivity in the late 30s and early 40s, followed by a decline.

There is a trend for scientists in government research institutions to increase in productivity between 40 and 55. In the university and other teaching institutes, the productivity increases constantly until the early 40s and then declines between 45 and 50. After that the curve shows an upward trend in the early 50s, before relapsing again. In spite of overall differences in slopes, there are some remarkable similarities among these curves. The ages of best achievement

Table 4 Average Number of Papers in Relation to Recognition in Various Age Groups

Age group	Recognition High	Recognition Low
21–25+	2.0	1.5
26–30+	2.4	1.9
31–35+	2.9	2.2
36–40+	2.8	2.2
41–45+	2.3	2.7
46–50+	3.5	2.3
51–55+	3.1	5.0
56–60+	NA	NA
Above 60	NA	NA

in national laboratories, private and industrial research institutions, and to some extent universities and other teaching institutions, are quite similar. The late 30s and early 40s are the most productive periods in the lives of scientists except for those working in government research institutions.

Recognition, Age and Productivity

Recognition is an indicator of scientists' merit and social worth. In science, two most general forms[6] of recognition are: the immediate supervisor's evaluation of current research work and the credits for research publications. For our study recognition was measured largely through self-evaluation by the respondents themselves. If the respondent felt that he was highly appreciated by his supervisor and given preference in authorship, it was taken to indicate high recognition, otherwise he was categorized as having low recognition.

When recognition is examined in relation to productivity, keeping age groups constant, the results are quite interesting (Table 4). Productivity is low for scientists with low recognition. Although recognition is important to all scientists, younger scientists are more concerned about it, for they feel that it is essential if they are to establish themselves. Therefore, one of the environmental condition conducive to higher productivity in younger scientists is recognition. Another important outcome of Table 4 is that after the age of 50 organizational recognition is not important in the productivity of scientists, perhaps because at this age they are well established.

Freedom and Productivity

We have inferred the extent of freedom in research from the extent of autonomy in the selection of projects. Like the index for recognition, this has been reduced to two classes—high and low degrees of freedom.

Table 5 Publication per Year as Related to Freedom in Various Organizations

Organizational setting	Freedom High	Freedom Low	P*
National laboratories	2.4	2.0	n.s.
Government research institutes	3.4	2.2	0.05
Private/industrial research institutes	3.2	1.6	n.s.
University/teaching institutes	2.8	2.1	n.s.

* Significant level.

Our chief concern was to find out how scientists perform in these two categories of freedom. Table 5 shows the publications per year in various organizations where freedom is variable. It shows that the average number of publications per year is greater when there is a high degree of freedom than when there is a low degree of freedom in all four organizational settings. The differences within the organization were not statistically significant, however, except in government research institutes (other than national laboratories). It seems paradoxical that professional freedom has no bearing on productivity. In our study the emphasis was on freedom to select research projects, whereas a scientist is probably more concerned with the freedom to attack the problem and comparatively less bothered who selects the project for him. It is quite likely that the scientist wants least interference in his day to day work.

Prospects for Future Productivity

The results of our study present an interesting picture of Indian science. For the total sample productivity increases until the age of 55, and then declines. This contrasts with the finding of Pelz and others[7], and so this aspect was studied in detail for each organization (Fig. 1). It can be seen that productivity increases with age for: (i) government research laboratories, and (ii) teaching institutes and universities. In the case of national laboratories and private industrial research institutes the trend follows the international pattern, but for government laboratories and universities it shows a secular increasing trend, for which there is no obvious reason. Perhaps in government research laboratories and universities and teaching institutes authorship is based on seniority in the administrative hierarchy. But there may be other reasons, and it would be interesting to identify the factor responsible for the continuing increase of productivity with age in these institutes. It might then be possible to replicate these conditions in other institutes to augment the productivity of Indian scientists.

Scientists in national laboratories are less productive than their counterparts in organizations such as government research institutes; private and industrial research institutes, universities and so on. It is also evident that scientists in government research institutes have the highest productivity. It is interesting that this correlates with increasing productivity with age. This again tends to support the hypothesis that in government organizations, it is the hierarchy that decides the authorship rather than the actual participation in the research project.

Recognition and freedom have been suggested as motivating environmental factors which influence the productivity of scientists. From the operative point of view, the influence of these factors varies with age. We have found that people in the higher age group are neutral to recognition while those in the younger age group seem to be very susceptible to it. The lack of influence of freedom to choose projects on productivity needs to be studied further in view of the fact that the conflict between administrators and scientists revolves round control and freedom. In another study (unpublished) we found that freedom was a neutral factor with regard to the commitment of scientists to organizational goals. This could be explained by a lack of precision in the definition of the term "freedom" in the questionnaire, or a difference of opinion among scientists about the meaning of the term. In India scientists seem to be indifferent to this type of freedom. They probably interpret freedom as the opportunity to attack a problem without hindrance.

We have attempted to isolate a few of the environmental variables which influence the productivity of scientists. If these factors are properly manipulated, it is possible to increase productivity, and so it is essential that policy makers should consider these aspects more seriously.

[1] Ahmed, A., and Gupta, S. P., *Opinion Survey of Scientists and Technologists*, Survey Rep. No. 9 (RSPO, CSIR, New Delhi, 1967).

[2] Crane, D., *Amer. Sociolog. Rev.*, **30**, 699 (1965).

[3] Hagstrom, W. O., *The Scientific Community*, 38 (Basic Books, 1965).

[4] Lehmann, H. C., *Age and Management* (Princeton University Press, 1954).

[5] Meltzer, L., and Salter, J., *Amer. Sociolog. Rev.*, **27**, 351 (1962).

[6] Glaser, B. G., *Social Problems*, **19**, 268 (1963).

[7] Pelz, D. C., *Trans. IEEE on Engineering Management*, **EM11**, 23 (1964).

Unique Bedrock and Soils associated with the Teesdale Flora

G. A. L. JOHNSON & D. ROBINSON

Department of Geology, University Science Laboratories, Durham

M. HORNUNG

Nature Conservancy Research Station, Bangor

This article describes the geological factors which have contributed to the formation of the unique bedrock geology and soils of the metamorphosed limestones on Widdybank and Cronkley fells in Upper Teesdale. The survival of the relict arctic–alpine flora is probably directly related to the distinctive geology of the area.

THE distinctive relict arctic–alpine flora of Upper Teesdale, Co. Durham and North Yorkshire, has been famous in biological circles for more than a century. In a subarctic environment, not quite warm enough for the growth of trees, there occur many species of flowering plants, pteridophytes, bryophytes and lichens which are rare in the British Isles and a few species which are not known in Britain outside Teesdale[1–4]. The flora includes a number of plants belonging to widely differing phytogeographical groups and has been interpreted as a relict flora from the vegetation that colonized Teesdale shortly after the end of the Quaternary glacial period. The flora has survived through the post-glacial forest and blanket bog intervals in Teesdale partly on rendzina soils associated with rotted crystalline marble on Widdybank and Cronkley fells[1,3].

Stratigraphy and Structure

The structure of Upper Teesdale is dominated by a north–south trending, east facing faulted monocline called the Burtreeford Disturbance[5] that has an eastward downthrow of some 200 m (Fig. 1). On the west, upfaulted side of this structure, Skiddaw Slates and tuffs of Ordovician age are exposed in the sides of the Tees at Pencil Mill, forming a small and poorly exposed inlier in the valley floor[5]. An angular unconformity separates the folded Ordovician from the overlying almost horizontal Carboniferous marine strata. The Carboniferous sequence[5] commences with a Basement Group composed of conglomerate and sandstone beneath dark limestones and shales about 25 m thick. The succeeding Lower Limestone Group is dominated by the Melmerby Scar Limestone, a light coloured limestone 38 m thick, which is separated into thick beds with thin shale and sandstone partings. Above the Melmerby Scar the upper beds of the Lower Limestone Group and the overlying Middle Limestone Group are composed of a rhythmic sequence of predominantly dark coloured limestone bands with intervening shales and sandstones. Significantly the Teesdale relict flora is found particularly in association with outcrops of metamorphosed light coloured Melmerby Scar and Robinson limestones and not with the overlying unaltered darker pigmented limestones.

It is probable that a full sequence of Carboniferous sediments was laid down in Teesdale followed possibly by Permian and Mesozoic deposits, but no evidence of these beds remains because of extensive Tertiary erosion which has removed any younger rocks.

Towards the end of the Carboniferous period uplift, compression and doming of the region took place and the Great Whin Sill quartz–dolerite intrusion was emplaced. The heat of intrusion baked and altered the sediments above and below the sill, producing new minerals and textures in the altered rocks contributing significantly to their present nature.

In Teesdale the Burtreeford Disturbance was active before the emplacement of the Great Whin Sill. The sill cuts across the faulted monocline from a position near the Tyne Bottom Limestone, within the Middle Limestone Group, on the east to a horizon near to the base of the Melmerby Scar Limestone on the west (Fig. 1). On the west of the disturbance the sill, 73 m thick, nears its maximum thickness and is at its lowest stratigraphical horizon. In all directions it tends to rise in the Carboniferous succession and decrease in thickness away from this centre[6,7].

After the emplacement of the Whin Sill, hydrothermal mineralization commenced in the Pennine region. This process is believed to have extended over at least 100 m.y. (ref. 8) producing economic galenabarytes veins in Upper Teesdale. The veins have been extensively worked in the region, notably at Cow Green Mine, and trial workings, chiefly for lead ore, are present on Widdybank and Cronkley fells.

A further important factor in the history of Upper Teesdale is the long period of erosion, perhaps 50 m.y. from the early Tertiary to the present day, that has removed the younger rocks and exposed the Whin Sill within the Melmerby Scar Limestone on the floor of the valley. The almost flat top of the sill forms a wide bench on Widdybank and Cronkley fells and the overlying crystalline marbles outcrop extensively though often masked by superficial glacial drift. The morainic drift and peat deposits, formed during Quaternary glacial and post-glacial times, have also played a significant part in the formation of the biologically important soils.

Whin Sill and Metamorphism

On Widdybank and Cronkley fells metamorphism of the Melmerby Scar Limestone and associated sediments occurs above and below the Great Whin Sill. The limestone is recrystallized for more than 25 m from the contact and the metamorphic effect can be detected to almost 40 m above and below the sill on the basis of spotting in shales. Elsewhere in northern England, where the sill is thinner, the effect of metamorphism is restricted to beds close to the contact.

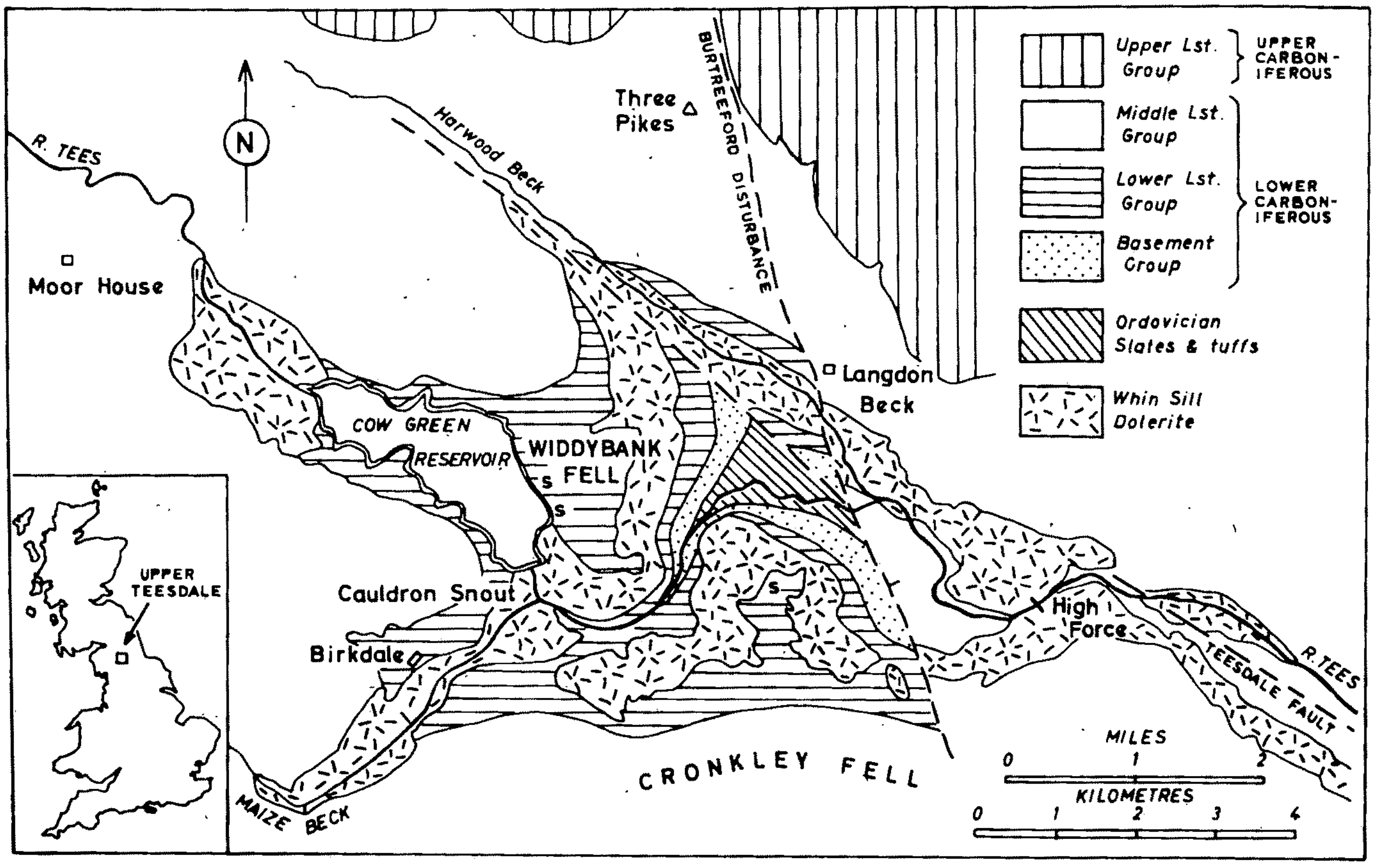


Fig. 1 Geological sketch map of part of Upper Teesdale showing the outcrop of the Great Whin Sill and the principal groups of sedimentary rocks. *S*, Well developed rotten marble and calcite sand rendzinas.

The alteration of pure limestones is limited to simple recrystallization. Impure limestones close to the contact form calc–silicate rocks in which a wide variety of minerals are present. Shales adjacent to the Whin Sill are converted into hard porcellaneous rocks (whetstones) in which the bedding is lost. Further from the contact spotted shales are commonly developed. Pure sandstones are converted into white quartzite at the contact and impure sandstones develop alteration patterns depending on the amounts of argillaceous or calcareous material present. The mineralogy of contact rocks associated with the Whin Sill in Teesdale has been described recently by Robinson[9].

The pure Melmerby Scar Limestone of Widdybank and Cronkley fells is recrystallized by the Whin Sill to a saccharoidal marble for about 25 m from the upper contact; a small thickness of marble is also present below the sill. The marble consists of dodecahedral to rounded grains of calcite varying in average grain size from 0.5 mm at the contact to 0.05 mm at a distance of 18 m above. The dark pigmented limestones, found principally in the Middle Limestone Group, are not recrystallized because the carbonaceous organic matter causes the suppression of marmarization[10]. Thus where dark limestones are found near the Whin contacts very little or no crystalline marble is found.

The formation of marble in Upper Teesdale is clearly the result of three factors combining to form a bedrock formation unique in northern England. First, the Whin Sill is near maximum thickness and lowest stratigraphical horizon. Second, the sill is injected into the thickest limestone in the Carboniferous succession of the region, the Melmerby Scar Limestone. Finally, the low carbon content of this pure, light coloured limestone allowed metamorphic recrystallization to take place readily, with the development of thick marble formations.

Weathering of Crystalline Marble

The marble outcrop is partly masked by patches and tongues of glacial drift on Widdybank and Cronkley fells. This is a pattern of exposure that has not changed greatly from the early post-glacial period when continuous vegetation cover stabilized the unconsolidated drift slopes. Since then peat has developed over impervious deposits, particularly the clay drifts, and thin soils have formed over exposed bedrock.

Weathering of bedrock crystalline marble takes place both at the surface, by processes of subaerial erosion, and below the surface at the bedrock drift or mineral soil interface. At the surface a hardened crust tends to form on the marble and erosion may be relatively slow except near to the Whin contact where the coarsely crystalline rock is often readily attacked by subaerial weathering processes. At the subsurface junction of bedrock marble and overlying drift or soil, percolating ground water causes solution weathering leading to loss of cohesion between calcite grains in the marble. Sections through the drift-marble interface show rotten saccharoidal marble or calcite sand deposits 0.25 to 3.0 m thick. The thickness of the layer and the rate of weathering of the marble are dependent on the amount of ground-water movement. Subsurface erosion sufficient to produce hollows in the land surface has taken place as the rotted marble is removed in solution.

From these observations it follows that the unconsolidated calcite sand, known to a succession of biologists as "sugar limestone", is chiefly a subsurface weathering product of saccharoidal marble formed beneath a cover of drift and soil. Its presence at the surface today is caused by contemporary erosion of the soil and drift cover. Calcite sand is a most unstable surface deposit which tends to erode by rain and wind action at any place where the protective drift, soil or vegetation cover is removed. A cycle consisting of subsurface weathering below thin soil followed by erosion of unstable calcite sand may have taken place on crystalline marble outcrops on Widdybank and Cronkley fells during much of the post-glacial time. This would provide a succession of open habitats suitable for the survival of the arctic–alpine flora. Because of its instability and secondary origin it is unlikely that calcite sand deposits were present on these outcrops during the period of initial plant colonization in late-glacial times.

Soil Development

The presence or absence and thickness of drift cover have been important factors in determining the soils developed over crystalline marble on Widdybank and Cronkley fells. Under continuous drift, more than 60 cm thick, the limestone acts chiefly as a platform on which soil formation takes place in the upper layers of the drift cover. The limestone exerts some influence on soil formation by maintaining free drainage in the subsoil and lower drift, even though the latter is often of heavy texture. Peaty gleyed podzols or deep peat are the resultant soils. Where the superficial drift cover is thinner (30 to 60 cm) the limestone maintains free drainage, but also exerts an influence on the chemistry of the soil so that brown earths or brown calcareous soils result. Where there is only a thin layer of superficial material, less than 30 cm thick, the bedrock marble dominates soil formation and rendzinas are the resulting soils.

The rendzinas form part of a complex in which three subtypes, for the purpose of this paper called α, β and γ, occur in association with small areas of brown earth soils. The brown earths are found over patches of deeper superficial material. The rendzinas are characterized by alkaline reaction and excessive drainage such that even short dry periods cause a water deficit[11]. Rendzina α is the simplest and forms *in situ* from rotten crystalline marble. The profile is divisible into a dark grey, humic but sandy surface horizon (A or A_h) which has a dense mat of fibrous roots, and an underlying C horizon of white or cream coloured calcite sand. Formation of α rendzinas requires the prior removal of any superficial drift with exposure of the underlying marble to subaerial erosion and soil forming processes.

Rendzina β is the most widespread in the rendzina complexes. It develops over crystalline marble bedrock in regions where drift parent materials play an important part in profile formation. A typical profile is composed of a thin horizon (up to 10 cm) of dark brown or reddish brown clay or silt loam over calcite sand resting on relatively unaltered marble. The colour and texture of the surface layer vary considerably. Colour variation reflects a number of factors but bright coloured reddish variants have a high content of total iron present as hydroxides. The bright red soils overlie marble cut by fine siderite veins which oxidize to hydroxides of iron including goethite. The soils inherit their coluration and high iron content from the vein material, fragments of which are normally present in the soils. Textural variation depends on changes in the proportions of sand and clay with the silt fraction remaining fairly constant. The soils also contain small stones which have been derived from the surrounding drift. The loam is clearly of mixed origin containing contributions from the drift and the underlying marble; mixing probably took place during pedogenesis.

Rendzina subtype γ is a closely related group of soils which have aeolian erosion and depositional characteristics as a uniting feature. They form a small part of the total rendzina complexes and show much variation in detailed profile morphology. These rendzinas are particularly well developed in the Thistle Green area, Cronkley fell, where they are associated with and abut against bare areas of calcite sand. Profiles of rendzina γ almost always contain a number of buried surface horizons and clear evidence of surface accretion. Observation on a windy day immediately demonstrates that aeolian movement and deposition are active and sufficient to produce profile accretion. These soils were formed under conditions of periodic erosion, aeolian transport and deposition of calcite sand. Readily identifiable old surface horizons within the rendzina γ profiles support an origin by periodic deposition rather than continuous accretion. Periodic erosion of limestone sand would produce areas available for plant colonization and would aid the survival of the Teesdale flora.

The typical rendzina profiles differ markedly from soils developed over unaltered Carboniferous limestone though the rendzina β subtype grades imperceptively into normal limestone soils away from the Whin Sill contact as the bedrock changes from coarse to fine grained marble. The marble rendzinas, though distinctive, form only a relatively small part of the soils of the botanically significant region of Upper Teesdale. The brown earths, brown calcareous soils and the various gleys developed on drift and other parent materials are also important.

Bedrock–Soil–Flora Association

The combination of lower Carboniferous clear water limestone, late Carboniferous Earth movements and the injection of the Whin Sill produced coarse grained crystalline marble in Upper Teesdale. Tertiary and recent erosion with the Quaternary glaciation exposed this unusual bedrock at the surface. Rendzinas developed on outcropping marble by soil forming processes. A geological setting of this type is unknown outside Upper Teesdale in Britain and comparable rendzinas are similarly restricted. In the vicinity of this narrow geological and pedological setting the Teesdale flora has survived since shortly after the end of the Quaternary glaciation. It is our contention that the survival of the relict flora in association with unique geology and soils is not coincidental. The inherent instability and excessive drainage of the calcite sand soils have provided a continuous series of precarious habitats suitable for the survival of the alpine plants. In the vicinity of these soils elements of the flora have colonized restricted adjacent regions of differing soil type giving the plant distribution found today. Other factors have clearly played their part in sustaining the flora over the period of about 10,000 years. Two factors are particularly noteworthy; first, the severe climate of the Teesdale fells provides a suitable environment, and second, continuous grazing of the small areas of limestone grassland from prehistoric times[12] to the present day has tended to maintain the required more open habitats.

We thank Drs W. A. Clark, A. W. Davison, J. Turner and D. F. Ball who have read the manuscript and offered helpful advice. This research forms part of a survey of Upper

Teesdale supported by the Natural Environment Research Council.

Received July 12, 1971.

[1] Pigott, C. D., *J. Ecol.*, 44, 545 (1956).
[2] Bellamy, D. J., Bridgewater, P., Marshall, C., and Tickle, W., *Nature*, 222, 238 (1969).
[3] Turner, J., in *Durham County and City with Teesside*, 123 (Brit. Assoc., Durham, 1970).
[4] Bradshaw, M., in *Durham County and City with Teesside*, 141 (Brit. Assoc., Durham, 1970).
[5] Johnson, G. A. L., *Trans. Nat. Hist. Soc. Northumb.*, **41**, 5 (1970).
[6] Dunham, K. C., *The Geology of the Northern Pennine Orefield*, 1 (Mem. Geol. Surv. Gt. Brit., 1948).
[7] Dunham, A. C., *Trans. Nat. Hist. Soc. Northumb.*, **41**, 92 (1970).
[8] Dunham, K. C., Firch, F. J., Ineson, P. R., Miller, J. A., and Mitchell, J. G., *Proc. Roy. Soc.*, A, **307**, 215 (1968).
[9] Robinson, D., *Trans. Nat. Hist. Soc. Northumb.*, **41**, 119 (1970).
[10] Robinson, D., *Cont. Min. Pet.* (in the press, 1971).
[11] Welch, D., and Rawes, M., *Trans. Nat. Hist. Soc. Northumb.*, n.s., **17**, 57 (1969).
[12] Johnson, G. A. L., and Dunham, K. C., *Geology of Moor House* (Nature Conservancy Monograph No. 2), 158 (1963).

Transitive Inferences and Memory in Young Children

P. E. BRYANT
Department of Experimental Psychology, University of Oxford

T. TRABASSO
Department of Psychology, Princeton University

Contrary to the conclusions of Piaget, young children can make transitive inferences if precautions are taken to prevent deficits of memory from being confused with inferential deficits.

FOR some time, it has been believed that young children are unable to form transitive inferences about quantity until they pass the stage of logical preoperations at about 7 yr old[1,2]. This argument has been very widely accepted[3,4]. Piaget and his colleagues propose that the young child cannot infer, for example, that $A > C$ from the information that $A > B$ and $B > C$. They assert that the child is unable to coordinate the first two, separate items of information in order to reach the correct inferential conclusion about A and C. If true, this claim has important educational implications, for a child who cannot combine this information must also be unable to understand the most elementary principles of measurement.

We report two experiments which show that the claim is unjustified. The experiments demonstrate that 4 yr old children can make transitive inferences about quantity, provided that they can remember the items of information which they are asked to combine.

One must ensure that the child has retained the comparisons which he has to combine, if one is to infer whether or not he can make transitive inferences. Otherwise, an error might simply be due to a failure in memory and have nothing to do with inferential ability. Yet experiments in which it is reported that children cannot make inferential judgments lack this elementary precaution. One can control for forgetting by taking two precautions. First, teach the child the initial comparisons ($A > B$ and $B > C$) very thoroughly. Second, test for memory of these comparisons at the same time as one asks the inferential question about A and C. The first precaution ensures retention of the initial comparisons and the second checks for this retention during the inferential problem.

We found it necessary to introduce another control in our experiments. This was against the transfer of "absolute" responses. The general procedure in prior experiments has been to use three quantities (A, B and C). If only three are used, the correct response to the two extreme quantities must be the same in both the inferential problem and in the initial direct comparisons. The quantity A is the "larger" when compared with B, and C is the "smaller" when compared with B, in the initial direct comparisons. Yet "larger" is the correct response to A and "smaller" is the correct response to C in the inferential AC comparisons. Thus a child who produces the correct response when asked the AC question may do so by parroting a verbal label picked up during the initial comparisons, and not, as has always been assumed, by making a genuine inference through the combination of two separate comparisons.

The correct control is to introduce more stimuli and thus more direct comparisons. If there are as many as five stimuli (in descending order of size they are A, B, C, D and E) four initial direct comparisons are possible, $A > B$, $B > C$, $C > D$, and $D > E$. Note that B, C, and D all feature in two of these comparisons and that each of them is the larger in one comparison and the smaller in the other. This means that no one absolute response can be transferred to any one of these three quantities, and it therefore follows that the crucial transitive comparison will be between B and D. This indirect comparison cannot be solved by transfer of absolute responses, since B and D were both larger and smaller in the initial direct comparisons.

Our experiments thus involved first a thorough training period with four direct comparisons, followed by a test period in which the children were tested for their ability to make transitive judgments *and* to remember the initial comparisons. In the first experiment, sixty children in three age groups of twenty each participated. The mean ages of the groups were

4.5, 5.6, and 6.7 yr respectively. The youngest group came from a local nursery school and the older groups from two local infants' schools.

The training and testing materials were identical. Five coloured wooden rods, each of a different length and colour, were used. The lengths were coloured differently in order that they could be identified and remembered. Thus the child learned, for example, that blue was larger than red and red larger than green. The colours were red, white, yellow, blue and green, and the lengths were 3, 4, 5, 6 and 7 inches. The particular colour–length combinations differed from child to child and were counterbalanced within each age group.

The rods were presented in pairs throughout the experiment. The pairs were always presented in such a way that each rod protruded from the top of a container by one inch. The equal protrusion was achieved by sinking bores of different lengths in the container box. This kind of presentation forced the child to use the colour differences in making a choice between different lengths.

In the initial training, each trial consisted of showing the child a pair of rods of different colours, each rod protruding one inch from the top of the box. The child was asked to indicate which rod was the "taller" or the "shorter" ("big" and "little" were the terms used with the youngest group, as they were more easily understood). When the child made his choice the experimenter drew out the rods and held them perpendicular to the table, so that the child could compare lengths directly. This was the feedback for the choices in the direct comparisons, which enabled him to learn to make the correct choice. The question asked (taller and shorter) and the positions of the rods (left and right) were varied in an irregular order from trial to trial.

The training was divided into two phases. In the first, the four comparisons, $A > B$, $B > C$, $C > D$, and $D > E$, were trained separately and in order from either the tallest to the shortest or vice versa; half of the children in each group were trained with each order. Each of the pairs was learned to a criterion of eight out of ten successful choices. In the second phase which followed immediately, all four pairs were presented in a random order on different trials. This interweaving continued until each child responded correctly to six successive presentations of each pair.

Testing followed immediately after the completion of the second training phase. The testing procedure was the same except that no feedback at all was given, that is, the lengths were not shown after a choice. Each child was tested four times on every one of the ten possible pairs of colours. These ten included the four, initial (direct) comparisons, and six, new, transitive (indirect) comparisons. The order in which the pairs were tested was random and varied between children. The type of questions and the position of the rods in each pair varied irregularly as in training.

All groups learned the initial (direct) comparisons rapidly. In the first phase of the training there seemed to be no age differences in speed of learning. In the second phase, however, the 4 yr old children made more errors than the other two age groups who were not significantly different from each other.

The test results are summarized in Table 1, which gives the average probability of a correct response on each pair of colours for each age group. The main diagonal cell entries (A, B: B, C: C, D: D, E) of each subtable in Table 1 show the correct choice probabilities for the initial direct comparisons. The top row and the right column off-diagonal entries (A, C: A, D: A, E: B, E: and C, E) are those for transitive tests where one or both of the rods included one of the end points, which were always either larger (A) or smaller (B). (These transitive comparisons may have been susceptible to the transfer of absolute responses.) The critical entry is the off-diagonal B, D pair, in which each rod had been both larger and smaller in the initial comparisons. The importance of this cell is stressed.

The evidence for transitivity is impressive at all age levels. All the transitive comparisons (AC, AD, AE, BD, DE, and CE) were well above chance level, since the upper 99% confidence bound on the hypothesis of chance is 0.65 with a sample of $n=20$. Turning back to the crucial BD comparison it should be noted that children at all three age levels were able to make genuine transitive inferences very well.

It should also be noted that the BD scores were consistently the lowest of all ten comparisons. Of particular interest is the fact that the children seemed to perform at a lower level with the BD comparisons than with BC or CD comparisons, and yet these last two provide the information on which the transitive BD judgment must be based. This conclusion is supported by an analysis of variance of the errors on these three comparisons, which produced a significant difference between pairs (F3.3%, d.f.2,114, $P<0.05$) and no interaction with age. Thus our conclusion from the first experiment was that young children can make genuine transitive inferences extremely effectively, but not perfectly. The reason for less than perfect inference is discussed later.

Table 1 Probability of Correct Choices on Tests for Transitivity and Retention (Experiment 1)

Stimulus	B	C	D		E
4 yr old children					
A	0.96	0.96	0.93		0.98
B	—	0.92	0.78 †	(0.83) *	0.92
C	—	—	0.90		0.94
D	—	—	—		0.91
5 yr old children					
A	1.00	0.96	1.00		0.98
B	—	0.86	0.88 †	(0.80) *	1.00
C	—	—	0.92		1.00
D	—	—	—		1.00
6 yr old children					
A	0.99	0.99	1.00		1.00
B	—	0.94	0.92 †	(0.92) *	0.99
C	—	—	0.98		1.00
D	—	—	—		1.00

* Predicted.
† Critical entry.

There is, however, one possible objection to our conclusion. In the training phase we showed the children the full length of the rods when we gave them feedback at the end of each trial. It is possible that the children solved the BD transitive question not by making a genuine transitive inference but simply by remembering that B was 6 inches and D was 4 inches. Such a solution to the problem is not an inferential one, and although we did not think that this kind of solution was likely, nevertheless we did not exclude its possibility in the first experiment.

We therefore carried out a second experiment on twenty-five 4 yr old and twenty-five 5 yr old children, in which our procedure was exactly the same as in the first experiment, except that we eliminated visual feedback during the training session. Instead of being shown which of the two rods was longer at the end of each training trial, the children were simply told which was the longer and which the shorter. The rods were never displayed completely until the whole experiment was finished.

The test results for this experiment are given in Table 2, which shows that the removal of visual feedback had no effect on children's ability to make transitive comparisons. Once again performance on all transitive comparisons was well above chance. The performance of both age groups was as good in the BD comparison in this experiment as the performance of the equivalent age groups in the first experiment. Again, however, the BD scores were somewhat lower than the BC and CD scores. We analysed the scores of the 4 and 5 yr olds on these three comparisons in the two experiments

Table 2 Probability of Correct Choices on Tests for Transitivity and Retention (Experiment 2)

Stimulus	B	C	D		E
4 yr old children					
A	0.98	0.98	0.93		0.97
B	—	0.89	0.82 †	(0.77) *	0.90
C	—	—	0.87		0.88
D	—	—	—		0.94
5 yr old children					
A	0.98	0.92	0.95		0.97
B	—	0.87	0.85 †	(0.85) *	0.98
C	—	—	0.97		0.95
D	—	—	—		0.98

* Predicted.
† Critical entry.

together, and again found a significant difference between pairs ($F3.89$, d.f.2,172, $P<0.05$). There was no interaction with age or with experiments. Thus in both experiments, children's transitivity, though considerable, was not perfect.

We shall now argue that the lower performance on the crucial BD pairs is not due to a failure to make inferences but to a failure of retention of the information contained in the initial comparisons. Let $P_{b,c}$ and $P_{c,d}$ be the respective probabilities of recalling the information on the BC and CD pairs. Assume that the recall of these information units is independent. Then the probability of making a correct inference on the BD test ($P_{b,d}$) is the probability of jointly recalling the information for each of the training pairs. Since these events are independent, their probabilities multiply; that is, $P_{b,d}=P_{b,c}\times P_{c,d}$.

These assumptions were tested by multiplying the observed probabilities for the BC and CD tests for each subtable of Tables 1 and 2. The predicted $P_{b,d}$ is shown in parentheses. It can be seen that these predictions are reasonably close to the observed values. Hence, the lower performance on the BD tests may be attributed to a failure in memory rather than to inferential difficulty.

This last experiment conclusively demonstrates that very young children are able to make transitive inferences extremely effectively. They can combine separate quantity judgments very well and they can do so at a far younger age than has generally been assumed. This is a conclusion which has practical as well as theoretical importance.

Received June 18, 1971.

[1] Piaget, J., and Inhelder, B., *The Child's Conception of Space* (Routledge and Kegan Paul, London, 1956).
[2] Piaget, J., Inhelder, B., and Szeminska, A., *The Child's Conception of Geometry* (Routledge and Kegan Paul, London, 1960).
[3] Smedslund, J., *Psychol. Bull.*, **71**, 237 (1969).
[4] Flavell, J. H., and Wohlwill, J. F., in *Studies in Cognitive Development* (edit. by Elkind, D., and Flavell, J. H.) (Oxford University Press, New York, 1969).

Optimum *P*% Library of Scientific Periodicals

B. C. BROOKES

School of Library Archive and Information Studies, University College London, Gower Street, London WC1E 6BT

A quantitative method is described for finding the optimum percentage of periodicals for a library in a given field. The appearance of new journals is apparently counterbalanced by the disappearance of the old.

By applying two empirical laws of informatics it is now possible to design the most compact selection of scientific periodicals to cover any specified percentage $P\%$, of the total periodical coverage of any well defined scientific subject. The two laws are the Bradford law of scatter and the negative exponential law of obsolescence.

Ideal and Practical Totalities

If all scientific periodicals are supposed to be arranged in order of decreasing productivity of papers relevant to some specified subject S, an ordered linear array which can be subdivided into three exclusive sets is obtained.

The set S_1 consists of all periodicals which contribute an average of at least r papers/yr which are relevant to S. The set S_2 consists of those periodicals which have contributed some papers relevant to S but with an average productivity which is less than r papers/yr. The set S_3 consists of those scientific periodicals which have not yet contributed papers relevant to S and are practically certain not to (Fig. 1).

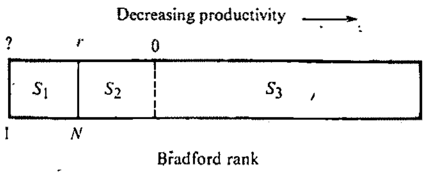


Fig. 1 The three sub-sets of periodicals.

From the point of view of a scientist interested only in the subject S, the periodicals of the set S_3 need not be identified. The periodicals of S_2 are identifiable by the fact that they have contributed some relevant papers: they must be regarded as candidates for inclusion in S_1. The periodicals of set S_1 contain almost all the literature relevant to S if r is made small enough. To make quantitative analysis possible, some numerical value has to be assigned to r.

Bradford observed[1] that if the periodicals which contribute papers relevant to any well defined scientific subject S are arranged in decreasing order of productivity and ranked 1, 2, 3, . . . , then the periodical ranks begin with a very productive "nuclear" zone (of ranks 1 to c, where c may be any number from 3 upwards and which increases with the generality of the subject) followed by a long tail of steadily less productive periodicals.

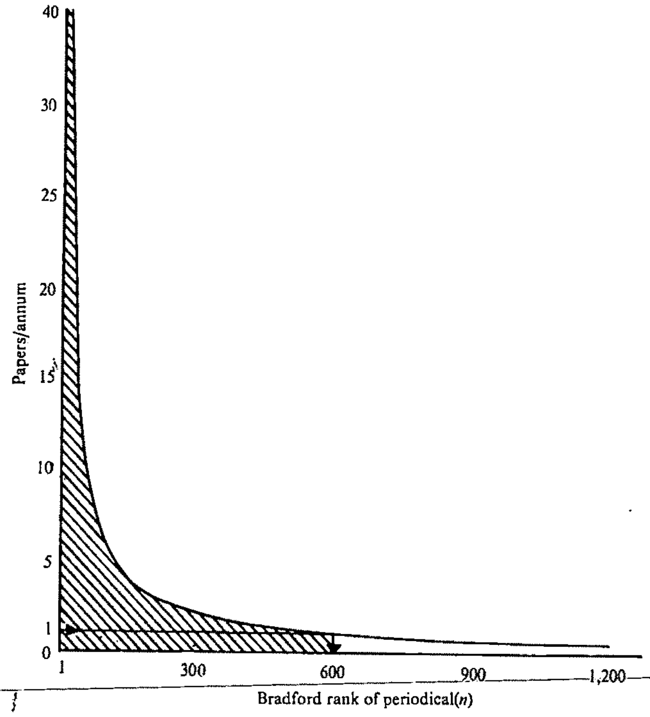


Fig. 2 The stipulated 100% totality (linear scales).

It has been shown[2] that the total number of relevant papers contributed by the periodicals of rank 1 to n is given by

$$R(n) = N \log_e n/s, \ (n \geqslant c) \tag{1}$$

where N is the number of periodicals which contribute an average of at least 1 paper/yr and s (>1) is a constant characteristic of the subject. It follows that the periodical of rank n contributes N/n relevant papers/yr. The total number of papers contributed by set S_1 will therefore be

$$R(N/r) = N \log_e N/rs \tag{2}$$

When $n \leqslant N$ or $r \geqslant 1$, the contributing periodicals can all be identified without difficulty. When $n > N$, the periodicals contribute only occasionally and apparently at random.

For the purposes of this analysis it is therefore stipulated that the practical annual totality of papers relevant to S be that contributed by the periodicals ranked 1 to N, so that $r = 1$. This total, henceforward regarded as the 100% totality, is given by

$$R(N) = N \log_e N/s \tag{3}$$

For example, if $N = 600$, $s = 1.5$, then

$$R(N) = 600 \log_e 400 = 600 \times 5.99$$
$$\approx 3{,}600$$

Admittedly, this totality is arbitrarily defined; some further papers will be contributed by periodicals outside the set S_1. But any other definition of totality which is also quantifiable would be open to similar criticism. The difficulty is illustrated by Fig. 2 which shows how the annual productivity decreases as n increases up to and beyond N for the case in which $N = 600$, $s = 1.5$. The practical totality proposed is indicated by the shaded area.

Obsolescence

It is known that the immediate or scientific usage of scientific periodicals declines with the age of the periodical volumes. Strictly, this decline conforms closely with a negative exponential law but, in practice, because reference is usually given to the year of publication, it is much more convenient to use instead the geometric distribution:

$$p(t) = (1-a)a^{t-1}, \ (1 < t < \infty) \tag{4}$$

where $p(t)$ is the probability of reference to an issue of the periodical of age t years and a is a constant characteristic of the literature and of the nature of its usage.

It will be assumed that a is constant for all values of t. For $a < 1$, the series

$$1 + a + a^2 + \ldots a^{t-1} + \ldots$$

converges to the sum $1/(1-a)$ as $t \to \infty$. If R references are made to a given periodical in a given library context during its first year of life, then aR references can be expected during its second year, a^2R references during its third year and so on. It is therefore possible to estimate the total number of references that will be made to it during its (infinite) lifetime. This total will be

$$R(1 + a + a^2 + \ldots a^{t-1} + \ldots) = R/(1-a) \tag{5}$$

If the issue of the periodical is already t years old, then the number of further references to it that can be expected is given by

$$R(a^t + a^{t+1} + a^{t+2} + \ldots)$$
$$= Ra^t(1 + t + t^2 + \ldots) \tag{6}$$
$$= Ra^t/(1-a)$$

which reduces to (5) when $t = 0$.

Within the given library context, expression (5) can be called the total utility of a periodical volume which has just been published; it will be denoted by $U(O)$. Its remaining utility at age t is given by (6) and can be denoted by $U(t)$. We then have

$$U(t) = Ra^t/(1-a) = a^t \ U(O) \tag{7}$$

For calculations on utilities the most convenient technique is to draw the graph of

$$a^t = U(t)/U(O) \tag{8}$$

using a logarithmic scale for $U(t)/U(O)$ (as a fraction or as a percentage) and a linear scale for t. Values of t corresponding to any fraction, for a given value of a, can then be read directly from the straight line graph, and vice versa.

The sum of the geometric series, $1/(1-a)$, decreases as a, the annual ageing factor, decreases; that is, short lived periodicals have a smaller total utility than periodicals in which interest declines more slowly. The factor $1/(1-a)$, which arises frequently in the analysis of obsolescence, will henceforward be called the utility factor of the periodicals and be denoted by u.

Application to a Bradford Periodical Set

It will now be assumed that all the relevant papers in the current issues of the set of N periodicals which constitute the totality are of equal significance. (It seems likely that the papers published in the periodicals of the nuclear zone are, on average, of greater significance than those in the more peripheral zones but this issue awaits empirical analysis.)

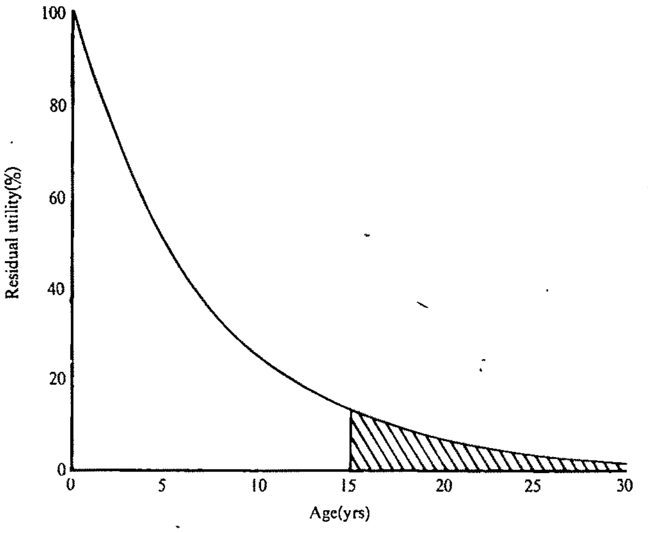


Fig. 3 The residual utility (linear scales).

Consider the utility of the periodical of rank n in a Bradford set. This periodical will have an annual average of N/n relevant papers and so the distribution of utilities over the successive years of its life from $t=0$ to $t=1, 2, \ldots$ is given by the series

$$N/n\,(1+a+a^2+\ \ldots\ a^{t-1}+\ \ldots\ )\doteq Nu/n \qquad (9)$$

So its initial utility is N/n (10*a*)

its utility at age t is $N\ a^{t-1}/n$ (10*b*)

its total utility is Nu/n (10*c*)

and its residual utility after t yr is Nua^t/n (10*d*)

(These measures are relative rather than absolute, but it is not necessary to multiply each by the same constant when only comparisons within a given library context are being made.) We have so far regarded the series (9) as expressing the declining utility of a single volume of the periodical as it ages from $t=0$ to $t=1, 2, 3, \ldots$ and so on (Fig. 3). But it can also be regarded as expressing the declining utilities of the ageing volumes of the periodical in its present complete run.

The sum of all the relevant papers in the Bradford set is given by

$$R(N)=N\log N/s$$

so that, for the complete Bradford set, the analogous utility measures are

initial utility is $R(N)=N\log N/s$ (11*a*)

utility at age t is $R(N)a^{t-1}=Na^{t-1}\log N/s$ (11*b*)

total utility is $R(N)u=uN\log N/s$ (11*c*)

and residual utility after t years is $R(N)\,ua^t=uNa^t\log N/s$ (11*d*)

We can now consider techniques of selecting $P\%$ of the total utility of a complete Bradford set of periodicals.

Some $P\%$ Solutions

There is an infinity of ways of selecting $P\%$ from the 100% totality. The simplest technique is to cut the complete Bradford set of N periodicals to the smaller set ranked $1, 2, \ldots M$, that is, to the M most productive periodicals. In this case M would be given by

$$N\log M/s=N\log N/s\times P/100$$

so that

$$M/s=(N/s)^{P/100} \qquad (12)$$

For $N=600$, $s=1.5$, $P=50\%$ and 75%, this result yields $M=30$ for $p=50\%$ and $M=90$ for 75% (Fig. 4).

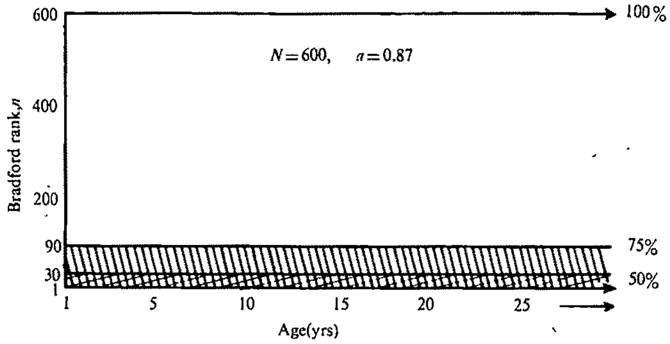


Fig. 4 The 50% and 75% "Bradford cut" solutions.

The solution reduces the expenditure on periodicals to the minimum for any specified value of P, but it has the disadvantage of leaving the shelves still occupied by the long little-used tails of the periodicals remaining so that the mean utility per metre shelf space is still low.

A second "cross cut" solution is to leave equal runs (in terms of years) on the shelves but to discard the ageing tails at the age given by

$$a^t=\frac{\text{discard utility}}{\text{initial utility}}=(1-P/100)=f \qquad (13)$$

For $a=0.87$ (half-life$=5.0$ yr) we have for $a^t=0.5$, $t=5.0$ yr and, for $a^t=0.75$, $t=10$ yr (Fig. 5).

Such solutions have the advantage of providing 100% coverage of papers published within some specified age limit, but the most productive periodicals are thus discarded when their residual utilities are still very high compared with the initial utilities of the peripheral periodicals.

A third solution, the "contour cut", is to discard tails of equal residual utility so that all the volumes remaining on the shelves have utilities above this critical discard level and yet give an overall $P\%$ coverage. This technique implies that

Table 1 Estimates of Shelf Space Required

Method	Bradford cut	Cross cut	Contour cut
50%	1,500	3,000	556
75%	4,500	6,000	2,570

Bradford set: $N=600$, $s=1.5$, $a=0.87$.

Shelf space is estimated in "periodical years", 100% required 30,000 periodical years for a 50 yr life.

periodicals of low initial utility will have much shorter runs than the more productive periodicals.

Consider the total loss of utility if the discard utility is equal to the initial utility of the periodical of rank M. We then have a discard utility level of N/M and for any periodical of rank n, where $c \leqslant n \leqslant M$, we have the age of discard, t, given by

$$a^t = \frac{\text{discard utility}}{\text{initial utility}} = \frac{N/M}{N/n} = \frac{n}{M} \qquad (14)$$

The corresponding value of t can be read from the graph of a^t. The residual utility of the nth periodical at discard, using (10d) and (14), will therefore be

$$Nua^t/n = Nun/nM = Nu/M \qquad (15)$$

Each of the M periodicals is, by this technique, discarding equal utilities, so the total utility thus discarded is Nu. Moreover, because the periodicals of rank $(M+1)$ to N have been discarded completely, we have the proportional loss of utility from the 100% totality, using (11c), given by

$$\frac{Nu + uN \log N/s - uN \log M/s}{uN \log N/s} = \frac{1 + \log N/M}{\log N/s} = f \quad (16)$$

from which the value of M for any specified value of f can be found. For $f=0.5$ and 0.25 and for the same numerical data used above, 50% utility would be provided by the most productive eighty-one periodicals and 75% utility by the most productive 364 periodicals when all are discarded as they decline to the same discard utility contours (Fig. 6). The technique is easy to apply because, when the contours are mapped on a graph on which n is logarithmic and t is linear, they appear as parallel straight lines for $c \leqslant n \leqslant N$.

Table 2 Costs per Relevant Paper

Range of set acquired	Yield of relevant papers per annum	Relative cost paper/yr
1–75	2,350	1
1–150	2,760	1.70
1–300	3,180	2.97
1–450	3,420	4.12
1–600	3,600	5.22

Bradford set: $N=600$, $s=1.5$.

The advantage of this contouring technique is that it provides the most compact solution of the P% library but costs more in the buying of periodicals than the Bradford cut method. (In practice it may be convenient or economical to use some hybrid form of two of these solutions but if the hybridization is rationally planned it is possible to estimate the corresponding value of P for any compromise that may be decided on or to design the hybrid to conform with any specified value of P.)

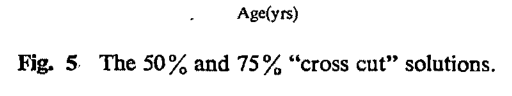


Fig. 5 The 50% and 75% "cross cut" solutions.

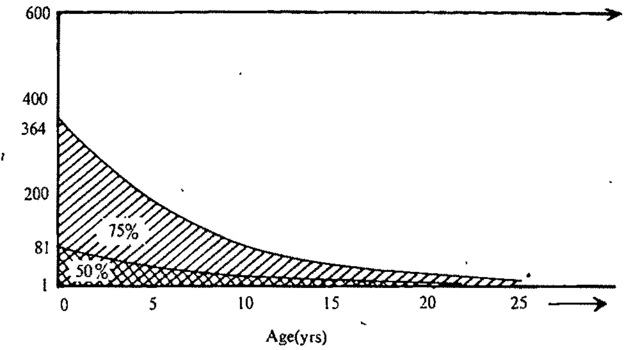


Fig. 6 The 50% and 75% "contour cut" solutions.

Estimates of the shelf space demanded by each of the three solutions, in terms of "periodical years", are given in Table 1. In these estimates a complete back run is reckoned at 50 periodical years so that the 100% totality would require 30,000 periodical years. It can be seen that the contour method offers the most compact solutions.

Effects of Growth

The cost per relevant paper acquired by buying periodicals increases steadily as the range of the periodicals ranked 1 to n of a Bradford set increases (Table 2). It is therefore uneconomic to attempt 100% coverage especially if the particular science library is backed by some regional or national library serving many science libraries from which it can borrow periodicals, acquire photocopies or particular papers or receive suitable video transmissions. The cut-off point at which it becomes more economic to borrow than to buy[3] then determines the value of P.

I have assumed that the literature of the particular scientific subject S grows uniformly year by year and that it conforms closely with the empirical laws applied. But even if these conditions are not fulfilled, the principles underlying the techniques can still be applied though more laborious graphical methods may have to be adopted. In practice, too, the Bradford set selected in any one year is also liable to change as new periodicals appear, as some die in their tracks and as others change the focus of their scientific interest. So once the required P% library has been established it will need regular review and revision. If the rate of growth of the literature is increasing, then the value of a can be expected to decrease from year to year. But increase in the rate of growth of the literature is more likely to arise from an increase in the number of scientific workers than from an increase in their output of scientific papers. An increase in the number of scientists working in the subject increases the utility of all the literature they depend on. And so the effects of the two kinds of growth act in opposite directions on the magnitude of a. It can be shown that if the two rates of increase are equal, then the value of a is unchanged[4].

It is interesting that the P% library designed by the contour technique discards, year by year, as many annual volumes as it acquires. The "information explosion" becomes less alarming if it is appreciated that the "explosion" of new literature is almost exactly counterbalanced by the discarding of the old. But the librarian is conservative; he has yet to learn how to be as ruthless as his scientific colleagues in the art of discarding the obsolete.

Received January 29, 1971.

[1] Bradford, S. C., *Documentation* (London, 1948).
[2] Brookes, B. C., *Nature*, **224**, 935 (1969).
[3] Brookes, B. C., *J. Doc.*, **26**, 22 (1970).
[4] Oliver, M. R., *J. Doc.*, **27**, 11 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Solar Proton Medium Flux Constancy over a Million Years

THE data of cosmogenic radionuclide activity measurements in lunar material from the Apollo 11 and 12 missions have now been published[1-8]. Unfortunately, the data concern chiefly the measurements of medium radioactivity of whole rock (by non-destructive gamma-spectrometry) or large volume samples (by radiochemical methods). The data provide no way to determine the overall depth distribution gradient of radionuclides, produced by galactic and solar cosmic rays, and so no information can be extracted about the intensity of cosmic rays. But ref. 1 describes how the radioactivities of some radionuclides have been measured as a function of depth in lunar sample 10,017. By analysing these data we have drawn a conclusion about possible constancy of solar proton medium flux for the last million years.

For our purpose the calculation of the depth distribution of cosmogenic isotopes in the surface layer of the Moon has been fulfilled, which allows accurate interpretation of the measurements. The calculation enables us to determine the distribution with depth of primary cosmic ray and secondary nuclear-active particles as well as the distributions of cosmogenic radionuclides[9-11]. The efficiency of the method has been proved by numerous calculations of radioactivities in meteorites. Our calculations have been carried out for a large body ($d \ll R$) with composition similar to that of the lunar crystalline rock B/17-33/ (ref. 6), for which depth radioactivity was measured[1]. The production cross sections of radionuclides from the different elements in the energy range 0.001 to 28 GeV have been taken from the experimental data of ref. 12. Unknown cross sections have been resolved from formulae (1) and (2) (*vide infra*) which link, by the ratios of Rudstam coefficients[13], unknown production cross sections of an isotope from a target with experimental production cross sections of this isotope from other targets

$$\sigma_i^{\mathrm{I}}(A_i Z_i) = \frac{K(A^{\mathrm{I}})}{K(A^{\mathrm{II}})} \exp\{[P(A^{\mathrm{I}}) - P(A^{\mathrm{II}})]A_i\}\, \sigma_i^{\mathrm{II}}(A_i Z_i)^{\mathrm{exper.}} \tag{1}$$

where I, II are the targets and *i*, *j* the isotopes, or, with that of other isotopes from the same target[14]

$$\sigma(A_i Z_i) = \exp[P(A_i - A_j) - R_i(Z_i - S A_i + T A_i^2)^{3/2} + R_j(Z_j - S A_j + T A_j^2)^{3/2}]\, \sigma(A_j Z_j)^{\mathrm{exper.}} \tag{2}$$

Thus, the same cross sections can be calculated from (1) and from (2) using different experimental data. The cross sections determined in this way were weighted according to the integral cosmic ray spectra and composition of the rock. The intensity and spectrum of the galactic cosmic rays were determined from the measurements in the stratosphere for the period 1958 to 1970 (refs. 15 and 16). The spectrum of solar protons with energy >20 MeV has been given in ref. 17, together with depth distributions of solar protons and secondary neutrons in the lunar surface layer. Because of the lack of data for the medium solar proton intensity over long periods of time we believe it is best for our purpose—to compare cosmic ray intensity for different time intervals—to determine the quantity of medium intensity solar protons by extrapolation of the measured activity data of one of the radionuclides (for example ^{22}Na) in lunar rock. Further calculations of the depth distributions of other radionuclides with different half lives, using the same value of solar proton intensity, will lead by comparison with measured activities of these isotopes to an estimate of the constancy of the solar proton flux.

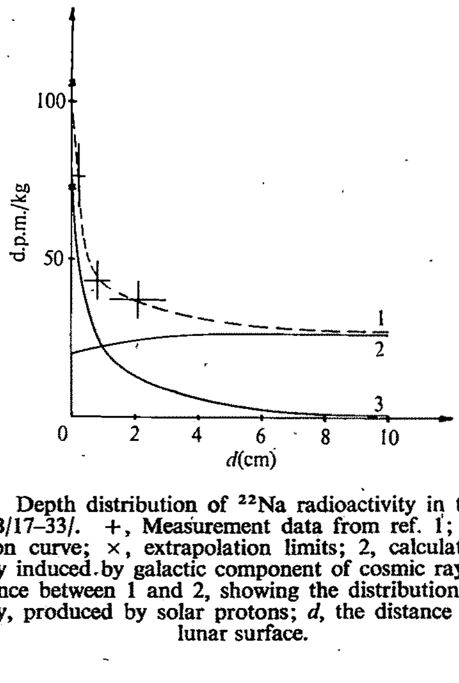


Fig. 1 Depth distribution of ^{22}Na radioactivity in the lunar rock B/17-33/. +, Measurement data from ref. 1; 1, extrapolation curve; ×, extrapolation limits; 2, calculated ^{22}Na activity induced by galactic component of cosmic rays; 3, the difference between 1 and 2, showing the distribution of ^{22}Na activity, produced by solar protons; *d*, the distance from the lunar surface.

In Fig. 1 the ^{22}Na activity measured as a function of depth[1] is plotted, with experimental errors in the depth ranges marked. Extrapolation of curve 1 intercepts the ordinate at 96 d.p.m. kg^{-1} with $\left\{\begin{matrix}+10\%\\-25\%\end{matrix}\right.$ error (extrapolation limits marked by crosses on ordinate). Curve 2 shows our calculated depth distribution of ^{22}Na produced by the galactic component of cosmic ray and secondary nuclear-active particles. The difference between these curves (curve 3) shows accumulation of ^{22}Na by solar proton and secondary neutron bombardment. According to ref. 17 the secondary neutron flux is three orders of magnitude smaller than incident solar proton flux on the lunar surface, so that the interception point of curve 3 with the ordinate defines the activity induced by solar protons with intensity $2.46^{+0.44}_{-0.74}$ protons cm^{-2} s^{-1} sterad^{-1} (that is, $\left\{\begin{matrix}+18\%\\-30\%\end{matrix}\right.$ error). This is the medium solar proton flux for 4 yr from 1966 to 1969 (1.5 $T_{1/2} \sim 4$ yr for ^{22}Na). With this value of medium intensity of solar protons the activities of other

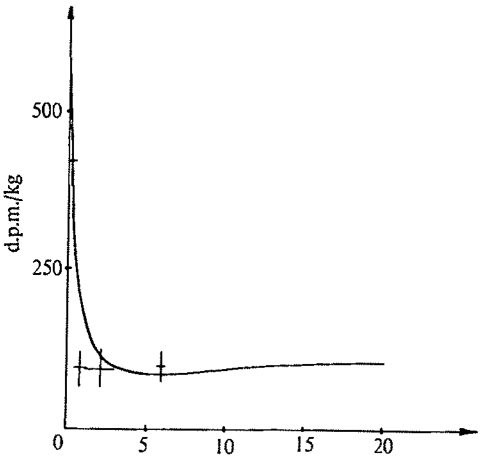


Fig. 2 Depth distribution of ^{55}Fe radioactivity in the rock B/17–33/.

long lived radionuclides in lunar rock B/17–33 have been calculated. In Fig. 2 the curve of calculated ^{55}Fe activity as a function of depth is plotted, with measured points from ref. 1. (The ^{55}Fe half life is the same as that of ^{22}Na.) In Fig. 3 the radioactivity data of ^{26}Al, with half life 7.4×10^5 yr, are plotted. The agreement between calculated (curve 1) and measured depth change of ^{26}Al activity is good. Extrapolation over ^{26}Al measured data, in the same way as for ^{22}Na above, gives medium solar proton intensity $2.46^{+0.34}_{-0.54}$ protons cm^{-2} s^{-1} sterad^{-1} (that is, $\begin{cases}+14\% \\ -26\%\end{cases}$ error). We have also calculated the depth distributions of ^{26}Al activity induced by solar protons with twice smaller intensity (curve 2) and with twice greater intensity (curve 3). For depth $d > 4$ cm all three curves draw nearer because of the fall in solar proton flux, but the corresponding ^{26}Al activities on the surface lie far outside the limits of possible extrapolation.

It follows from this analysis that at least within ~30% error we can say that the mean intensity of solar cosmic rays with the energy >20 MeV has been constant for the last million years at about $31.1^{+5.6}_{-9.4}$ protons cm^{-2} s^{-1}. This conclusion is confirmed by calculations of radioactivities of ^{53}Mn ($T_{1/2} = 2 \times 10^6$ yr) and ^{54}Mn ($T_{1/2} = 303$ d).

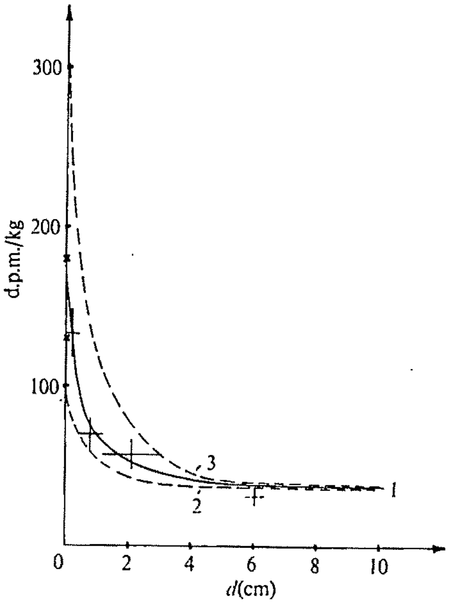


Fig. 3 Depth distribution of ^{26}Al in the rock B/17–33/. +, Measurement data from ref. 1; 1, ^{26}Al activity calculated using the medium solar proton intensity 31.1 protons cm^{-2} s^{-1}; 2, depth distribution of ^{26}Al, calculated with twice smaller solar proton flux; 3, ^{26}Al distribution, calculated with twice greater solar proton flux; ×, the limits of possible extrapolation.

A. K. LAVRUKHINA
G. K. USTINOVA

Vernadsky Institute of Geochemistry and
Analytical Chemistry,
Academy of Sciences of the USSR, Moscow

Received April 6, 1971.

[1] Shedlovsky, T. P., Honda, M., Reedy, R. C., Evans, J. C., Lal, D., Lindstrom, R. M., Delany, A. C., Arnold, J. R., Loosli, H., Fruchter, J. S., and Finkel, R. C., *Science*, **167**, 574 (1970).
[2] Perkins, R. W., Rancitelli, L. A., Cooper, J. A., Kaye, J. H., and Wogman, N. A., *Science*, **167**, 577 (1970).
[3] O'Kelley, G. D. O., Eldridge, J. S., Schonfeld, E., and Bell, P. R., *Science*, **167**, 580 (1970).
[4] Fireman, E. L., D'Amico, J. C. D., and DeFelice, J. C., *Science*, **167**, 566 (1970).
[5] Stoenner, R. W., Lyman, W. J., and Davis, R., *Science*, **167**, 553 (1970).
[6] Wänke, H., Begemann, F., Vilcsek, E., Rieder, R., Tescka, F., Born, W., Quijano-Rico, M., Voshage, H., and Wlotzka, F., *Science*, **167**, 523 (1970).
[7] *Lunar Sample Preliminary Examination Team: Apollo-11, Science*, **165**, 1211 (1969).
[8] *Preliminary Examination of Lunar Samples from Apollo-12, Science*, **167**, 1325 (1970).
[9] Lavrukhina, A. K., and Ustinova, G. K., *Astronomichesky J.*, **44**, 1081 (1967).
[10] Lavrukhina, A. K., and Ustinova, G. K., *Dokl. Acad. Sci. USSR*, **179**, 1448 (1968).
[11] Lavrukhina, A. K., Ustinova, G. K., Ibraev, T. A., and Kuznetzova, R. I., *Meteorite Research*, 227 (Reidel, Dordrecht, 1969).
[12] Bruninx, E., *High-Energy Nuclear Reaction Cross-Sections*, I–III, CERN 64–17, Nucl. Phys. Div., Geneva, 1961, 1962, 1964.
[13] Rudstam, G., *Z. Naturforsch.*, **21a**, 1027 (1966).
[14] Lavrukhina, A. K., and Ustinova, G. K., *Izvest. Acad. Sci. USSR*, **34**, 2401 (1970).
[15] Charahchjan, A. N., and Charahchjan, T. N., *Trans. Sixth All-Union Winter School on Cosmic Phys.*, Part II, 36 (Apatity, 1969).
[16] Bazilevskaya, G. A., Kvashnin, A. N., Svirzevskaya, A. K., Stozkov, Ju. I., Charahchjan, A. N., and Charahchjan, T. N., *The Report on All-Union Conf. on Cosmic Rays* (Moscow, 1970).
[17] Ebeoglu, D. B., and Wainio, K. M., *J. Geophys. Res.*, **71**, 5863 (1966).

Extragalactic Faraday Rotation

IN three very important and groundbreaking papers, Sofue, Fujimoto and Kawabata[1], Kawabata *et al.*[2], and Reinhardt and Thiel[3] call attention to evidence which indicates that Faraday rotation is taking place in space outside our own Galaxy. Essentially they show that above low galactic latitudes, where material in the disk of our own Galaxy is known to produce Faraday rotation, extragalactic radio sources exhibit Faraday rotations which generally increase with the redshift of the source, and that these rotations behave similarly in certain regions of the sky.

They attribute this extragalactic Faraday rotation to a metagalactic magnetic field which is ordered on the scale of the observable universe (the scale observable on the assumption that the redshifts of the quasars are cosmological). It seems to me, however, reasoning in analogy with our own Galaxy, that this latter model is not the most likely one with which to explain the extragalactic Faraday rotations. It would not, for example, be logical to look to the low-density polar regions and possible ordered magnetic field in our own Galaxy for Faraday rotation. Instead we look to the plane where we know there are concentrations of ionized matter and magnetic

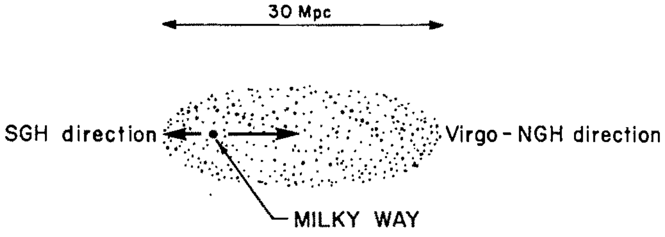


Fig. 1 Schematic cross-section of the local supercluster showing location of Local Group and approximate dimensions.

field in the form of gas, H II regions, and spiral arms. In fact, this is where the galactic Faraday rotation is observed.

When we look outside our own Galaxy, however, we see something analogous in the local supercluster of galaxies as outlined by de Vaucouleurs[4]. In that supercluster there is a concentration of galaxies in the supergalactic plane. Those galaxies represent the concentrations of ionized matter and magnetic field which we know about—and it is here that the authors of refs. 1–3 find the maximum extragalactic Faraday rotation.

That direction of maximum Faraday rotation was designated originally by Sofue *et al.* as $l^{\mathrm{II}}=100°$, $b^{\mathrm{II}}=-30°$ and the opposite direction in the sky $l^{\mathrm{II}}=280°$, $b^{\mathrm{II}}=30°$. In the latest paper by Kawabata *et al.*, the direction is designated as $l^{\mathrm{II}}=115°$, $b^{\mathrm{II}}=-5°$. Perhaps this difference is an indication of the uncertainty of the determination. In any case, as Sakashita and Kaneko pointed out[2], this direction lies in the plane of the supercluster. It is close to the direction of the Virgo cluster $l^{\mathrm{II}}=280$ to 300, $b^{\mathrm{II}}=+50$ to $+70$, which marks the approximate centre of the local supercluster as seen from our own Galaxy.

In order to test further the alternatives represented by the two models, we should indicate that the local supercluster is supposed to be a flattened system of galaxies and clusters of galaxies about 30 Mpc in diameter, as shown in Fig. 1. Our own Local Group of galaxies is located somewhere out near the edge, so that we see the bulk of the supercluster in the direction of Virgo, but there is also a lesser amount of supercluster which we look out through in the opposite direction.

Table 1 Faraday Rotations for Sources with $b^{\mathrm{II}} > 42°$, $0.1 < z < 1.2$

North Galactic Hemisphere			South Galactic Hemisphere		
Source	z	RM	Source	z	RM
1353+19	0.72	+7	2115−30	0.98	+28
1328+254	1.06	−60	2135−14	0.20	+19
1545+21	0.26	+20	2313+03	0.22	+7
1622+23	0.93	+31	2309+09	0.23	+3
3C286	0.85	+4	0035−02	0.22	+1
3C254	0.73	−23	0056−00	0.72	−1
3C219	0.17	−10	0159−11	0.67	+4
1040+12	1.03	+31	0336−01	0.85	+23
1127−14	1.19	+31	0350−07	0.96	+23
1136−13	0.55	−22	0403−13	0.57	+4
1229−02	0.39	+19	$\langle z\rangle=0.56\pm0.06$ (p.e.)		
1241+16	0.56	−18	$\langle\lvert RM\rvert\rangle=11.3\pm2.1$ (p.e.)		
1253−05	0.54	+24			
1335−06	0.63	−13			
1330+02	0.22	+2			
$\langle z\rangle=0.66\pm0.05$ (p.e.)					
$\langle\lvert RM\rvert\rangle=21.0\pm2.4$ (p.e.)					

Data from Reinhardt and Thiel, Table 1. (Note their value of b^{II} for 3C390 should be changed from +60 to +6.) 3C273 is excluded from this table as anomalous.

Table 1 shows that the Faraday rotations in the north galactic hemisphere (the general direction of the centre of the supercluster) average about twice the absolute value of Faraday rotations in the direction of the south galactic hemisphere (opposite the centre of the supercluster). Another interesting result is that the mean value of the redshifts of these quasars and allied objects is about 0.1 greater in the supercluster centre direction as opposed to the supercluster anticentre direction. I have previously suggested that most quasars and quasarlike compact galaxies are at distances closer than their redshifts would indicate[5] and are, in fact, generally members of the Local Group and local supercluster. In that case we would expect more distant quasars, on the average, in the direction of the bulk of the supercluster and fewer lower-redshift objects in the anticentre direction. This kind of distribution would be expected to give Faraday rotation from material within the local supercluster roughly as indicated in Table 1.

Finally, it is necessary to test the increase in the mean absolute value of the Faraday rotation as a function of the redshift of the source. None of the previous papers have shown such a plot, but Fig. 2 shows the Faraday rotations for high-latitude radio sources as a function of redshift. Although there is considerable scatter, it is clear that the sources with very low redshifts have $\langle\lvert RM\rvert\rangle\sim10$ rad/m^2, the sources with $z\sim0.5$ have $\langle\lvert RM\rvert\rangle$ between 10 and 20, and the sources with $z\sim1.0$ have $\langle\lvert RM\rvert\rangle\sim30$. I agree with the previous authors[2] that there is a general rise in the mean absolute value of the Faraday rotation with increasing redshift. But a most important result now appears after redshifts of about $z=1.2$. The five quasars with redshifts $z\sim2$ definitely have $\langle\lvert RM\rvert\rangle\sim10$ rad/m^2 again. Kawabata *et al.* seem to explain this as arising from a disordered metagalactic magnetic field which dominates beyond a distance which corresponds to a redshift (cosmological) of about $z=1.4$. But on their picture, regardless of how disordered the field is outside $z=1.4$, the radiation must traverse the region between $z=1.4$ and the observer, and it should therefore be rotated by at least the ~30 rad/m^2 in that region. Although there are few data available they certainly indicate, using this model, that the $z\sim2$ quasars are closer than the $z\sim1$ quasars and are in fact at distances corresponding to the $z\sim0.1$ to 0.3 quasarlike objects.

This last result seems particularly significant because the evidence discussed earlier[5] indicated that the quasars with redshifts of the order of $z=0.2$ are low-luminosity Local Group objects, and that with higher redshifts the quasars become of higher luminosity and generally distributed throughout the local supercluster of galaxies. But for redshifts around $z\sim2$ the evidence indicated that they are once again low-luminosity objects distributed in space in the Local Group and nearby regions of the supercluster.

It is interesting to note from Table 1 that the Faraday rotations in the north galactic hemisphere are about evenly divided between plus and minus. We would conclude that this occurs as the radiation encounters concentrations of plasma in the local supercluster which have no preferred alignment. On the other hand, the ten Faraday rotations in the south

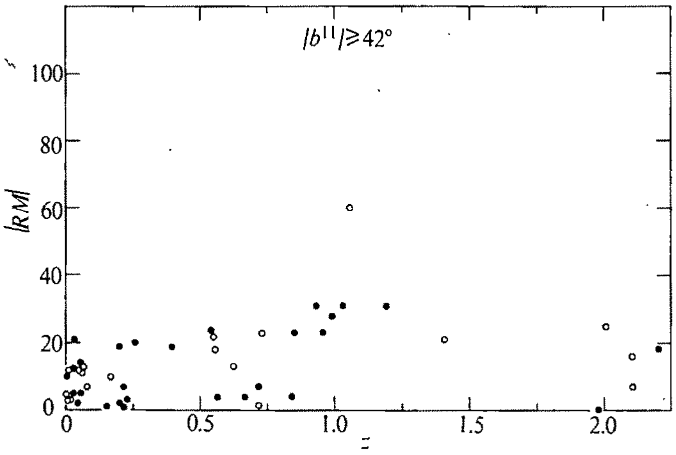


Fig. 2 Plot of Faraday rotation (●, positive; ○, negative) against redshift of source. Data from Reinhardt and Thiel, Table 1.

galactic hemisphere are almost all at least slightly positive. This may indicate there is some preferential alignment of magnetic field within our own Local Group of galaxies, the centre of which lies in the direction of M31 in the south galactic hemisphere. An additional point can be made about the quasars with very high redshifts. Of the seventeen quasars with redshifts greater than $z \doteq 1.5$ known in 1968, thirteen lie in the south galactic hemisphere. This would support the inference that they are Local Group objects concentrated in the general direction of M31.

Finally, it can be remarked that the previous authors[1-3] estimated a mean electron-density-magnetic-field component of $n_e B_{\parallel} \sim 10^{-14}$ along the path length to quasars if they were at their cosmological distances. If I use a distance on the average of 1/100 of this for the quasars, an $n_e B_{\parallel} \sim 10^{-12}$ would be derived for the local supercluster. Because we are dealing with the same order of extragalactic Faraday rotation as galactic, we can scale the observed galactic Faraday rotation near the plane in our own Galaxy (about 300 pc path length with $n_e B_{\parallel} \sim 10^{-7}$ to 10^{-8} gauss/cm^3) to the scale of the supercluster (about 30 Mpc). This yields $n_e B_{\parallel} \sim 10^{-12}$ to 10^{-13}, in agreement with the observations for the supercluster. This mean value of $n_e B_{\parallel}$ is reasonable, so the question arises of how might it be distributed along the line of sight. It must be between the two extremes of completely even, homogeneous spatial distribution and complete concentration into regions the size of the optical diameters of galaxies. If we indeed have some intermediate distribution of this Faraday rotating plasma—say on the scale of groups or clusters of galaxies—then we would expect the radio emission from quasars and compact galaxies to intersect regions of higher and lower $n_e B_{\parallel}$ as it crosses the supercluster, and suffer Faraday rotations of either plus or minus sense with an increasing dispersion as the distance across the supercluster increases.

HALTON ARP

Hale Observatories,
Carnegie Institution of Washington,
California Institute of Technology,
Pasadena, California 91101

Received May 28, 1971.

[1] Sofue, Y., Fujimoto, M., and Kawabata, K., *Publ. Astron. Soc. Japan*, **20**, 368 (1969).
[2] Kawabata, K., Fujimoto, M., Sofue, Y., and Fukui, M., *Publ. Astron. Soc. Japan*, **21**, 293 (1969).
[3] Reinhardt, M., and Thiel, M., *Astrophys. Lett.*, **7**, 101 (1970).
[4] Vaucouleurs, G. de, *Soviet A. J.*, **3**, 897 (1959).
[5] Arp, H., *Astron. J.*, **75**, 1 (1969).

Evidence for Black Holes in Binary Star Systems

ONE of the most exciting predictions of the general theory of relativity is that a star of more than about 1.4 $M_{\odot}$ may undergo gravitational collapse to produce a black hole when it has exhausted its nuclear fuel. It would therefore be a very important confirmation of the theory if such holes could be detected. Unfortunately their very small size (a few km) makes it virtually impossible to observe them directly, but their existence could be inferred from the motion of other objects in their gravitational field. The natural place to look for black holes therefore is in binary star systems in which only one object is observed (single line binaries). Thorne and Trimble[1] gave a list of all single line spectroscopic binaries in Batten's[2] catalogue in which the mass of the unseen object was estimated to be 1.4 $M_{\odot}$ or over. In most of these binaries the unseen secondary was less massive than the primary observed star and therefore could have been a normal star which is not observed in comparison with its brighter companion. In a smaller number of cases the secondary was heavier than the primary, but in each of these cases Trimble and Thorne were able to suggest a possible reason why it might not have been observed. They concluded therefore that while some of these single line binaries might contain black holes there was no compelling reason for believing that any of them did.

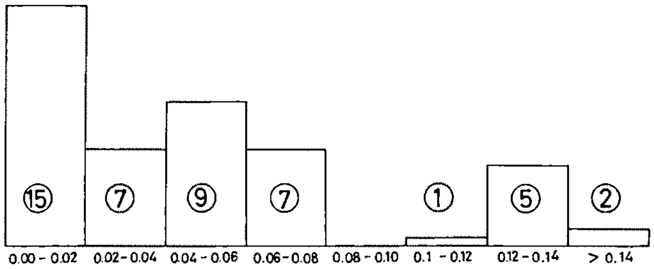


Fig. 1 The eccentricities of forty-six short period double line spectroscopic binary systems.

Here we would like to present an additional argument not considered by Trimble and Thorne. This suggests that at least one or two of these binaries may contain black holes. The evidence for this comes from studying the eccentricities of the shortest period binaries.

In a short period binary system (say, with a period of less than 5 days) one would expect any initial eccentricity of the orbits to be damped out. This is because of dissipation processes such as tidal friction, mass exchange and magnetic breaking which will reduce the energy of the system but not its angular momentum. The orbit therefore tends to that with the lowest energy for a given angular momentum, that is, a circular orbit. Suppose now that one of the stars collapses to form a black hole. Then in a time short compared with the orbital period it might lose a significant fraction of its mass by the emission of gravitational waves or the ejection of a shell of matter. In addition, in unpublished work by one of us (S. W. H.), it is shown that in the Brans–Dicke theory the object will lose the fraction of its mass which arises from the scalar interaction (~7%). This sudden mass loss will cause the orbit which was initially nearly circular to acquire an eccentricity of $e = \dfrac{\Delta M_2}{M_1 + M_2}$ where M_1 and M_2 are the final masses of the two stars and ΔM_2 is the mass loss of the second star. This eccentricity will eventually be damped away but at a rather slower rate because tidal dissipation will occur only in the star which has not collapsed. It will be observable if the collapse did not occur too long ago.

To test this idea we have compared the eccentricities of short period double line binaries—that is, binaries where both stars are observed—with those of short period single line binaries. We examined all the binaries in Batten's catalogue[2] with a period less than 5 days and where the masses of both objects were above 1.4 $M_{\odot}$ and where the quality of the data was not considered bad (class *a* to *d* in Batten's classification). In the case of the double line binaries we excluded those eleven where the eccentricity was assumed zero. In fact these probably have very low eccentricities. The distribution of eccentricities for double line binaries is shown in Fig. 1. From this it can be seen that most of them (38 out of 46) have very low eccentricities (below 0.08). There are eight binaries with eccentricities above 0.08. Seven of these have spectral types B3 or earlier and are probably younger than 10^8 yr. This indicates that the damping time for loss of eccentricity is probably of the order of 10^8 yr in a close binary. The eighth binary is exceptional in that its spectral type is AO which is considerably later and its eccentricity is 0.29 whereas the next highest eccentricity is 0.19. This spectroscopic binary is in fact one visual component of the visual double system γ Andromeda (ADS

Table 1 Single Line Binaries (M_1, $M_{2\min} \geq 1.4\ M_\odot$, Period < 5 days) (taken from ref. 1)

No. in Batten's catalogue	Star	α_{1900}	δ_{1900}	Magnitude	Spectral type	Period	e	$f(M)$	$\frac{M_1}{M_\odot}$	$\frac{M_{2\min}}{M_\odot}$
195	HD 40005	05 h 51.1 m	+16° 21′	6.91	B3V	3.036	0.0	0.157	10.0	3.0
544	HD 176318	18 h 54.6 m	+38° 07′	5.70	B6V	2.912225	0.169	0.150	5.0	1.9
567	201 G Sgr	19 h 27.3 m	−40° 15′	5.9	A3III	4.625	0.09	0.180	3.0	1.6
596	HD 191473	20 h 05.0 m	+36° 57′	8.6	B0.5V	4.2876	0.0447	0.535	15.0	6.2
617	HD 194495	20 h 20.8 m	+21° 10′	7.09	B7	4.9052	0.133	0.275	6.0	2.8
634	HD 198754	20 h 47.6 m	+37° 36′	6.97	B3	3.30353	0.018	0.082	10.0	2.3
683	HD 209961	22 h 02.0 m	+47° 45′	6.27	B2V	2.1721	0.0	0.47	12.0	5.2

1630). The other visual component of this system has a highly eccentric orbit which at periastron will pass fairly close to the spectroscopic binary. At each close passage it will be likely to change the eccentricity of the binary by a small amount. In 10^8 yr there will be more than a million such passages and so the present observed eccentricity could have been produced by a random walk process.

Table 1 shows all single spectroscopic binaries in Batten's catalogue which have periods less than 5 days and where Trimble and Thorne estimated that the masses of each object were greater than 1.4 $M_\odot$. They estimated the mass, M_1, of the primary from its spectral type and then obtained a lower limit on the mass M_2 of the secondary from the observationally determined quantity $f(M) = M_2 \sin^3 i/(M_1+M_2)^2$ where i is the angle between the line of sight and the normal to the plane of the orbit. Of the seven systems in this table one (HD 209961) has an eccentricity which was assumed to be zero. Of the remainder three (HD 176318, 201 G Sgr and HD 194495) have eccentricities greater than 0.08. On the basis of seven double line binaries out of forty-six with high eccentricities one would expect three or more out of a sample of seven with a probability of less than 8%. This is suggestive but it is not good enough for one to be confident that one or more of these binaries contains a black hole. But there is some supporting evidence for the black hole hypothesis in the fact that, whereas in the case of the double line binaries those with high eccentricities are among the earliest in spectral type, those single line binaries with high eccentricities are all distinctly later and are not among the earliest types in their class. We therefore suggest that it would be worth examining these three binary systems more carefully to see whether one could rule out the possibility that the secondary was a normal star which has not been observed to date because it is less luminous than the primary. In the case of 201 G Sgr the primary is a giant and so overluminous for its mass; one could therefore not hope to detect the secondary in this system; however, in HD 176318 and HD 194495 the secondaries, if they are normal stars, should be less than 3.4 and 2.7 magnitudes respectively down on the primaries. With the use of image intensifiers it might be possible to detect them. It might also be worth looking for these objects in other wave bands—Zel'dovich and Guseynov[5], for example, have pointed out that matter falling into a black hole could cause the emission of X-rays.

In 201 G Sgr and HD 176318 the lower limits on the mass of the secondary are just below the highest estimates of the maximum mass of neutron stars[6]. It is therefore just possible that these two could be neutron stars. In the case of HD 194495, however, the lower limit on the mass of the secondary is 2.8 $M_\odot$ which is too high to be a neutron star. If the present observed eccentricities of these systems resulted from mass loss,

$$\frac{\Delta M_2}{M_2 + \Delta M_2}$$

would be less than 0.39 for HD 176318, 0.21 for 201 G Sgr and 0.29 for HD 194495.

To summarize, there is suggestive but not conclusive evidence for the presence of black holes in binaries. The evidence could be strengthened by careful observations which could rule out the possibility that the secondaries in these binaries are normal stars.

We thank Dr Virginia Trimble for help and advice.

G. W. GIBBONS

Department of Applied Mathematics and
Theoretical Physics,
University of Cambridge

S. W. HAWKING

Institute of Theoretical Astronomy,
University of Cambridge

Received June 30, 1971.

[1] Trimble, V. L., and Thorne, K. S., *Astrophys. J.*, **156**, 1013 (1969).
[2] Batten, A. H., *Pub. Dom. Astrophys. Obs. Victoria*, **13**, 119 (1967).
[3] Maestre, L. A., and Wright, J. A., *Astrophys. J.*, **131**, 119 (1960).
[4] Muller, P., *J. des Observateurs*, **40**, 48 (1957).
[5] Zel'dovich, Ya. B., and Guseynov, O. H., *Astrophys. J.*, **144**, 841 (1965).
[6] Tsuruta, S., "The Effects of Nuclear Forces on the Maximum Mass of Neutron Stars" in *The Crab Nebula* (edit. by Davies, R. D., and Smith, F. G.) (Reidel, Dordrecht, in the press).

Arctic Palaeotemperatures in Late Caenozoic Time

RECONSTRUCTION of the climatic and hydrologic history of ocean basins has been based on either oxygen isotope palaeothermometry or studies of foraminiferal assemblages preserved in deep sea sediments. We report here the application of both methods to deep sea sediments collected from the Arctic Ocean by the Lamont–Doherty Geological Observatory. Oxygen isotope determinations were made on two selected cores from the central Arctic basin to test the validity of the palaeoceanographic interpretations based on microfaunal analysis[1–3]. Details of the cores are given in Fig. 1 and Table 1. Analyses of the cores have already been published in detail: here we wish to summarize what we have discovered about the history of the Arctic Ocean[1–4].

Biostratigraphic and lithologic correlations between cores accompanied by $^{18}O/^{16}O$ determinations and radiometric

Table 1 Location, Depth and Length of Cores

Core	Latitude	Longitude	Depth	Length
D.st.A.2	83° 52′ N	168° 12′ W	1,521	206
D.st.A.3	84° 12′ N	168° 33′ W	2,409	95
D.st.A.4	84° 21′ N	168° 49′ W	2,041	116
D.st.A.5	84° 28′ N	169° 04′ W	1,934	125
D.st.A.6	85° 15′ N	167° 54′ W	1,842	88
Arlis II, 1	81° 57′ N	168° 07′ E	2,816	44
Arlis II, 2	82° 00′ N	168° 04′ E	2,864	80
T3-67-4	79° 22.7′	174° 46′	1,760	272
T3-67-9	79° 37.9′	172° 07′	2,237	356
T3-67-11	79° 34.9′	172° 30′	2,810	250
T3-67-12	80° 21.9′	173° 33′	2,867	374

dating were used to estimate ages and sedimentation rates as well as to reconstruct the climatic and oceanographic history of the Arctic[3,4]. The time interval of the longest core (T3-67-12, Fig. 3) is thought to exceed 3.3. m.y. Ages were estimated by interpolations and extrapolations based on radioisotope determinations[5] and close interval faunal and lithologic correlations with neighbouring cores that have an established palaeomagnetic stratigraphy. Age assessments have been modified from those presented in a previous publication[3], due to reinterpretation of the palaeomagnetic data of core 224 by Clark and his colleagues[6]. The earlier palaeomagnetic ages[7,8] had apparently been greatly over-estimated.

On the basis of faunal and sediment characteristics three climatic units can be distinguished. The oldest, unit III, was deposited between about 3.3 m.y. and 3 m.y. ago; it consists of foraminifera-poor, Fe and Mn oxide-rich sediments. Calcareous foraminifera, in part corroded, are thick shelled forms. Arenaceous foraminifera (*Glomospira gordialis*, *Cyclammina pusilla* and *Alveolophragmium subglobosum*) dominate the benthic assemblages. Lower than present sedimentation rates could account for the selective solution of the less resistant limy tests and impoverished character of the fauna.

Unit II was deposited between approximately 3 and 0.7 m.y. ago and is poor in both Fe, Mn oxides and in foraminifera. It contains one foraminifera-rich layer: the predominant foraminifer in this layer is temperate–arctic, euryhaline *Globigerina quinqueloba* ($^{18}O/^{16}O$ determinations). This species occurs with sinistral *Globigerina pachyderma* throughout unit II. Fragile calcareous benthonic foraminifera (*Stetsonia horvathi*, *Sphaerodina bulloides* and *Bolivina arctica*) periodically abound, constituting up to 88% of the fauna. Concentration of thin walled benthonic foraminifera suggests that the near absence of planktonic forms was due to adverse environmental conditions rather than to dissolution of the less resistant tests. It has been suggested that the Arctic was essentially free of permanent pack ice during the deposition of units II and III (refs. 2, 3).

Unit I was deposited in the past 0.7 m.y., a time of conspicuous climatic fluctuations as indicated by the temporal variations in fauna and in mineral/fauna ratio (Fig. 2 and Fig. 3). Beds rich in both foraminifera and in Mn, Fe oxides alternate with layers poor in both foraminifera and Mn, Fe oxides. The former represent conditions similar to present (permanent pack ice cover) and contain sinistral *G. pachyderma* almost exclusively[3,4]. *G. quinqueloba* attained high frequencies at the beginning and end of some of these cold periods. In the foraminifera-poor beds, sinistral *G. pachyderma* is accompanied by *G. quinqueloba*. Pteropods (*Limacina helicina*) are preserved principally in core tops. Unit II/unit I boundary approximately corresponds to the last major polarity reversal in the Earth's magnetic field conveniently marking the Brunhes/Matuyama boundary[3]. Ice rafted débris throughout the cores indicates that high latitude glaciation commenced before 3.3 m.y. ago.

Among the factors determining surface water salinity in the Arctic, precipitation, evaporation, ocean currents, convective mixing and the presence of drift ice are considered most important and the oxygen isotopic composition of Arctic surface water is determined by the same factors[9]. Evaporation results in concentration of ^{18}O while precipitation and river inflow cause its dilution. The range of present day surface water temperature and salinity is $-1.6°$ C to 0° C and $<29^{0}/_{00}$–$33^{0}/_{00}$ respectively[10].

As a result of slow vertical mixing, present day surface salinities are about $4.8^{0}/_{00}$ lower than those of the bottom water[10]. Analysis of $^{18}O/^{16}O$ for Arctic water[11] indicates that the ^{18}O

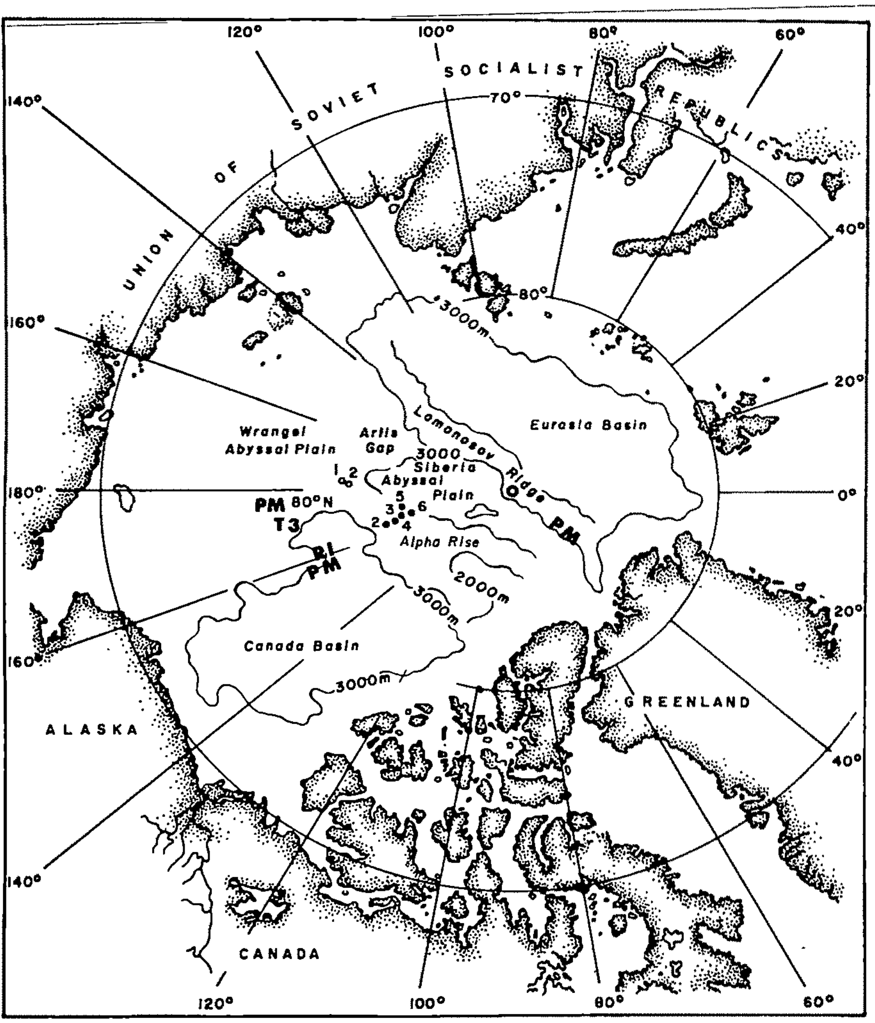


Fig. 1 Bathymetric map of the Arctic Ocean based on the geological map of the Arctic (1960). T3 shows the location of cores T3-67-4,9,11,12 while PM shows the location of cores with known palaeomagnetic stratigraphy and RI locates the radioisotopically dated core. Dark and light circles show location of previously studied cores[1-3].

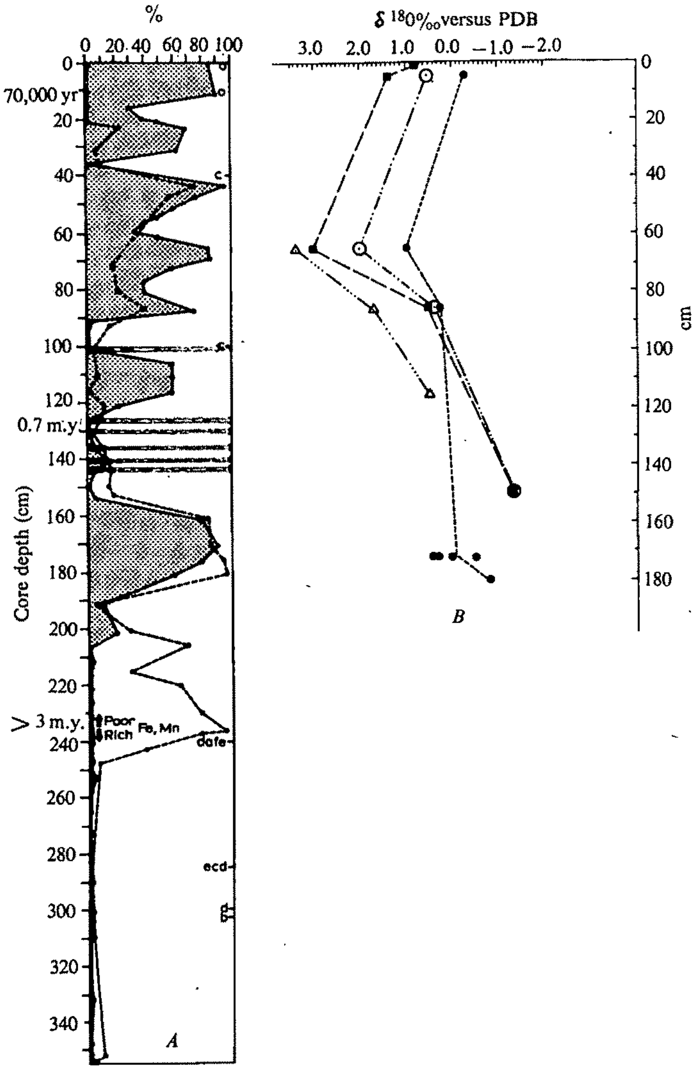


Fig. 2 Core T3-67-9. *A*, Variation of microfauna to clastics in the coarse fraction (>62 μm). The shaded areas represent volume percentages of microfauna. The occurrence of low latitude foraminifers is as follows: *a*, *Globorotalia menardii—tumida*; *b*, *Globorotalia scitula*; *c*, *Globorotalia* sp.; *d*, *Globigerinoides* sp.; *e*, *Globigerinoides ruber*; and *f*, *Sphaeroidinella dehiscens*. The circles represent pteropods and the dashed line gives the percentage of *G. quinqueloba* complex out of the total planktonic population. Dark horizontal lines are horizons where benthonic foraminifers are in excess of 10%. *B*, The variation in $\delta^{18}O$ for: ■, *G. pachyderma*; ●, *G. quinqueloba*; ○, average planktonic species; and △, total planktonic and benthonic foraminifers.

depletion closely follows the salinity profile, with $\delta^{18}O$ changing by 0.8‰ with 1‰ change in salinity in the upper 350 m. This surface seawater is depleted in ^{18}O by about 4‰, while at greater depth $\delta^{18}O$ approaches values close to SMOW.

The $^{18}O/^{16}O$ ratios of calcareous tests from foraminifera-rich and foraminifera-poor layers were determined (Figs. 2 and 3). The measurements were made on an Atlas-Werke M86 mass spectrometer developed by Nier. The foraminifers were hand picked, and treated with commercial 'Clorox' solution; the CO_2 was extracted from the $CaCO_3$ according to the method described by McCrea[12].

To calculate the temperatures from the $^{18}O/^{16}O$, the formula of Epstein *et al.*[13], modified by Craig[14], was used

$$t = 16.9 - 4.2\,(\delta s - \delta w) + 0.13\,(\delta s - \delta w)^2$$

The established relation

$$\left(\delta s\ ^{18}O = \frac{^{18}O/^{16}O \text{ sample}}{^{18}O/^{16}O \text{ standard}} - 1 \times 1{,}000\right)$$

depends on the water temperature (t° C) as well as on the isotopic composition of the water in which the organisms lived.

When sufficient shell material was available, planktonic tests were analysed separately from the benthonic forms. Moreover, when samples yielded adequate amounts of the two dominant planktonic foraminifers, *G. quinqueloba* and *G. pachyderma*, their tests were analysed separately. As indicated in Figs. 2 and 3 the former registered consistently lower $\delta^{18}O$ values than the latter. This can readily be explained by their differing life habitats: *G. quinqueloba*, an epiplanktonic form, incorporates the $CaCO_3$ necessary for test building at surface, in the isotopically lighter, low salinity water, whereas the thick shelled *G. pachyderma*, known to inhabit greater water depth[15], constructs the early part of its shell at surface, and the outer, thick cortex at greater depth, in denser, more saline water, hence its great $^{18}O/^{16}O$ value. Determinations of $\delta^{18}O$ made on composite samples of benthonic and planktonic foraminifers indicate that, with one exception, the bottom water was enriched in ^{18}O (Figs. 2 and 3) attesting to the restricted vertical mixing and subsequent density stratification of the Arctic water.

As mentioned previously, changes in the $\delta^{18}O$ of the seawater are reflected in the oxygen isotopic ratios of the calcareous foraminiferal tests. The greatest shift towards positive values occurs at 65 cm depth in core T3-67-9 and at 40 cm depth in core T3-67-12 (Figs. 2 and 3); both samples come from foraminifera-rich horizons and were deposited during a period when seawater was colder and/or more saline than today.

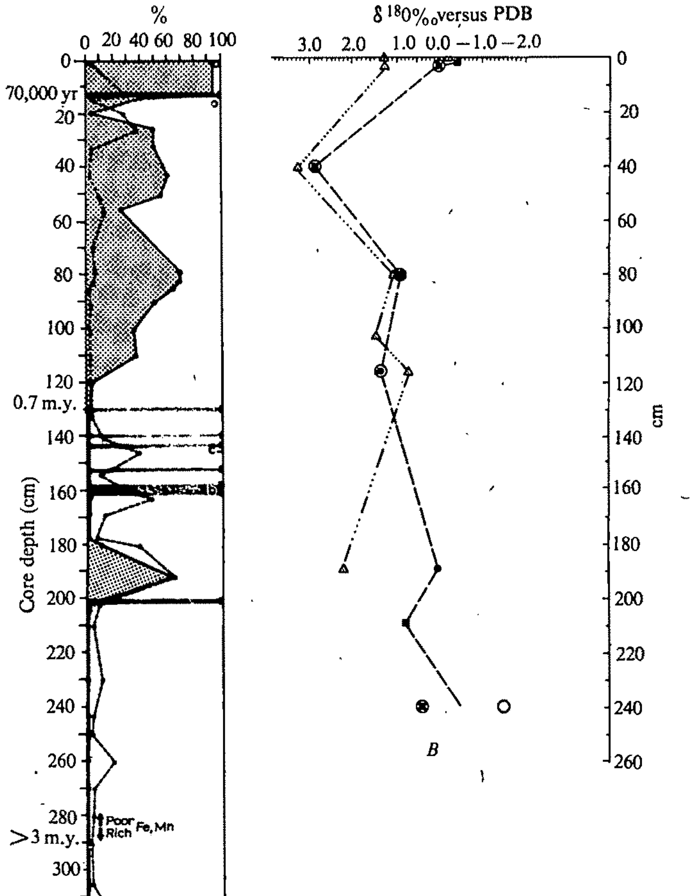


Fig. 3 Core T3-67-12. *A*, Variation in microfauna to clastics. Same legend as for Fig. 2. *B*, Variation in $\delta^{18}O$. Same legend as for Fig. 2.

Below 85 cm $\delta^{18}O$ values are more negative than in the core "tops" suggesting slightly warmer and/or less saline water in the Arctic basin between 3.3 and 0.7 m.y. ago than those of the present.

In conclusion, oxygen isotope and palaeontological determinations of planktonic foraminifers allow us to make several generalizations on the palaeoceanographic history of the Arctic Ocean back to Mid Pliocene. We conclude that the surface and deep water underwent periodic fluctuations and that the average surface water temperatures have varied between about $-2°$ C and $+0.5°$ C, with salinities ranging from approximately $36^0/_{00}$ to $<29^0/_{00}$, during the time interval represented by the cores investigated. This assumption is based on the $^{18}O/^{16}O$ data combined with the estimated environmental requirements of the extant planktonic foraminiferal species[15].

Temperature minima correspond to the upper Pleistocene, probably the Riss (Illinoian) or Würm (Wisconsin) Glacial, whereas higher temperatures and/or lowest salinities were registered by foraminifers between about 3.3 million and 0.7 million years BP.

We thank P. E. Rosenberg and J. R. O'Neil for valuable suggestions, K. Hunkins for collecting the cores and R. Capo for shipping the samples. Coring operation was supported by ONR and NSF grants to Lamont-Doherty Geological Observatory.

YVONNE HERMAN

Department of Geology,
Washington State University,
Pullman, Washington 99163

C. VERGNAUD GRAZZINI

Université de Paris,
Faculté des Sciences,
Laboratoire de Geologie Dynamique et Centres,
de Recherches Geodynamiques,
Paris

C. HOOPER

Department of Geology,
Washington State University,
Pullman, Washington 99163

Received May 3, 1971.

[1] Herman, Y., *Nature*, **201**, 386 (1964).
[2] Herman, Y., *Palaeogeog., Palaeoclimatol., Palaeoecol.*, **5**, 251 (1969).
[3] Herman, Y., *Science*, **169**, 474 (1970).
[4] Herman, Y., *Second Intern. Symp. on Arctic Geol.*, San Francisco, Abst., 25 (1971).
[5] Ku, T. L., and Broecker, W. S., *Prog. Oceanog.*, **4**, 105 (1967).
[6] Clark, D. L., *Geol. Soc. Amer. Bull.*, **81**, 3139 (1970).
[7] Clark, D. L., *Arctic*, **22**, 233 (1969).
[8] Steurwald, B. A., Clark, D. L., and Andrew, J. A., *Earth Planet. Sci. Lett.*, **5**, 79 (1968).
[9] Craig, H., and Gordon, L. I., *Symp. in Marine Geochem.*, **3**, 277 (1965).
[10] Treshnikov, A. F., *Intern. Oceanog. Cong.*, New York, Abst., 522 (1959).
[11] Van Donk, J., and Mathieu, G., *J. Geophys. Res.*, **74**, 3396 (1969).
[12] McCrea, J. M., *J. Chem. Phys.*, **18**, 849 (1950).
[13] Epstein, S., Buchsbaum, R., Lowenstam, H., and Urey, H. C., *Geol. Soc. Amer. Bull.*, **64**, 1315 (1953).
[14] Craig, H., *Stable Isotopes in Oceanog. Studies and Palaeotemp.*, Spoleto (1965).
[15] Boltovskoy, E., *Micropalaeontol.*, **15**, 237 (1969).

Palaeoclimatic Significance of the Stagnation of the Cariaco Trench

ALMOST all available information about the past thermal behaviour of the oceans has been obtained by studying the oxygen isotope ratios of cores from the deep sea[1]. One basic limitation of that method is that to derive a palaeotemperature curve from oxygen isotope data one must assume some initial value for the oxygen isotope ratio in the ocean at the time each sample was formed. There has been little independent evidence of past oceanic temperatures to provide a check on these assumed values, and some questions have been raised[2] about the significance of the $^{16}O/^{18}O$ palaeotemperature record. I suggest that the deep water of the Cariaco Trench may provide an estimate of the late glacial surface temperature of the Caribbean Sea which is completely independent of the oxygen isotope method.

The Cariaco Trench is a small closed basin off the coast of Venezuela with a sill depth of about 146 m and a maximum depth of 1,400 m[3,4]. The water in the trench is presently anaerobic below a depth of 375 m and isothermal below 250 m, but sediment cores indicate that during late glacial time the bottom of the trench was ventilated and that the present stagnant condition developed about 10,900 radiocarbon years ago[4,5–7].

I suggest that during the late glacial period of ventilation of the deep waters of the Cariaco Trench, oceanic cooling and overturning enabled oxygenated surface waters to descend to the bottom of the trench; the isothermal and isohaline conditions must then have extended to the surface, because of vigorous mixing. These conditions ended when surficial warming at the beginning of the postglacial established a stable stratification; the deep water of the trench has been isolated since that time. I therefore regard the deep water as "fossil" late glacial age surface water in some respects. In particular, the deep water can hardly have cooled since the late glacial, because the overlying water is warmer than the deep water, and the geothermal heat flux prevents loss of heat downwards. Therefore, I regard the present deep water temperature of 16.9° C as the maximum possible surface temperature for the waters of the Cariaco Trench at the end of the last glaciation. This is in contrast to the modern values of 24° C to 26° C (ref. 3), and implies a temperature difference of at least 7° C between the late glacial period and the present.

This interpretation provides an unusual opportunity to check some of the assumptions which must be made in oxygen isotope work.

DAVID P. ADAM

Laboratory of Tree Ring Research,
University of Arizona,
Tucson, Arizona 85721

Received May 3, 1971.

[1] Emiliani, C., *Science*, **171**, 571 (1971).
[2] Shackleton, N., *Nature*, **218**, 79 (1968).
[3] Richards, F. A., and Vaccaro, R. F., *Deep Sea Res.*, **3**, 214 (1956).
[4] Lidz, L. L., Charm, W. B., Ball, M. M., and Valdes, S., *Bull. Marine Sci. Gulf Caribbean*, **19**, 1 (1969).
[5] Broeker, W. S., Ewing, M., and Heezen, B. C., *Amer. J. Sci.*, **258**, 429 (1960).
[6] Heezen, B. C., Menzies, R. J., Broeker, W. S., and Ewing, M., *Bull. Geol. Soc. Amer.*, **69**, 1579 (1958).
[7] Athearn, W. D., *Woods Hole Oceanog. Inst. Tech. Rep.*, 65–37 (1965).

Monolithic Light Emitting Diode Arrays using Gallium Phosphide

THE high diode packing densities that can be achieved on single crystal slices of semiconductors mean that monolithic arrays of light emitters offer the possibility of displaying typescript signed characters from a 7 × 5 matrix, either for direct visual applications or for data recording. Previously this has only been achieved using the direct band gap semiconductors gallium arsenide and gallium arsenide phosphide, which emit radiation in the infrared or extreme red parts of the spectrum. Two problems have prevented gallium phosphide being used in monolithic arrays; they are the low electroluminescent efficiencies obtained from junctions formed by diffusion into indirect band gap materials and the lack of an adequate mask for use on gallium phosphide to define the diode areas during gaseous zinc diffusion.

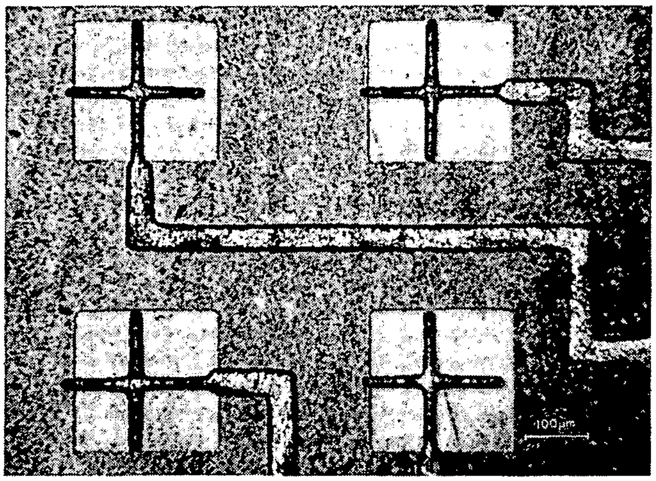


Fig. 1 Photograph of four diodes in a monolithic array. The diode squares are light emitting zinc diffused areas, the remainder of the surface being covered by the dielectric mask. Contact is made to the devices by aluminium interconnectors, the cross structure being used on top of the diode to facilitate current spreading.

We have fabricated green emitting monolithic arrays from epitaxial layers of gallium phosphide grown from gallium solution onto substrates cut from ingots produced by the liquid encapsulated Czochralski process[1] or grown by the vapour transport method[2]. These layers were doped with sulphur so as to have a carrier concentration in the range 5×10^{16}–10^{17} cm^{-3} and had nitrogen added so as to give a concentration in the semiconductor of approximately 2×10^{18} atoms cm^{-3}. The nitrogen substitutes for the phosphorous atom in the lattice, so acting as a shallow trap which is responsible for the green emission process[3]. In order to produce a p–n junction we have then diffused zinc into the layer at 650° C resulting in a surface zinc concentration of 3×10^{18} atoms cm^{-3}. The relatively low zinc level produced in the material enables the conditions for good electroluminescent efficiencies in an indirect band gap material to be established. The diode areas have been defined by diffusing through holes cut by photolithographic techniques in a triple mask of silica, silicon nitride and silica. This structure has excellent adhesion to the gallium phosphide and also has a low porosity to zinc atoms, so enabling it to act as an effective diffusion mask.

The monolithic arrays produced by this process have had electroluminescent efficiencies for green emission up to 0.1% at a current density of 20 A cm^{-2}. This compares with the best reported figure of 0.35% for individual diodes produced by the more complex double liquid epitaxial process grown on Czochralski substrates[4].

This work was carried out in part under a CVD contract and is published by permission of the UK Ministry of Defence (Navy Department).

M. A. Carter
A. Mottram
A. R. Peaker
P. D. Sudlow
T. White

Ferranti Ltd,
Chadderton,
Lancashire

Received June 7, 1971.

[1] Bass, S. J., and Oliver, P. E., *J. Crystal Growth*, **3**, 4, 286 (1968).
[2] Mottram, A., Peaker, A. R., and Sudlow, P. D., *J. Electrochem. Soc.*, **118**, 318 (1971).
[3] Dean, P. J., Gershenzon, M., and Kaminsky, G., *J. App. Phys.*, **38**, 5332 (1967).
[4] Logan, R. A., White, H. G., and Wiegmann, W., *Solid State Electron.*, **14**, 55 (1971).

Drag Reduction arising from Adhesive Tapes

Cossar has reported[1] that the presence of a uniform layer of double-sided adhesive tape ('Scotch' brand) on the floor of a water channel can have a significant influence on the mean-velocity profile in the boundary layer of a turbulent flow. Indeed, Cossar's experiments indicate that, in his operating conditions, the velocity distribution above a layer of adhesive tape is almost the same as that obtained in flow over rough boundaries for which the mean height of roughness is about 0.040 inches (0.116 cm). He accordingly suggested that 'Scotch' tape is physically "sticky" towards water. But Cossar's interpretation of his results is open to question: it may be that, rather than increasing the resistance to the flow, the influence of the adhesive tape reduces it. This conclusion can in fact be inferred from Cossar's results. For flows past smooth boundaries, the shear stress τ exerted on the boundary is related to the viscosity η and the normal velocity gradient by

$$\tau = \eta\left(\frac{\partial u}{\partial y}\right)_{\text{wall}} \tag{1}$$

where u is the tangential velocity and y is the normal coordinate. But this is a good approximation only for boundaries that are hydraulically smooth, in the sense that the surface irregularities lie within the viscous sublayer of the flow. If, on the other hand, the roughness elements at the boundary are large enough, they can contribute to the shear stress exerted on the boundary through an effective form drag (see ref. 2, page 376), and then equation (1) is invalid. Indeed, it is usual for values of $\partial u/\partial y$ near the wall to decrease as the surface roughness increases (compare Cossar's results), although the actual shear stress exerted on the boundary increases. If we now make the assumption that the adhesive tape in Cossar's experiments was hydraulically smooth, it follows that the observed reduction in $\partial u/\partial y$—which presumably extends right up to the wall—is accompanied by a reduction in shear stress, compared with what it would be on a smooth insoluble wall.

If these conclusions are correct they might have some interesting implications, for it would seem that the drastic changes in flow properties observed by Cossar emanate from the absorption into solution of some adhesive from the tape. But the solubility in water of the gum of such adhesive tapes is rather low, which suggests that only extremely small quantities of additive are needed to change the structure of the turbulent boundary layer, provided the additive is supplied in "sensitive" areas.

Naturally these ideas are speculative. To check them some solutions of adhesive were made by soaking pieces of tape in water, and then the drag reduction properties of these solutions were measured in turbulent pipe flows.

The apparatus used consisted of a hypodermic syringe to which a 93 cm length of hypodermic tubing of 1 mm bore was attached. The syringe was filled with the solution to be tested, and a known pressure was applied to the plunger of the syringe, forcing the liquid through the small-bore tubing. An electronic counter was used to measure the time for a given quantity of liquid (usually 10 ml.) to be ejected. The ejection times determined in this way were easily reproducible, for a given fluid, to within ±2%. The drag reduction was taken

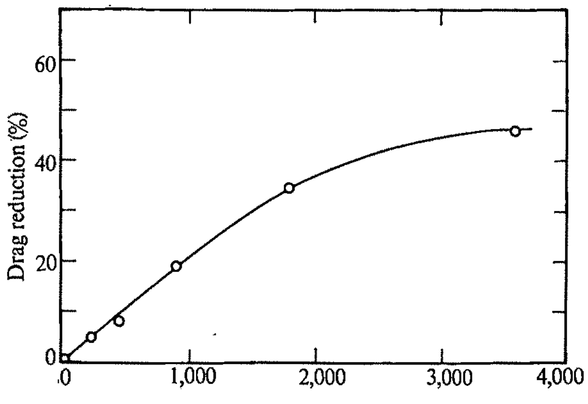


Fig. 1 Drag reduction of a solution of 'Scotch' tape in petroleum ether. Solvent Reynolds number was 60,000.

to be the fractional increase in the flow rate of the sample over that obtained for the solvent alone.

In view of Cossar's results it was initially anticipated that an extremely dilute solution, obtained from soaking 'Scotch' tape in water, would give rise to a large drag reduction. But early attempts to observe the Toms effect with such solutions proved unfruitful, and it was only after prolonged soaking of the tape that any effect was observed. For example, after soaking 0.02 m^2 of 'Scotch' tape in 200 ml. of water for 40 days, the resulting solution showed a drag reduction of 19% compared with water when ejected at a Reynolds number of 8,200. With another proprietary brand of adhesive tape ('Sellotape') the same procedure gave a drag reduction of 8% which rose to 20% after 80 days of immersion. In connexion with these measurements it is perhaps worth noting that: (i) the viscosity of the 'Scotch'-tape solution, measured under laminar flow conditions, was about 6% greater than that of water at the same temperature; and (ii) the drag reduction remained unchanged when the adhesive tape solutions were passed through filter paper (Whatman No. 1 grade) before being tested.

To obtain a solution of known concentration, the gum from a sample of 'Scotch' tape was completely dissolved in a "good" solvent (petroleum ether). The properties of this solution are not expected to differ greatly from those of the corresponding aqueous solution. The values of the drag reduction obtained at various dilutions are shown in Fig. 1. At a concentration of 3,600 p.p.m.w., when the viscosity of the solution was 50% greater than that of the solvent, the drag reduction was 46%. The results in Fig. 1 suggest that, compared with some drag reducing compounds, the concentrations of the present solutions need to be quite large in order to produce substantial drag reduction. On the other hand, if our suggestions regarding Cossar's experiments are correct, it would seem from his results that only extremely small quantities of additive can have large influences on a turbulent boundary layer when it is supplied from the wall. Accordingly it might be highly effective, for drag reduction, to coat surfaces with such compounds, which, because of their poor solubility in water, could have a fairly long period of effectiveness.

B. J. S. BARNARD
W. G. PRITCHARD

Fluid Mechanics Research Institute,
University of Essex

Received June 25, 1971.

[1] Cossar, A. E., *Nature*, **227**, 1044 (1970).
[2] Goldstein, S., *Modern Developments in Fluid Dynamics* (Clarendon Press, Oxford, 1938).

BIOLOGICAL SCIENCES

Quantitative Evidence for Protein Denaturation as the Cause of Thermal Death

THE specific cause of the death rates of unicellular organisms and poikilothermic animals at high temperatures is not known for certain[1]. We wish to report a good numerical correlation between certain thermodynamic parameters in protein denaturation and death rates, where these have been reported, for these organisms. A similar correlation may exist between the thermal denaturation of other biopolymers, such as DNA and RNA, and thermal death, but in the absence of adequate data we cannot consider this possibility further.

At sufficiently high temperatures (>315 K) most proteins denature with pseudo-first order kinetics. This implies that the renaturation rate is slow enough to be negligible, and the rate is described by $\mathrm{d}n/\mathrm{d}t = -k_D n$. The first order denaturation rate constant, k_D, varies exponentially with temperature and is usually described by the absolute rate theory equation[2]

$$k_D = \kappa \frac{k_B T}{h} \exp[\Delta S^\ddagger/R] \times \exp[-\Delta H^\ddagger/RT] \qquad (1)$$

where $\Delta S^\ddagger$ and $\Delta H^\ddagger$ are the activation entropy and activation enthalpy respectively.

We have found evidence from the work of Williams and Milby[3] on the thermal denaturation of rhodopsins that, for the proteins tested, the $\Delta S^\ddagger$ and $\Delta H^\ddagger$ values were related by the simple linear equation[4]

$$\Delta S^\ddagger = a\Delta H^\ddagger + b \qquad (2)$$

The existence of such a relationship constitutes a compensation law behaviour, because variations of $\Delta H^\ddagger$ in the negative exponential of equation (1) are partially compensated by parallel variations of $\Delta S^\ddagger$ in the positive exponential in their effects on k_D. (At the temperature $T = 1/a$, the compensation is exact and the denaturation rate is independent of the values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$. This temperature, T_c, is the compensation law temperature.) Such compensation law behaviour occurs in a number of physical and chemical processes[5-9] for which *ad hoc* explanations have been suggested. So far there is no compelling evidence that such a rule as equation (2) can be derived from general thermodynamic principles. Exner[10] has pointed out that, in certain limited conditions, a compensation law may occur accidentally, and he has provided a set of criteria for judging the reliability of the rule. In some of the cases described here, such criteria have been used and satisfied.

We tested the generality of such a compensation law using the compilation of thermodynamic data on protein denaturations from Joly[11]. Fig. 1 presents all these data, plotted as $\Delta S^\ddagger$ versus $\Delta H^\ddagger$, with no selection. All the data cluster about a linear regression line. From a least squares fit of the data points, the line yields a value of $T_c (= 1/a)$ of 329 K and a value of $b = -64.9$ calories/mol × K (an equally valid intercept could be the value of $\Delta H^\ddagger$ when $\Delta S^\ddagger = 0$; this yields $\Delta H^\ddagger = 21.3$ kcalories/mol). The corresponding values for the rhodopsins are: $T_c = 334$ K and $b = -66$ calories/mol × K. Several of the proteins are represented by a number of points, each referring to a specific *p*H or ionic strength of the solution. Therefore, although the $\Delta S^\ddagger$ and $\Delta H^\ddagger$ values for a given protein may vary depending on the experimental conditions, the values always fall on the same line.

Sukhorukov and Likhtenshtein[12] have published a plot similar to Fig. 1 for a large number of proteins, which overlap only partially the proteins of our figure. The values we have estimated from their plot are: $T_c \simeq 325$ K, $b \simeq -70$ calories/mol × K, which are closely similar to ours. They have also included proteins and viruses in the dry state, as well as proteins in alcohol–water mixtures, and in D_2O solution, all of which still fall on the compensation law line.

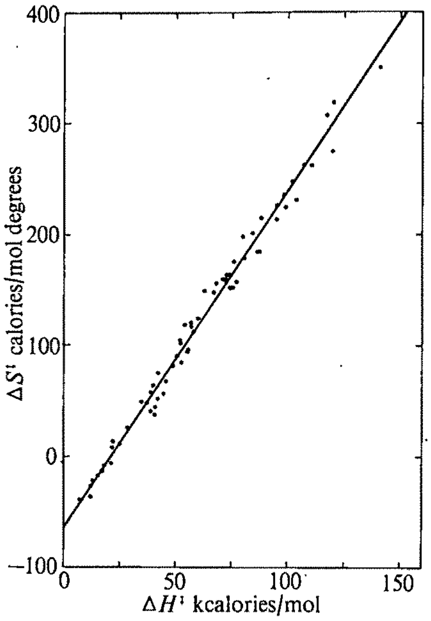


Fig. 1 A plot of the activation entropy, $\Delta S^\ddagger$, versus the activation enthalpy, $\Delta H^\ddagger$, for protein denaturation (after Joly[11]). The regression line is a least squares fit to the data, and the close clustering to the line indicates the validity of the compensation law (equation 2). The constants of the line are: $T_c=329$ K, and $b=-64.9$ calories/mol × K.

We conclude that virtually all proteins[7], in a variety of conditions, denature at elevated temperatures according to the compensation law of equation (2), with the same value of T_c and b.

Lumry (personal communication) has called our attention to the work of van Uden and his co-workers[13] who have reported compensation law behaviour in the thermal killing of yeasts. Here again (1) the death rates followed pseudo-first order kinetics with respect to time; (2) the specific thermal death rate, k_D, depended exponentially on temperature; and (3) the $\Delta S^\ddagger$ and $\Delta H^\ddagger$ values obtained were correlated according to equation (2). From their data we have calculated the values of the compensation law constants as $T_c=325$ K and $b=-64.5$ calories/mol × K.

We have attempted to pursue this correlation with other unicellular organisms such as viruses and bacteria. Usable data in the literature are surprisingly scant, but Barnes *et al.*[14] have carefully investigated this compensation law in the thermal inactivation of the Sindbis virus. Their data again meet the three criteria described above, and they calculate $T_c=330$ K; while we calculate $b=-64$ calories/mol × K. In the case of bacteria, data are available on *Pseudomonas fragi*[15], *Staphylococcus aureus*[16,17], and two strains of *Salmonella*[17] which also meet our three criteria. The values we have calculated for the compensation law constants are $T_c=331$ K and $b=-65$ calories/mol × K.

We have plotted all the points for the yeasts, virus and bacteria in Fig. 2. In Table 1 we collect all the values of the constants for comparison. The unicellular organisms have a specific death rate for thermal killing which follows a compensation law with constants which are in very good agreement with the constants for thermal denaturation of proteins. We suggest that this is good quantitative support for the general hypothesis that protein denaturation is the cause of thermal death in unicellular organisms. Such a statement, however, does not allow the identification of any specific protein as the limiting cause, because the cells will have different $\Delta S^\ddagger$, $\Delta H^\ddagger$ values in different environmental conditions, and any specific protein will exhibit a class of $\Delta S^\ddagger$, $\Delta H^\ddagger$ values in different environmental conditions *in vitro*. It may be possible, though difficult, to find an isolatable protein, or group of proteins, the $\Delta S^\ddagger$, $\Delta H^\ddagger$ values of which *in vitro* coincide with those of the cell for the same conditions, and draw the inference that this (these) is (are) the limiting protein(s). The viruses, constituted with a small number of proteins, recommend themselves for such a study.

Table 1 Comparison of Compensation Law Constants, T_c and b, from : $\Delta S^\ddagger = a\, \Delta H^\ddagger + b$; $T_c = 1/a$

	T_c (K)	b (calories/mol × K)
Proteins	329	−64.9
Virus	330	−64
Yeasts	325	−64.5
Bacteria	331	−65

In a cell, a protein i is manufactured with a creation rate c_i, and has a denaturation rate, $k_{D_i} n_i$ (again we ignore renaturation, although it could be subsumed under c_i); therefore we have:

$$\frac{dn_i}{dt} = c_i - k_{D_i} n_i \qquad (3)$$

If a steady state could occur, then the number of molecules of protein i is $n_i = c_i/k_{D_i}$. The creation rate c_i must be assumed to be a complex function of both the genetic structure and environmental conditions. For a population of cells to die with pseudo-first order kinetics, the temperature must be high enough that the denaturation rate overwhelms the ability of the cell to create the proteins that are limiting, and $dn_i/dt = k_{D_i} n_i$. If N is the number of viable cells, then our hypothesis requires that $dN/dt = -k_{D_i} N$. It is not obvious why the k_{D_i} for the cell population should be identical with k_{D_i} for the protein population. For example, the assumption that a threshold number of proteins, i, is essential for cell viability would lead to a single time instant of death for all the cells of the population, rather than a constant probability of death as the kinetics require. It can be shown mathematically that at least three hypotheses connecting protein denaturation with cell death could lead to the appropriate kinetics and thermodynamics. These are (*a*) that there is a statistical distribution of protein concentration thresholds in the population of cells; (*b*) that the stress on the cell to create protein at a rate sufficient to compensate the denaturation rate is the cause of death; and (*c*) that the stress on the cell to prevent the accumulation of denatured protein

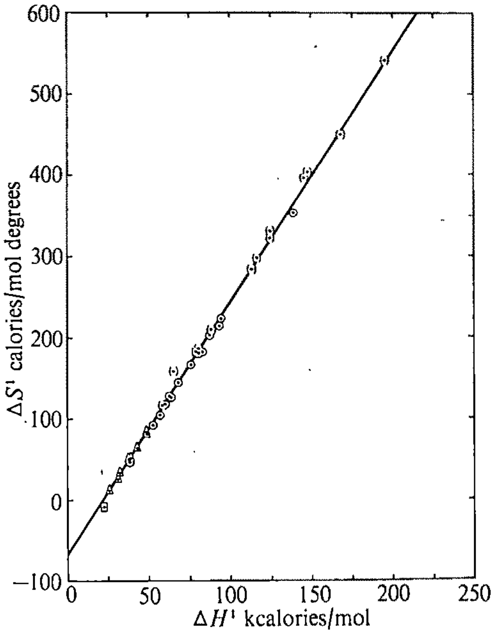


Fig. 2 A plot of the activation entropy, $\Delta S^\ddagger$, versus the activation enthalpy, $\Delta H^\ddagger$, for thermal killing of various organisms: (·), yeasts (after van Uden *et al.*[13]; ⊙, bacteria (after Luedecke[15], Walker[16], and Beamer and Tanner[17]); △, virus (after Barnes *et al.*[14]); and ⊡, *Drosophila m.* (after Strehler[18]). The constants of the line are: $T_c=325$ K and $b=-66$ calories/mol × K.

debris kills the cell. There are undoubtedly other possible hypotheses which should be tested.

In Fig. 2 we have plotted a single point on the line which refers to the multicellular poikilotherm, *Drosophila melanogaster*, after the data of Strehler[18]. We have replotted his death rate curves to obtain the first order kinetic plots versus time. These k_Ds do show an exponential dependence on the temperature; and using equation (1), we have calculated the $\Delta S^\ddagger$ for these data. The datum point falls on the line close to the $\Delta S^\ddagger \simeq 0$ value. Strehler[1] has pointed out that a number of other studies on arthropods (*Drosophila*, *Pinus tectus* and *Daphnia*) have shown almost identical activation energies for the rate of ageing (as measured by the mortality rate) and these would yield data points close to $\Delta S^\ddagger \approx 0$, according to our estimates. We can now speculate that, if this is so, it has at least two explanations. First, the evolutionary jump from unicellular to multicellular organisms can be represented on the compensation law plot as the convergence of the $\Delta H^\ddagger$ values to the lowest value consonant with $\Delta S^\ddagger \geq 0$. That means that the arthropods have accepted a higher thermal death rate still consistent with survival of the species, for a smaller variation of the thermal denaturation rate of the limiting proteins with ambient temperature changes. Second, Sukhorukov and Likhtenshtein[12] have shown that many dry proteins fall on the compensation law plot at $\Delta S^\ddagger \approx 0$, and that hydration of these proteins causes the $\Delta S^\ddagger$, $\Delta H^\ddagger$ values to move up the line. $\Delta S^\ddagger \approx 0$ may be the smallest value that can be reasonably expected in biological circumstances; therefore one could argue that in going up the evolutionary scale, "dry" proteins are first encountered in vital roles at the level of multicellular organisms. Because these proteins denature fastest, they represent the rate limiting step in the death of these organisms. These latter arguments are purely speculative.

This work was supported in part by contracts from the Office of Naval Research and the Atomic Energy Commission.

Note added in proof. A fourth hypothesis connecting protein denaturation and cell death was proposed by Rahn in 1929 (Rahn, O., *Bact Rev.*, **8**, 1; 1944). According to him, "a logarithmic order is possible only when death is due to the destruction of a single protein molecule in the cell". We find this the most attractive alternative, with some interesting ramifications which we will develop later.

BARNETT ROSENBERG
GABOR KEMENY
ROBERT C. SWITZER
THOMAS C. HAMILTON

Department of Biophysics, Michigan State University,
East Lansing, Michigan 48823

Received February 8, 1971.

[1] Strehler, B. L., *Time, Cells and Aging* (Academic Press, New York, 1962).

[2] Glasstone, S., Laidler, K. J., and Eyring, H., *The Theory of Rate Processes* (McGraw-Hill, New York, 1941).

[3] Williams, T. P., and Milby, S. E., *Vision Res.*, **8**, 359 (1968).

[4] Rosenberg, B., and Williams, T. P., *Vision Res.* (in the press).

[5] Eley, D. D., *J. Polymer Sci.*, Pt C, **17**, 73 (1967).

[6] Rosenberg, B., Bhowmik, B. B., Harder, H. C., and Postow, E., *J. Chem. Phys.*, **49**, 4108 (1968).

[7] Lumry, R., and Rajender, S., *Biopolymers*, **9**, 1125 (1970).

[8] McCreary, J. R., and Thorn, R. J., *J. Chem. Phys.*, **50**, 3725 (1969).

[9] Leffler, J., and Grunwald, E., *Rates and Equilibria of Organic Reactions* (Wiley, New York, 1963).

[10] Exner, O., *Nature*, **201**, 488 (1964).

[11] Joly, M., *A Physico-Chemical Approach to Denaturation of Proteins* (Academic Press, New York, 1965).

[12] Sukhorukov, B. I., and Likhtenshtein, G. I., *Biofizika*, **10**, 935 (1965).

[13] van Uden, N., Abranches, P., and Cabeca-Silva, C., *Archiv. Mikrobiol.*, **61**, 381 (1968).

[14] Barnes, R., Vogel, H., and Gordon, I., *Proc. US Nat. Acad. Sci.*, **62**, 263 (1969).

[15] Luedecke, L. O., thesis, Michigan State Univ. (1962).

[16] Walker, G. C., thesis, Michigan State Univ. (1964).

[17] Beamer, P. R., and Tanner, F. W., *Zentralblatt Bakt.*, **100**, 81 (1939).

[18] Strehler, B. L., *J. Gerontol.*, **16**, 2 (1961).

Preparation and Characterization of an Arylsulphatase insolubilized on Porous Glass

THE enzyme sterol sulphate sulphohydrolase (EC 3162) is used for the hydrolysis of steroid conjugates before analysis of body fluids for total oestrogens; but it is rather costly. The availability of a re-usable derivative of this enzyme which would be stable for a long time might be of value to the clinical chemist. I therefore decided to prepare an insolubilized derivative of this enzyme and determine its properties.

The enzyme was purchased as a mixture of β-glucuronidase/arylsulphatase (Boehringer). The arylsulphatase activity of the commercial preparation was 2.6 IU/ml. of enzyme solution. The insoluble enzyme was prepared as previously described[1-3]. Porous glass used for this study was 96% silica particles, pore diameter 550 Å, 40/60 mesh particle size. The enzyme was coupled through azo linkage to the glass. The insoluble enzyme was assayed with 0.001 M *p*-nitrophenylsulphate dissolved in 0.25 M Na_2CO_3 buffer (*p*H 9.0). The assay was carried out using 25 ml. of substrate to which was added a known weight of enzyme derivative. The reaction was continued for 10 min after which a sample was taken and read spectrophotometrically at 410 nm. The reaction was carried out at 25° C. Activity of the final product was 0.31 IU/g of glass. All other studies were carried out by the column method.

One gram of derivative was packed into a small, glass, 1.0 cm wide, jacketed column to a height of 5.0 cm. Temperature was maintained at 6° C throughout these studies.

A *p*H profile was obtained by passing 1×10^{-5} M *p*-nitrophenylsulphate through the column at a flow rate of 0.67 ml./min. The column effluent was continuously monitored at 410 nm in a flowthrough colorimeter. The buffers used were 0.25 M acetate between *p*H 5.0 and 6.7 and carbonate between *p*H 7.8 and 10.5. The *p*H optimum lies between *p*H 9.0 and 9.5 (Fig. 1).

By passing substrate at increasing concentrations through the column while maintaining constant flow rate (0.67 ml./min), the values for K_m and V_{max} were obtained (Fig. 2). The apparent V_{max} for the insoluble enzyme was 1.67×10^{-8} mol/s. The apparent K_m was 1.57×10^{-3} M. These values were

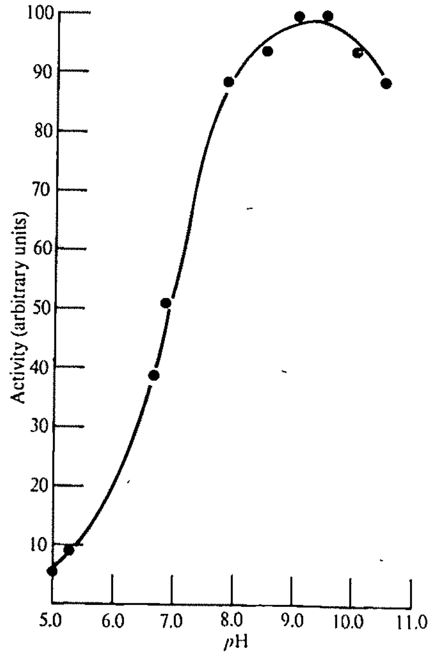


Fig. 1 *p*H profile using 1×10^{-5} M *p*-nitrophenylsulphate as substrate.

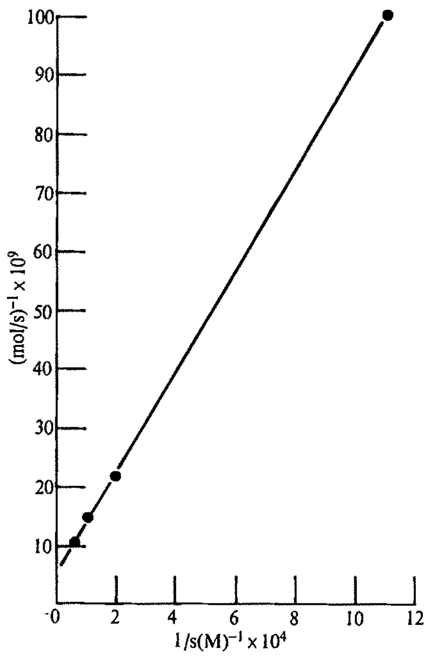


Fig. 2 Plot of 1/v against 1/(S). Substrate was *p*-nitrophenylsulphate in 0.25 M $NaCO_3$ (*p*H 9.0).

within 20% of values we obtained experimentally for the soluble enzyme at the same enzyme concentration.

The hydrolytic rate using 2.5×10^{-4} M substrate was found, as expected, to be inversely related to flow rate (Fig. 3). The relationship is not inversely proportional, however, and the curve seems very quickly to become asymptotic. Although the difference in flow rate between 0.67 ml./min and 3.0 ml./min is approximately 4.5 ×, the product concentration is decreased only 70%. The actual enzyme velocity of the insoluble derivative thus seems to increase with increasing flow rate, causing the asymptotic curvature. The apparent increased velocity is probably caused by the increased turbulent flow which decreases the film layer resistance at the surface of the particles. Because the substrate concentration used for the experiment was less than K_m, the decreased film resistance allowed more rapid diffusion of substrate. The enzyme, seeing substrate concentrations below that necessary to maintain V_{max}, produced more product when exposed to higher substrate concentrations.

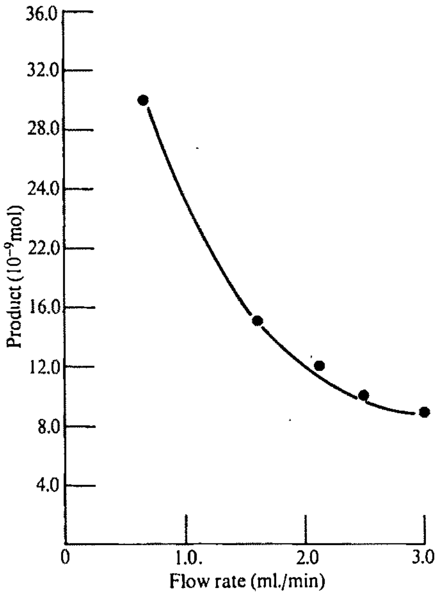


Fig. 3 Flow rate against product formation. Substrate was 2.5×10^{-4} M *p*-nitrophenylsulphate in 0.25 M $NaCO_3$ (*p*H 9.0).

The same insoluble enzyme preparation was used for all the above described experiments. The entire study took 4 weeks. At the end of this time the derivative was removed from the column and assayed. No losses of activity had occurred.

This study has shown that an arylsulphatase can be insolubilized. The insolubilized enzyme is active, stable and can be re-used many times within at least 30 days.

H. H. WEETALL

Research and Development Laboratories,
Corning Glass Works,
Corning,
New York 14830

Received March 12, 1971.

[1] Weetall, H. H., *Nature*, **223**, 959 (1969).
[2] Weetall, H. H., *Science*, **166**, 615 (1969).
[3] Weetall, H. H., *Biochim. Biophys. Acta*, **212**, 1 (1970).

Possible Role of Cyclic AMP in the Short Term Regulation of Adrenal Steroidogenesis

THE most significant short term physiological effect of adrenocorticotrophic hormone (ACTH) is to increase the rate of adrenal steroid hormone synthesis and secretion[1,2]. There is a great deal of evidence for adenosine 3′,5′-monophosphate (cyclic AMP) as intracellular mediator of this acute steroidogenic response[3–9]. The following steps are probably involved[2]: ACTH increases adrenocortical adenyl cyclase activity, thus raising the intracellular concentration of cyclic AMP which in turn enhances the conversion of cholesterol to pregnenolone, normally the rate-limiting reaction. Much of the evidence for this concept comes from experiments on intact cells though the enhancement of a variety of steroid hydroxylations in cell-free adrenal preparations by cyclic AMP has been reported[10–13].

Recently Koritz *et al.*[14] found that cyclic AMP and several chemically related compounds inhibited the conversion of cholesterol to progesterone in beef adrenocortical mitochondria; and that, in rat adrenal homogenates, cyclic AMP diminished overall corticosteroid formation by inhibiting the conversion of pregnenolone to progesterone. These observations have been confirmed by others (ref. 15 and unpublished results of J. D. Jones and myself). The conversion of Δ^5-3β-hydroxysteroids to their Δ^4-3-ketosteroid products is essential in the formation of the active steroid hormones. It is catalysed by the consecutive action of a 3β-hydroxysteroid dehydrogenase (3β-HSD) and 3-ketosteroid isomerase (3-KSI) present in both the microsomal and mitochondrial subcellular fractions[15,16]. NAD^+ is the preferred cofactor for the dehydrogenase[17] while both NAD^+ and NADH are potent activators of the 3-KSI[18]. Cyclic AMP and chemically related compounds inhibit the NAD^+ and NADH-dependent activation of beef adrenocortical 3-KSI[16,18] and inhibit 3β-HSD activity competitively with respect to the cofactor NAD^+[15,16]. Cyclic AMP is the most potent inhibitor; the suppression of corticosterone synthesis in crude adrenal homogenates is observed with concentrations of cyclic AMP above 0.2 mM and the Ki for cyclic AMP inhibition of the microsomal 3-HSD activity has been estimated to range (according to my unpublished results) from 19 to 270 μM and from 121 to 192 μM[16]. In the absence or in the presence of varying concentrations of cyclic AMP 3β-HSD activity is rate-limiting in the conversion of Δ^5-3β-hydroxysteroids to their Δ^4-3-ketosteroid products[16].

These data from cell-free preparations may reflect an unsuspected inhibitory role for cyclic AMP in a relatively intact system. The experiments of Grahame-Smith *et al.*[6] on intact rats are compatible with this contention: administration of large amounts of ACTH ($\geq$ 50 mU) to rats caused an increase of the adrenal cyclic AMP to a maximum of 0.45 mmol/kg tissue. But the injection of only 2 mU of ACTH, resulting in a cyclic AMP concentration of about 25 μmol/kg adrenal tissue, proved maximally stimulatory for corticosteroidogenesis. This suggests that with larger doses of ACTH the rising adrenal level of cyclic AMP prevented a continued increase in the rate of corticosterone formation.

The following hypothesis is an attempt to reconcile the concept of cyclic AMP as intracellular mediator of the acute steroidogenic response to ACTH with its inhibitory action described here.

I propose that a small increase in plasma ACTH results in a correspondingly small increase in the intra-adrenal concentration of cyclic AMP, sufficient to enhance the conversion of cholesterol to pregnenolone and promote steroidogenesis. The ratio of C21 to C19 steroid hormone formation will depend on intracellular compartmentation and on the kinetic parameters and activities of the enzymes involved in the two pathways. With further increase in plasma ACTH the adrenal cyclic AMP level rises sufficiently to cause significant inhibition of the conversion of Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids by competing with NAD$^+$ for binding to the 3β-HSD. Fig. 1 shows that in these conditions steroidogenesis is specifically directed towards dehydroepiandrosterone (DHEA) rather than corticosteroid and androgen formation. Thus ACTH, depending on its plasma level, may invoke at least two patterns of adrenal steroid hormone synthesis for both of which cyclic AMP is the intracellular mediator. These may overlap considerably with a gradually increasing emphasis on DHEA secretion with respect to the Δ^4-3-ketosteroids. Finally, high adrenal cyclic AMP concentrations may, by raising the intracellular level of pregnenolone, inhibit the side-chain cleavage of cholesterol and hence overall steroidogenesis.

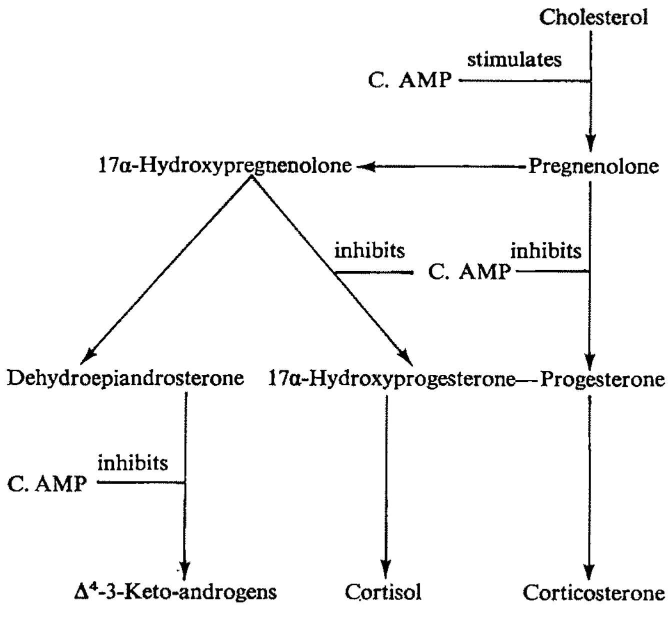


Fig. 1

The following points are relevant to a consideration of my thesis. ACTH exerts a variety of long term effects on adrenocortical function including the enhancement of specific enzyme activities[20,21] and the overall promotion of adrenal growth[22]. While these actions undoubtedly bear on the steroidogenic response to prolonged ACTH stimulation, they are not material to my hypothesis which is concerned with the acute regulation of steroid biogenesis. The validity of the hypothesis is independent of the mechanism whereby cyclic AMP stimulates the conversion of cholesterol to pregnenolone; nor is it necessarily affected by evidence[23] that 17α-hydroxyprogesterone rather than progesterone is the quantitatively more significant intermediate of cortisol biosynthesis. The validity of the hypothesis largely rests on whether, in physiological conditions, cyclic AMP reaches concentrations capable of effectively competing with available NAD$^+$ for binding to adrenal 3β-HSD. This may depend on some degree of intracellular compartmentation of cyclic AMP (ref. 16, and unpublished results of J. D. Jones and myself). Regulation of adrenal steroidogenesis by cyclic AMP is probably modified by the capacity of some C19 steroid intermediates to inhibit 3β-HSD activity[19], by the participation of steroid sulphates in biosynthesis and by the intracellular compartmentation of substrates, cofactors, and enzymes.

The hypothesis is compatible with some data derived from more physiological systems. The intravenous administration of large doses of ACTH to human subjects elicits a rapid and marked increase in adrenal DHEA secretion[24]. Urquehart *et al.*[25], in a study of the mechanisms underlying the regulation of corticosteroidogenesis, included a saturable enzyme step subsequent to cholesterol side-chain cleavage. According to my hypothesis stimulation by ACTH could cause the adrenal concentration of pregnenolone and 17α-hydroxypregnenolone to rise to levels at which zero order kinetics would hold for their conversion into their respective Δ^4-3-keto products.

The biological significance of the hypothesis remains to be determined. In species such as rodents, lacking significant 17-hydroxylase activity, adrenal dehydroepiandrosterone secretion would be negligible regardless of adrenal cyclic AMP levels. In other species, including man, DHEA (sulphate) secretion may be considerable at high plasma ACTH and adrenal cyclic AMP concentrations. As well as possessing insignificant intrinsic androgenic activity DHEA seems to be a limited peripheral precursor of the more potent sex hormones[26], except in the placenta[27]. Thus the diversion of steroid biosynthesis towards DHEA production may be a means of avoiding excessive and inappropriate androgen, and possibly corticosteroid, secretion in response to ACTH. Yang and Deal[28] have reported that cyclic AMP and related compounds inhibit yeast glyceraldehyde 3-phosphate dehydrogenase by a mechanism analogous to cyclic AMP inhibition of 3β-HSD. The direct inhibition of key dehydrogenases by cyclic AMP may have hitherto unsuspected significance in metabolic regulation.

G. M. B. BERGER

Red Cross War Memorial
Children's Hospital,
Rondebosch,
Cape,
South Africa

Received June 17; revised September 19, 1970.

[1] Robison, G. A., Butcher, R. W., and Sutherland, E. W., *Ann. Rev. Biochem.*, **37**, 149 (1968).
[2] Catt, K. J., *Lancet*, i, 1279 (1970).
[3] Haynes, jun., R. C., *J. Biol. Chem.*, **233**, 1220 (1958).
[4] Haynes, jun., R. C., Koritz, S. B., and Peron, F. G., *J. Biol. Chem.*, **234**, 1421 (1959).
[5] Karaboyas, G. C., and Koritz, S. B., *Biochemistry*, **4**, 462 (1965).
[6] Grahame-Smith, D. G., Butcher, R. W., Ney, R. L., and Sutherland, E. W., *J. Biol. Chem.*, **242**, 5535 (1967).
[7] Taunton, O. D., Roth, J., and Pastan, I., *Biochem. Biophys. Res. Commun.*, **29**, 1 (1967).
[8] Hilton, J. G., Kruesi, O. R., Nedeljkovic, R. I., and Scain, L. F., *Endocrinology*, **68**, 908 (1961).
[9] Imura, H., Matsukura, S., Matsuyama, H., Setsuda, T., and Miyaka, T., *Endocrinology*, **76**, 933 (1965).
[10] Roberts, S., Creange, J. E., and Fowler, D. D., *Nature*, **203**, 759 (1964).
[11] Creange, J. E., and Roberts, S., *Biochem. Biophys. Res. Commun.*, **19**, 73 (1964).
[12] Roberts, S., Creange, J. E., and Young, P. L., *Biochem. Biophys. Res. Commun.*, **20**, 446 (1965).
[13] Roberts, S., McCune, W. R., Creange, J. E., and Young, P. L., *Science*, **158**, 372 (1967).
[14] Koritz, S. B., Yun, J., and Ferguson, jun., J. J., *Endocrinology*, **82**, 620 (1968).

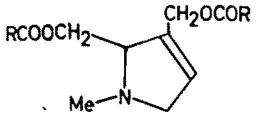
[15] Sulimovici, S., and Boyd, G. S., *Europ. J. Biochem.*, **7**, 549 (1969).
[16] McCune, R. W., Roberts, S., and Young, P. L., *J. Biol. Chem.*, **245**, 3859 (1970).
[17] Koritz, S. B., *Biochemistry*, **3**, 1098 (1964).
[18] Oleinick, N. L., and Koritz, S. B., *Biochemistry*, **5**, 715 (1966).
[19] Kowal, J., Forchielli, E., and Dorfman, R. I., *Steroids*, **4**, 77 (1964).
[20] Kowal, J., *Biochemistry*, **8**, 1821 (1969).
[21] Kowal, J., Simpson, E. R., and Estabrook, R. W., *J. Biol. Chem.*, **245**, 2438 (1970).
[22] Bransome, jun., E. D., *Ann. Rev. Physiol.*, **30**, 171 (1968).
[23] Deshpandu, N., Jensen, V., and Doouss, I. W., *J. Endocrinol.*, **43**, XIV (1969).
[24] Vaitukaitis, J. L., Dale, S. I., and Melby, J. C., *J. Clin. Endocrinol.*, **29**, 1443 (1969).
[25] Urquehart, J., Krall, R. L., and Li, C. C., *Endocrinology*, **83**, 390 (1968).
[26] Baird, D. T., Horton, R., Longcope, C., and Tait, J. F., *Rec. Prog. Hormone Res.*, **25**, 611 (1969).
[27] Villee, D. B., *New Engl. J. Med.*, **218**, 473 (1969).
[28] Yang, S. T., and Deal, jun., W. C., *Biochemistry*, **8**, 2806 (1969).

Synthetic Compounds with Toxic Properties similar to those of Pyrrolizidine Alkaloids and their Pyrrolic Metabolites

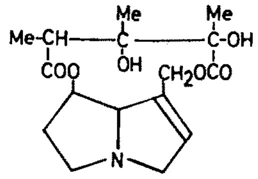
FOR the first time, some of the toxic effects of unsaturated pyrrolizidine alkaloids on rats have been reproduced by dosing animals with synthetic compounds. Many of the naturally occurring hepatotoxic alkaloids are esters of the isomeric amino-alcohols retronecine or heliotridine with various branched chain hydroxy-acids[1]. The most toxic alkaloids are cyclic diesters of retronecine[1], which are particularly resistant to enzymic hydrolysis. Because hepatotoxicity of the pyrrolizidine alkaloids has been associated with the metabolic conversion of the pyrroline to a pyrrole ring[2,3], with retention of the ester groups[4], it was expected that simpler pyrroline esters would have similar toxic properties provided that the ester groups were resistant to enzymic hydrolysis.

Of the compounds I have prepared and tested, the most effective are carbamate esters of 1-methyl-2,3-*bis*-hydroxymethyl-3-pyrroline. The N-ethyl carbamate diester (I) of this amino-alcohol is rather more toxic than the natural alkaloid monocrotaline (IV). In preliminary experiments, pyrrolic metabolites were demonstrated[5] in the livers, lungs and urine of male rats 2 h after intraperitoneal injection of this synthetic compound. Most animals given doses of more than 60 mg/kg body weight died 2–6 days later and had bloodstained ascites and massive haemorrhagic centrilobular liver necrosis. Histologically, this was characteristic of the type of damage caused by pyrrolizidine alkaloid poisoning[6]. Similar effects were seen after oral administration of the compound. Rats surviving for 3 weeks had the characteristic giant liver cells (megalocytes)[7] and these were abundant in livers of rats which had been given the compound intraperitoneally (30–40 mg/kg) at the age of 4 days. After very large doses (160–250 mg/kg) the animals usually died on the second or third day with their chests full of pale yellow fluid. It has been suggested[8,9] that lung damage due to pyrrolizidine alkaloids may be caused by some of the metabolite formed in the liver escaping to the lungs.

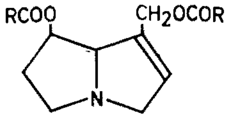
The analogous diacetyl and di-isovaleryl esters (II and III) were not toxic to rats at much larger doses than the carbamate, and were not metabolized to significant amounts of pyrrole derivatives. The choice of carbamate esters was made from previous experience with semisynthetic retronecine diesters. Hepatotoxicity has been demonstrated[10] in esters such as di-isovaleryl retronecine (V)[11] when doses larger than 250 mg/kg were administered to rats, but diacetyl retronecine (VI) was hepatotoxic only in much larger doses and after the animals had been treated with an esterase inhibitor[9]. It was thought that rapid hydrolysis by esterases to the highly water soluble amino-alcohol might be responsible for the low toxicities of semisynthetic esters and that carbamates might be more resistant to such hydrolysis, or even inhibitory to esterases[12]. The semisynthetic N-ethyl carbamate diester of retronecine (VII) was therefore prepared, and its toxicity to the rat was similar to that of monocrotaline (IV)[13]. The synthetic carbamate (I) subsequently proved to be even more toxic.



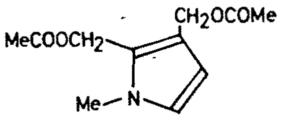
I R=EtNH
II R=Me
III R=Me_2CHCH_2



IV



V R=Me_2CHCH_2
VI R=Me
VII R=EtNH



VIII

Characteristic toxic effects in the lungs of rats have been found after intravenous injection of pyrrolic esters derived from natural pyrrolizidine alkaloids, such as monocrotaline pyrrole[14], and semisynthetic esters such as diacetyl retronecine pyrrole[9]. We have now obtained the same effects using the entirely synthetic compound, 1-methyl-2,3-diacetoxymethyl pyrrole (VIII). Injections of 5–12 mg/kg of this compound into the tail veins of rats of either sex often caused death after 20–35 days and produced changes in the lungs similar to those described earlier for monocrotaline pyrrole[14]. Some centrilobular liver necrosis was also seen. Larger doses caused massive pleural effusions and death within a few days.

The results described here are consistent with the view that chemically reactive pyrrolic metabolites are responsible for cytotoxic effects of pyrrolizidine alkaloids, and show for the first time that such effects can be caused by compounds with a single five membered ring. It follows that studies of the types of experimental liver and lung lesions described here need no longer depend on supplies of natural pyrrolizidine alkaloids. Chemically modified and radioactive labelled compounds will be more readily available for investigations of mechanisms of toxic action. Tumour-inhibitory activity has been observed with some of the natural alkaloids[15] and their pyrrolic derivatives[3], and the use of synthetic analogues might enable this property to be exploited more effectively. Further studies of the toxic and other effects of these and related synthetic compounds are in hand and will be reported in detail elsewhere.

I thank Drs J. M. Barnes and W. H. Butler for help with the pathology and R. Jones for technical assistance.

A. R. MATTOCKS

Toxicology Unit,
MRC Laboratories,
Woodmansterne Road,
Carshalton, Surrey

Received March 11; revised April 30, 1971.

[1] Schoental, R., *Israel. J. Med. Sci.*, **4**, 1133 (1968).
[2] Mattocks, A. R., *Nature*, **217**, 723 (1968).
[3] Culvenor, C. C. J., Downing, D. T., Edgar, J. A., and Jago, M. V., *Ann. NY Acad. Sci.*, **163**, 837 (1969).
[4] Mattocks, A. R., and White, I. N. H., *Nature New Biology*, **231**, 114 (1971).
[5] Mattocks, A. R., and White, I. N. H., *Anal. Biochem.*, **38**, 529 (1970).
[6] Davidson, J., *J. Path. Bact.*, **40**, 285 (1935).
[7] Schoental, R., and Magee, P. N., *J. Path. Bact.*, **74**, 305 (1957).

[8] Barnes, J. M., Magee, P. N., and Schoental, R., *J. Path. Bact.*, **88**, 521 (1964).
[9] Mattocks, A. R., *Nature*, **228**, 174 (1970).
[10] Schoental, R., and Mattocks, A. R., *Nature*, **185**, 842 (1960).
[11] Mattocks, A. R., *J. Chem. Soc.* (C), 2698 (1969).
[12] Baron, R. L., Casterline, J. L., and Orzel, R., *Toxicol. Appl. Pharmacol.*, **9**, 6 (1966).
[13] Schoental, R., and Magee, P. N., *J. Path. Bact.*, **78**, 471 (1959).
[14] Butler, W. H., Mattocks, A. R., and Barnes, J. M., *J. Path.*, **100**, 169 (1970).
[15] McLean, E. K., *Pharmacol. Rev.*, **22**, 429 (1970).

Tolerance to Opioid Narcotics: Time Course and Reversibility of Physical Dependence in Mice

REPEATED administration of an opiate narcotic leads to the tolerant-dependent state. Previous communications[1,2] from this laboratory have described the relationship between the degree of tolerance in mice and the dose, interval and duration of exposure to an opiate. Recently, jumping behaviour elicited by the opiate antagonist, naloxone, was shown to be a sensitive indicator of the degree of dependence[3]. We have used this method to follow the time courses of the onset and reversal of dependence in various conditions of exposure to opiates.

Male Swiss-Webster mice weighing 25–30 g were used. Water and Purina laboratory food were freely available. Levorphanol tartrate, a synthetic congener of morphine, was supplied by Hoffmann-LaRoche Inc. Naloxone hydrochloride was a gift of Endo Laboratories and morphine alkaloid was purchased from Mallinckrodt Chemicals Inc. Doses are expressed in terms of the free bases. All injections were intraperitoneal.

Physical dependence on morphine was produced by implanting a specially formulated 75 mg morphine pellet[4], without anaesthesia, in the subcutaneous tissue of the back. Animals so implanted display analgesia and vigorous running activity for less than 24 h. Release of morphine from the pellet proceeds linearly for longer than the time of our experiments[5].

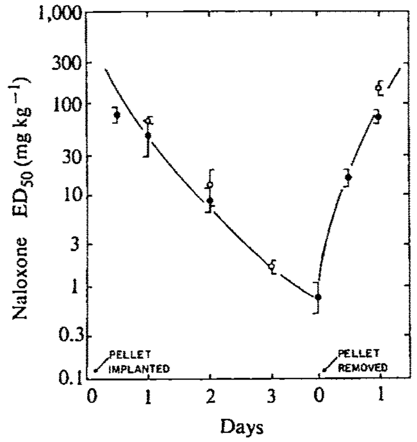


Fig. 1 Development and reversibility of physical dependence. The ED_{50} for naloxone-induced jumping is plotted on a logarithmic scale against time. Mice were implanted with 75 mg morphine pellets. Groups were tested with naloxone at various times, then discarded; pellets remained in place during testing. After 3.7 days pellets were removed from all remaining animals in order to follow the recovery process and 2 days later groups of mice were re-implanted on the opposite side with fresh pellets, which were also removed after 3.7 days. Solid circles represent the first cycle of dependence and recovery, open circles represent the second cycle. Standard errors of the ED_{50} estimates are shown by brackets above and below each data point. In these experiments 325 mice were implanted initially; each point is based on twenty-four to thirty-three animals.

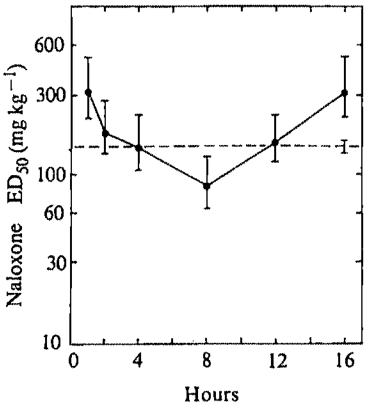


Fig. 2 Initiation of physical dependence by a single dose of levorphanol. At time zero, mice were given a single intraperitoneal 20 mg kg^{-1} levorphanol injection. Groups were tested with several doses of naloxone at various times thereafter to determine the ED_{50} for precipitated jumping. The dashed line indicates the ED_{50} for naloxone-induced convulsions in control mice. Each ED_{50} above the dashed line is an extrapolation from the lower dose segment of a quantal log dose-response curve obtained at the given time. Standard errors of the ED_{50} estimates are shown by brackets above and below each data point. Each point is based on forty-five animals.

Naloxone was used to precipitate withdrawal jumping, as described by Maggiolo and Huidobro[5] and Way *et al.*[3]. The ED_{50} (median effective dose) of naloxone was determined according to Litchfield and Wilcoxon[6] or by the more economical "up and down" method of Dixon[7], in which five mice were injected and placed on the platform together. If a mouse jumped off within 10 min, the dose for its replacement was halved; otherwise, the dose for its replacement was doubled. Thus, five replicate series were obtained. Groups of mice were tested at different times, but each group was tested once only, then discarded.

In untreated mice, no dose of naloxone would induce jumping, but in opiate-dependent mice, doses of about 150 mg kg^{-1} or less were effective: the smaller the degree of dependence, the higher the necessary dose of naloxone. The method was limited by the fact that naloxone itself, in dependent as well as untreated mice, causes convulsions at high doses (ED_{50} = 150 ± 13 mg kg^{-1} in untreated mice) so that a very small degree of dependence could not be measured.

After implantation of a morphine pellet, the sensitivity to naloxone became progressively greater for more than 3 days (Fig. 1). The time course of the approach to a steady state is evidently measured in days, although in these experiments a constant level of dependence was not quite reached. The process was completely reversible after removal of the pellet, and the return to normal was complete in 24 h. A group of the same animals was re-implanted and the entire cycle of onset and disappearance of dependence observed. The high degree of dependence produced in the first cycle clearly had no modifying effect on the second cycle, which was, within the limits of experimental error, identical to the first. The disappearance of dependence so much faster than it develops has interesting theoretical implications[8–10].

A single moderate dose of levorphanol (20 mg kg^{-1}) produced a definite but small degree of dependence (Fig. 2). (The ED_{50} of levorphanol for running activity and analgesia is 2.3 mg kg^{-1}; see ref. 1.) The process is evidently initiated immediately, reaches its peak after about 8 h, then wanes and disappears after 16 h. Naloxone can elicit jumping 16 h after a single injection although levorphanol has a very short half-life—less than 1 h—in plasma and brain of the mouse (unpublished observations of A. G.). As might be expected, injection of the same dose of levorphanol every 12 h produced only a small degree of dependence at the steady state, whereas injection every 4 h produced an increasing degree of dependence (Fig. 3).

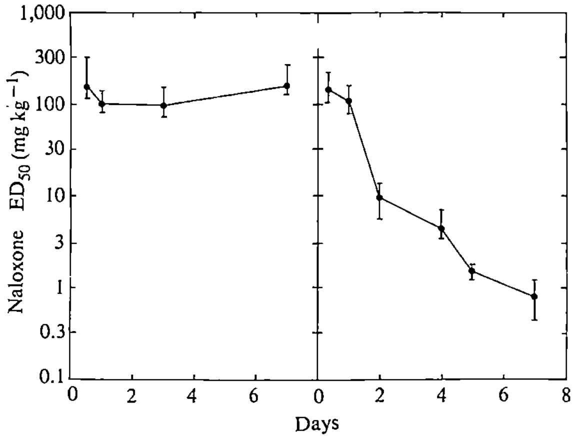


Fig. 3 Development of physical dependence on 12 h (left) and 4 h (right) schedules of levorphanol injections (20 mg kg^{-1}). Groups were tested with several concentrations of naloxone 12 h or 4 h, respectively, after the previous injection of levorphanol to determine the ED_{50} values for naloxone-induced jumping. Symbols are as in Fig. 2.

We have shown here that in the mouse, a single dose of opiate produces a certain quantum of physical dependence, which dissipates entirely in about 24 h. A small degree of dependence can also be produced by a single dose of opiate in the spinal dog[11]. In our experiments, frequent injections of levorphanol or implantation of a morphine pellet caused a cumulative build up of dependence. Injections at a long enough interval caused no build up of dependence, regardless of the duration of administration of the drug. These findings agree with our observations on tolerance[1,2]: the frequency of administration of a fixed dose controls the level of the steady state attained. Anecdotal evidence suggests a similar phenomenon in human narcotic addiction; tolerance and dependence probably do not develop at dose frequencies lower than about 0.5–1.0/day[12,13].

We have also shown that physical dependence in the mouse, like tolerance, is completely and rapidly reversible, and that one cycle of dependence and recovery does not modify the course of a subsequent cycle. On the other hand, Wikler and Pescor[14] demonstrated long lasting residual effects of an addiction cycle in rats. Goldberg and Schuster[15] showed, in monkeys, that naloxone could precipitate certain physiological disturbances months after morphine withdrawal, and in man Martin and co-workers[16,17] found that abnormalities similar to those of the withdrawal syndrome persisted for months after the principal withdrawal effects had terminated. We are not able to explain the differences between these results and our own.

This investigation was supported by a research grant from the National Institute of Mental Health and by a training grant from the National Institute of General Medical Sciences. We thank Dr R. D. Gibson for morphine pellets. (Some of the findings reported here were the subject of a preliminary communication[18].)

D. L. CHENEY
AVRAM GOLDSTEIN

Department of Pharmacology,
Stanford University School of Medicine,
Stanford, California

Received December 23, 1970; revised March 16, 1971.

[1] Goldstein, A., and Sheehan, P., *J. Pharmacol. Exp. Ther.*, **169**, 175 (1969).
[2] Richter, J. A., and Goldstein, A., *Proc. US Nat. Acad. Sci.*, **66**, 944 (1970).
[3] Way, E. L., Loh, H. H., and Shen, F., *J. Pharmacol. Exp. Ther.*, **167**, 1 (1969).
[4] Gibson, R. D., and Tingstad, J. E., *J. Pharmac. Sci.*, **59**, 426 (1970).
[5] Maggiolo, C., and Huidobro, F., *Acta Physiol. Latin Amer.*, **11**, 70 (1961).
[6] Litchfield, jun., J. T., and Wilcoxon, F., *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
[7] Dixon, W. J., *J. Amer. Stat. Assoc.*, **60**, 967 (1965).
[8] Goldstein, D. B., and Goldstein, A., *Biochem. Pharmacol.*, **8**, 48 (1961).
[9] Goldstein, A., and Goldstein, D. B., *Assoc. Res. Nerv. Mental Dis. Res. Pub.*, **46**, 265 (1968).
[10] Goldstein, A., Aronow, L., and Kalman, S. M., *Principles of Drug Action* (Harper and Row, New York, 1968).
[11] Wikler, A., and Carter, R. L., *J. Pharmacol. Exp. Ther.*, **109**, 92 (1953).
[12] Kolb, L., *Drug Addiction* (Thomas, Springfield, Illinois, 1962).
[13] Chein, I., Gerard, D. L., Lee, R. S., and Rosenfeld, E., *The Road to H* (Basic Books, New York, 1964).
[14] Wikler, A., and Pescor, F. T., *Psychopharmacologia*, **10**, 255 (1967).
[15] Goldberg, S. R., and Schuster, C. R., *Science*, **166**, 1548 (1969).
[16] Martin, W. R., Jasinski, D. R., Sapira, J. D., Flanary, H. G., Kelly, O. A.; Thompson, A. K., and Logan, C. R., *J. Pharmacol. Exp. Ther.*, **162**, 182 (1968).
[17] Martin, W. R., and Jasinski, D. R., *J. Psychiat. Res.*, **7**, 9 (1969).
[18] Cheney, D. L., Goldstein, A., and Sheehan, P., *Fed. Proc.*, **29**, 685 (1970).

Serological Demonstration of H-Y (Male) Antigen on Mouse Sperm

WE have recently overcome certain technical difficulties in applying the cytotoxicity test to mouse spermatozoa and have been able to show directly that H-2 antigens are expressed on these cells[1], thus confirming less direct evidence leading to the same conclusion[2]. We have since applied the cytotoxicity test to sperm with antisera directed against a number of other systems of mouse alloantigens, TL[3], θ[4], Ly-A[5] and Ly-B[5], but none of these has given a positive reaction with sperm. This is not surprising because these are all "differentiation antigens"[6] expressed primarily on lymphoid cells. Another antigenic system which distinguishes one mouse from another is H-Y. The H-Y antigen is carried by male cells only and is responsible for the rejection of male tissues by females of the same inbred strain[7].

In the case of H-Y, in contrast to the other antigenic systems referred to above, there has so far been no conclusive serological demonstration of the antigen *in vitro*. This obviously limits further analysis of the H-Y system and leaves much uncertainty as to the nature of the anti H-Y immune response in terms of humoral and cellular immune factors.

The following data establish three things: (*a*) H-Y antigen can be detected serologically by the cytotoxicity test *in vitro*.

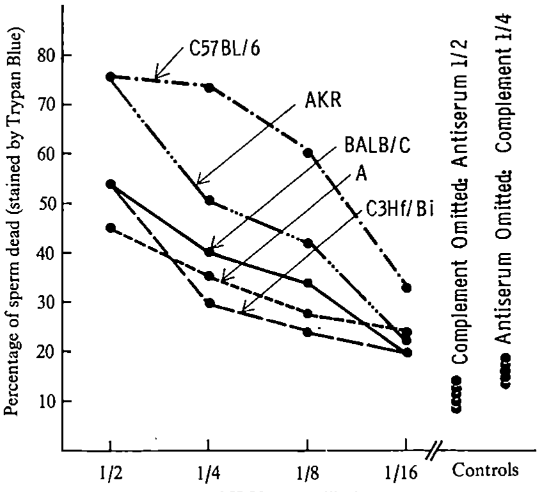


Fig. 1 Detection of H-Y antigen on epididymal sperm of A, C57BL/6, C3Hf/Bi, BALB/c and AKR mice by the cytotoxicity test with serum of C57BL/6 female mice which had rejected C57BL/6 male skin grafts.

(*b*) H-Y antigen is present on sperm as suggested by indirect evidence *in vivo*[8]. (*c*) Very probably, at least some *X*-bearing sperm as well as *Y*-bearing sperm carry H-Y antigen, indicating that at least some of the surface membrane of sperm is conserved from antecedent cells of an earlier stage in differentiation when both *X* and *Y* chromosomes were present in each cell.

Serum for the cytotoxicity test (referred to as anti H-Y serum) was obtained from C57BL/6 female mice 5–14 days after they had rejected a fourth or fifth skin graft from C57BL/6 males. Complement for the cytotoxicity test was provided in the form of serum from individual rabbits that had been selected for both low toxicity to mouse thymocytes and a high level of complement[9]. Before use, this selected rabbit serum was absorbed twice with mouse cells (eight parts to one by volume), including cells from the testis, in the absence of free calcium, to remove natural hetero-antibody. The object of excluding calcium is to prevent loss of complement during absorption[9].

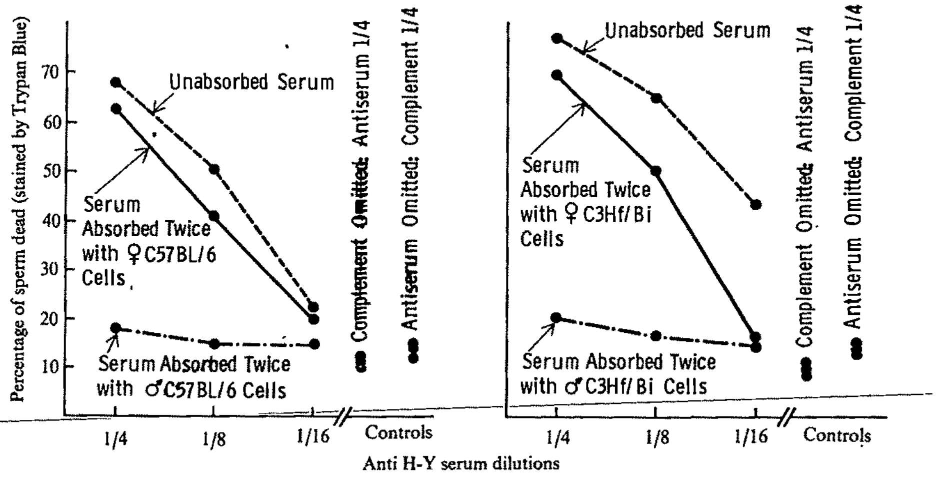


Fig. 2 Specificity of sperm lysis by anti H-Y serum(¼). Titration of cytotoxic anti H-Y serum absorbed twice with either male or female cells, and then tested on C57BL/6 sperm. First experiment (left): absorption with C57BL/6 cells; second experiment (right): absorption with C3Hf/Bi cells.

Mice of the inbred strains C57BL/6, A, AKR, BALB/c and C3Hf/Bi were obtained from colonies maintained by one of us (E. A. B.). Epididymal sperm were obtained by cutting the entire epididymis into slices in a few drops of phosphate-buffered saline (*p*H 7.0) containing 0.5% fructose and 5% foetal calf serum. After 5 min, the medium containing free sperm was pipetted off and the suspension kept at room temperature until use[1].

Table 1 Cytotoxicity Tests on Mouse Epididymal Sperm with anti H-Y Serum

Strain donating sperm	Experiment (i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)
		% sperm dead (stained by trypan blue)*						
C57BL/6	71	64	73	81	78	76	88	68
AKR					75			
A	37	57	45	62				
BALB/c	54	55						
C3Hf/Bi	54							

Summary of eight experiments: every test included a control in which complement was omitted and another with antiserum omitted; these showed consistently fewer than 18% sperm dead.

* In antiserum diluted by one half, at which dilution the greatest proportion of sperm were killed (see Fig. 1; undiluted antiserum was not tested).

Doubling dilutions of anti H-Y serum (half to one-sixteenth) were prepared for the cytotoxicity test. Equal volumes (0.05 ml.) of (*a*) diluted anti H-Y serum, (*b*) sperm suspension ($4–5 \times 10^6$/ml.) and (*c*) absorbed rabbit serum diluted one quarter, were incubated for 45 min at 37° C. Sperm counts were made in a haemacytometer after addition of 0.1 ml. of a freshly prepared solution of trypan blue (stock solution of 1% trypan blue in distilled water, diluted immediately before use with 42.5 g/l. of NaCl, one part in five, to render it isotonic).

The results summarized in Table 1 show that sperm from all strains tested were lysed by anti H-Y serum. Representative tests are illustrated more fully in Fig. 1. Sperm from strains A, C3Hf/Bi and BALB/c were less susceptible than C57BL/6 sperm indicating either (*a*) that there are strain differences in the amount of H-Y antigen on sperm, or (*b*) that these strains have different alleles at the H-Y locus[10] so that their H-Y antigens cross-react but are not identical. Known strain differences in rate and incidence of male skin rejection by females may be accounted for on these lines, and this is now amenable to serological investigation.

To confirm that the cytotoxic reaction was specific for H-Y antigen, aliquots of anti H-Y serum (diluted one-quarter) were absorbed twice with equal numbers of either male or female C57BL/6 cells from spleen, lymph node, and thymus (four volumes serum to one volume packed cells for 30 min in an ice bath), and the absorbed sera were then tested on C57BL/6 epididymal sperm. There was only minimal loss of cytotoxic activity after double absorption with female cells, no more than is to be expected from non-specific causes, and essentially complete loss of activity after double absorption with male cells: see Fig. 2, which includes a similar experiment in which C3Hf/Bi cells were used for absorption.

After due allowance for background levels of non-specific death of sperm during the cytotoxicity test, it is clear that the percentage of sperm specifically lysed by anti H-Y serum often exceeds 50. It is probable therefore that as in the case of H-2 (ref. 1) the surface phenotype of at least some sperm reflects the diploid complement of their precursor cells, rather than their own haploid constitution. It must be emphasized that there is no justification for assuming that cells surviving a cytotoxicity test, particularly in a relatively weak system like H-Y, necessarily lack the antigen being tested for; the probability of lysis for each cell of a uniform population probably depends on many randomly variable factors. It would therefore be rash to conclude that the surviving sperm are H-Y$^-$.

Anti H-Y sera were not positive in cytotoxicity tests with male thymocytes, lymph node cells or spleen cells. Nevertheless the presence of some H-Y antigen on these cells, or of some H-Y$^+$ cells in these populations, is implied by their capacity to absorb H-Y antibody (Fig. 2) and by tests *in vivo*[11–13]. In further absorption experiments, we have found that each of these cell populations individually is H-Y$^+$; and we shall report elsewhere that the cytotoxicity test is positive with enzymatically dissociated male (but not female) epidermal cells from skin.

E. H. G. is a National Institutes of Health trainee. E. A. B. and E. A. C. were supported in part by a National Cancer Institute grant. D. B. is a career investigator of the Health Research Council of New York and was supported by a National Institutes of Health grant. M. S. was the recipient of a research fellowship from the Stiftung Volkswagenwerk.

Ellen H. Goldberg
Edward A. Boyse
Dorothea Bennett
Margrit Scheid
Elizabeth A. Carswell

Department of Anatomy,
Cornell University Medical College, and
Division of Immunology,
Sloan-Kettering Institute,
New York 10021

Received February 4, 1971.

[1] Goldberg, E. H., Aoki, T., Boyse, E. A., and Bennett, D., *Nature*, **228**, 570 (1970).
[2] Vojtiskova, M., Polackova, M., and Pokorna, A., *Folia Biol.*, **15**, 322 (1969).
[3] Boyse, E. A., Stockert, E., and Old, L. J., *Intern. Convoc. on Immunol.* (Karger-Basel, New York, 1968).
[4] Reif, A. E., and Allen, J. M. V., *J. Exp. Med.*, **120**, 413 (1964).
[5] Boyse, E. A., Miyazawa, M., Aoki, T., and Old, L. J., *Proc. Roy. Soc.*, B, **170**, 175 (1968).
[6] Boyse, E. A., and Old, L. J., *Ann. Rev. Genet.*, **3**, 269 (1969).
[7] Eichwald, E. J., and Silmser, C. R., *Transplant. Bull.*, **2**, 148 (1955).
[8] Katsh, G. F., Talmage, D. W., and Katsh, S., *Science*, **143**, 41 (1964).
[9] Boyse, E. A., Hubbard, L., Stockert, E., and Lamm, M. E., *Transplantation*, **10**, 446 (1970).
[10] Hildemann, W. H., and Cooper, E. L., *Transplantation*, **5**, 707 (1967).
[11] Feldman, M., *Transplant. Bull.*, **5**, 15 (1958).
[12] Hirsch, B. B., *Transplant. Bull.*, **4**, 58 (1957).
[13] Billingham, R. E., Silvers, W. K., and Wilson, D. B., *Proc. Roy. Soc.*, B, **163**, 61 (1965).

Effects of Steroid Pharmaceuticals on Plasma Zinc

ORAL contraceptives have been found to cause large changes in the levels of copper and iron in the blood[1]. Their effects on the level of plasma zinc, however, are uncertain, for although in one case it was reported that the level increased[2], in another significant decreases were found[3]. Apart from testosterone propionate, which was found to increase the level of plasma zinc[4], other widely used steroid pharmaceuticals have not been investigated. We therefore undertook to determine the effects of such substances, including oral contraceptives, on the plasma zinc of healthy human subjects.

All volunteers were free of any obvious disease and were of families with no histories of conditions likely to influence the study. None had taken steroids before. Fasting blood samples were obtained by venepuncture on several occasions before the steroids were taken. No pharmaceuticals, other than the substance under test, were taken during the trial period. For the women in the study, the day of the menstrual cycle was recorded at the collection of each sample.

The steroids and the daily dose levels used were ethinylestradiol (0.05 mg), norethisterone acetate (1.0 and 0.5 mg), ethinylestradiol plus norethisterone acetate ('Minovlar' 0.05 mg + 1.0 mg), *d*(–)-norgestrel (1.0 mg and 0.25 mg), *d*(–)-norgestrel plus ethinylestradiol (Eugynon, 0.25 mg + 0.05 mg), mesterolone (Proviron, 100 mg), and prednisolone (5 mg and 10 mg). The steroids were taken once a day at the same time each day, for 30 consecutive days, except for the oral contraceptives which were taken for 21 days, and for prednisolone which was taken for only 10 days. Twenty-five subjects were investigated, twenty women and five men, ranging in age from 18 to 38 yr. Some received two courses of treatment separated by a suitable interval. Zinc was assayed in plasma by atomic absorption spectrophotometry, using methods similar to those of Davies *et al.*[5].

Table 1 Effects of Steroids on Plasma Zinc

Steroid	Daily oral dose (mg)	Sex	Plasma zinc (μg/100 ml., mean ±s.d.)	*P*
None	None	F	99±14	—
None	None	M	98±15	—
Ethinylestradiol	0.05	F	83±16	<0.01
Norethisterone acetate	1.0	F	99±15	N.S.
Norethisterone acetate	5.0	F	89±15	<0.02
Ethinylestradiol plus norethisterone acetate	0.05+ 1.0	F	82±15	<0.01
d(–)-Norgestrel	1.0	F	98±14	N.S.
d(–)-Norgestrel	0.25	F	100±15	N.S.
Ethinylestradiol plus *d*(–)-norgestrel	0.05+ 0.25	F	84±12	<0.01
Mesterolone	100	M	100±17	N.S.
Prednisolone	5	M	98±16	N.S.
Prednisolone	10	M+F	101±15	N.S.

The results are given in Table 1. The significance of any changes was assessed by a *t* test of each set of results against the control values. There was no difference in plasma zinc levels between untreated men and women. The stage of the menstrual cycle had no influence on the levels. There was no significant difference between concentrations in blood collected during the follicular or luteal phases, or at the time of menstruation. This finding strongly suggests that changes in endogenous steroid hormones are without effect on plasma zinc. Of the various steroid pharmaceuticals, only oestrogenic preparations had any significant action. Ethinylestradiol, 0.05 mg, given alone or in combination with a progestogen, produced a marked decrease in plasma zinc. This is in contrast to the effect of this steroid on blood iron and copper, both of which are increased[1].

The largest dose of norethisterone acetate (5 mg) also produced a small decrease in plasma zinc; this could be a consequence of the action of oestrogenic metabolites of this progestogen formed *in vitro*. Ethinylestradiol has been identified as a metabolite in the human[6]. That the effect is not due to progestogenic influences is shown by the lack of effect of the other progestogen, norgestrel. The latter is more than five times as progestogenic as norethisterone acetate, but lacks any significant oestrogenic metabolites[7].

Neither prednisolone nor the oral androgen mesterolone had any effect in contrast to the reported action of testosterone propionate on plasma zinc in rats[4]. To determine whether the action of ethinylestradiol on plasma zinc is reversible, we studied women who had ceased to take the steroid. Normal values were restored after 15 to 20 days. We have also studied levels of plasma zinc in a group of women which had taken oral contraceptives continuously for 5–8 yr. They also showed significantly depressed plasma zinc levels, indicating that the body cannot adopt to exogenous oestrogens and restore the level to normal.

The clinical significance of chronically low plasma zinc is unknown, but zinc has been implicated in a number of liver diseases and in diabetes mellitus. Both these have been suspected as long term side effects of oral contraceptives.

M. H. Briggs
Maxine Briggs

University of Zambia,
Lusaka

J. Austin

College of Technology,
Belfast

Received March 10; revised May 4, 1971.

[1] Briggs, M. H., *et al.*, *Adv. Steroid Biochem. Pharmacol.*, **2**, 111 (1970).
[2] O'Leary, J. A., and Spellacy, W., *Amer. J. Obstet. Gynecol.*, **103**, 131 (1969).
[3] Halstead, J. A., and Smith, J. C., *Lancet*, i, 322 (1970).
[4] Leonov, V. A., and Dubina, T. L., in *Mikroelm. Biofere* (edit. by Makeev, O. V.), **2**, 562 (Moscow, 1967).
[5] Davies, I. J. T., Musa, M., and Dormandy, T. L., *J. Clin. Pathol.*, **21**, 359 (1968).
[6] Breuer, H., *Intern. J. Fertil.*, **9**, 181 (1964).
[7] Edgren, R. A., *Steroids*, **2**, 319 (1963).

Meiotic Studies of the Ejaculated Seminal Fluid of Humans with Normal Sperm Count and Oligospermia

So far, all known studies of human meiosis have been performed on germinal cells obtained from testicular biopsies[1,2]. As this involves a surgical procedure to obtain the material, we wish to draw attention to the statement, first made by Michael and Joel[3], that immature germ cells can regularly be seen in the seminal fluid of the human male. We have found that in smear preparations of the ejaculate of subjects with a normal sperm count, nearly 3% of the cells consist of spermatogonia, spermatocytes and spermatids (Table 1), the maximum percentage being 5%. A similar result was obtained by Heinke and Doepfmer[4]. As the sperm count decreases, however, the percentage of immature cells generally increases and, in some pathogenic cases, the percentage may be 40% or more[5]. We have characterized the cells in material in which a high percentage of the cells were immature as a preliminary to cytogenetic studies of human male meiosis.

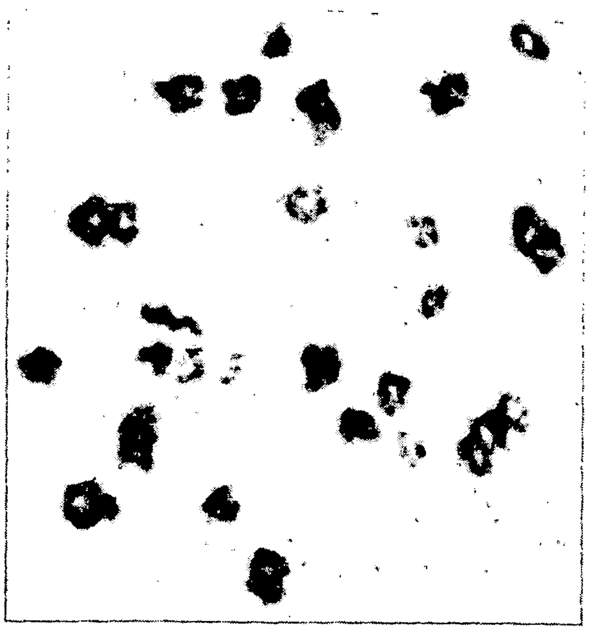


Fig. 1 Primary spermatocyte at diakinesis, from the ejaculated seminal fluid of a human male with oligospermia (× 1,800).

The cells were stained for viability by the trypan-blue method[6]. In both normal and oligospermia seminal fluid, 75–85% of the cells—the viable cells—remained unstained. Furthermore, autoradiographs of spermatogonia and preleptotene spermatocytes indicated that some could incorporate tritiated thymidine. Preparations of the meiotic chromosomes in diakinesis and metaphase-I were made after hypotonic treatment by the air-dry method of Evans *et al.*[7] (Fig. 1). The best results with cells at the pachytene stage were achieved by the squash method without any pretreatment with the fixative proposed by Annerén *et al.*[8]. The relative frequency of these different stages varies in each case but leptotene and pachytene cells are predominant, possibly because of the long duration of the meiotic prophase[9]. Nevertheless, cells in diakinesis occur in oligospermia preparations and also in the ejaculate of normal males. These preliminary results suggest that the seminal fluid is a useful source of immature germ cells for cytogenetic studies of human male meiosis.

KARL SPERLING
RUDOLF KADEN

Institute of Genetics, and Department of Dermatology,
Division of Andrology, Free University, Berlin

Received August 11, 1970.

[1] Kjessler, B., in *Modern Trends in Human Genetics* (edit. by Emery, A. E. H.) (Butterworth, London, 1970).
[2] Ohno, S., in *Methoden in der Medizinischen Cytogenetik* (edit. by Schwarzacher, H. G., and Wolf, U.) (Springer, Berlin, 1970).
[3] Michael, M., and Joel, K., *Schweiz. med. Wschr.*, **33**, 757 (1937).
[4] Heinke, E., and Doepfmer, R., in *Handbuch der Haut- und Geschlechtskrankheiten VI/3* (edit. by Schuermann, H., and Doepfmer, R.) (Springer, Berlin, 1960).
[5] Vasterling, H. W., in *Praktische Spermatologie* (Thieme, Stuttgart, 1960).
[6] Pertoft, H., Bäck, O., and Lindahl-Kiessling, K., *Exp. Cell Res.*, **50**, 355 (1968).
[7] Evans, E. P., Breckon, G., and Ford, C. E., *Cytogenetics*, **3**, 124 (1964).
[8] Annerén, G., Berggren, A., Stahl, Y., and Kjessler, B., *Hereditas*, **64**, 211 (1970).
[9] Heller, C. G., and Clearmont, Y., *Science*, **140** (1963).

Table 1 Mean Percentage of Immature Germ Cells in the Ejaculated Seminal Fluid of Human Males with Different Sperm Qualities

Sperm quality	No. of sperm (million/ml.)	No. of subjects investigated	Spermatogonia (%)	Spermatocytes (%)	Spermatids (%)	Total (%)
Normospermia	≥ 40	139	0.5	0.3	2.0	2.8
Oligospermia I	$\geq 5 < 40$	93	2.0	1.4	4.4	7.8
Oligospermia II	$\geq 1 < 5$	24	7.0	3.4	11.0	21.4

Mandibular Gland Secretion of Larvae of the Flour Moth, *Anagasta kuehniella*, contains an Epideictic Pheromone and elicits Oviposition Movements in a Hymenopteran Parasite

Hassell and Huffaker[1] demonstrated some density-dependent regulation of numbers in populations of the flour moth, *Anagasta kuehniella* (Zeller). Fighting between newly hatched larvae[2] contributes to the regulation of numbers in this species but is not the only controlling mechanism: Smith[3] showed that crowding from the end of the first instar onwards led to delayed emergence of adults and a reduction in body weight. Since smaller moths lay few eggs[4], both these effects would contribute to the density-dependent regulation of numbers in succeeding generations. I describe here a mechanism which could bring about these effects. I suggest that last-instar larvae of *A. kuehniella*, when they meet, deposit on the substratum drops of secretion from their mandibular glands; and that the response of other last-instar larvae to this secretion results not only in the regulation of the total numbers of

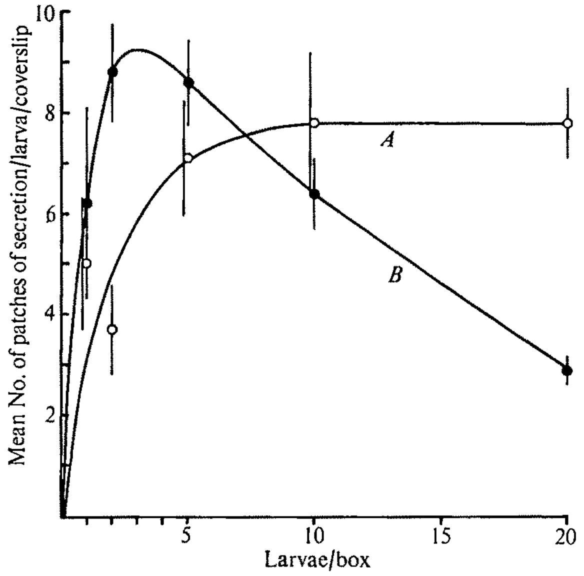


Fig. 1 Mean numbers of patches of mandibular gland secretion per larva on coverslips that were on the floors of boxes in which 1, 2, 5, 10 or 20 larvae were confined for 1 h (*A*) or for 24 h (*B*) (experiment 2). Each vertical line represents twice the standard error.

A. kuehniella in a finite system (as studied by Smith[3]), but also in the control of the dispersion of individuals within that system.

The mandibular glands of last-instar larvae of *A. kuehniella*, like those of *Galleria mellonella* (L.)[5], are tubular structures lying folded in the haemocoele. One opens at the base of each mandible. They contain a clear oily fluid which is deposited in droplets on the substratum beside the silken thread and can be stained red with Nile blue inside the gland or after deposition. Larvae that meet each other head to head show a characteristic response involving sudden withdrawal and the separation of the mandibles. My experiment 1 showed that this behaviour is sometimes (and perhaps always) accompanied by the release of mandibular gland secretion, which is sometimes deposited on the substratum. In experiment 1*A*, a larva was allowed to crawl over a 6 cm square surface marked with 1 cm squares, and at recorded locations the withdrawal response was elicited by touching it, head to head, with another larva held in forceps. After three, four or five such encounters the surface was treated with Nile blue and examined for red-staining material. In ten trials of this kind, involving forty-two encounters, all but one of the thirty-six patches of red-staining material were within 1 cm of meeting places or of the places where the larvae were put down at the beginning of each trial; larvae often made the withdrawal response when first put down. Another ten trials (experiment 1*B*), in which the response was elicited by prodding with forceps instead of by contact with another larva, gave similar results; the larvae were prodded thirty-three times, and all but two of the twenty-three patches of secretion were within 1 cm of a place where a larva had been prodded, or put down at the beginning of a trial. Evidently the withdrawal response, elicited by mechanical stimulation or by contact with another larva, often leads to the deposition on the substratum of secretion from the mandibular glands.

Fig. 1 shows the results of experiment 2 in which larvae were confined for 1 h (*A*) or 24 h (*B*), singly or in groups, in standard boxes of internal dimensions 56×38×13 mm. On the floor of each box was a coverslip (22×50 mm) which was subsequently treated with Nile blue so that patches of red-staining secretion on its surface could be counted. The number counted was assumed to be proportional to the number of patches on the inner surfaces of the box. Fig. 1*A* shows that individual larvae released more secretion when crowded than when alone, as would be expected if the relase of secretion is stimulated by meetings between larvae. Fig. 1*B* also shows this effect at low densities, although at high densities the number of patches of secretion appears smaller after 24 h than after 1 h, because crowded larvae, walking over the coverslips, picked up the secretion on their bodies. Dissections of larvae that had been crowded for 24 h showed that the percentage with one or both mandibular glands shrivelled and empty increased with the density of crowding from 8% (or 25) for solitary larvae to 48% (or 255) for larvae in groups of twenty per box.

Experiment 3 concerned the response of last-instar larvae of *A. kuehniella* to crowding (3*A*) and to mandibular gland secretion of other last-instar larvae (3*B* and *C*). The response was assessed in terms of the duration of the wandering period, during which fully fed larvae crawl about, away from their food, and which ends with the spinning of the cocoons in which the larvae will pupate. For each experiment a batch of larvae was selected from their stock cultures on the first day of their wandering stage. They were kept in standard transparent boxes at 25±0.5° C and for 10 (3*C*) or 12 (3*A* and 3*B*) days they were examined every morning so that the dates of cocoon-spinning and pupation could be recorded. Individuals (about 1–4%) that failed to spin up during the 10 or 12 day experiments were not included in the results. In each experiment the patterns of variation in the dates of cocoon-spinning and of pupation were alike, and only the dates of pupation are shown here.

In experiment 3*A* larvae were kept singly or "crowded" in groups of two, five, ten or twenty per box, and the results (Fig. 2*A*) show a pattern that is repeated in the results of experiments 3*B* and 3*C*: the larvae at an intermediate density pupated sooner than those that were either at very low density or very densely crowded. The results of experiment 3*B* (Fig. 2*B*) indicate that this effect of crowding was mediated

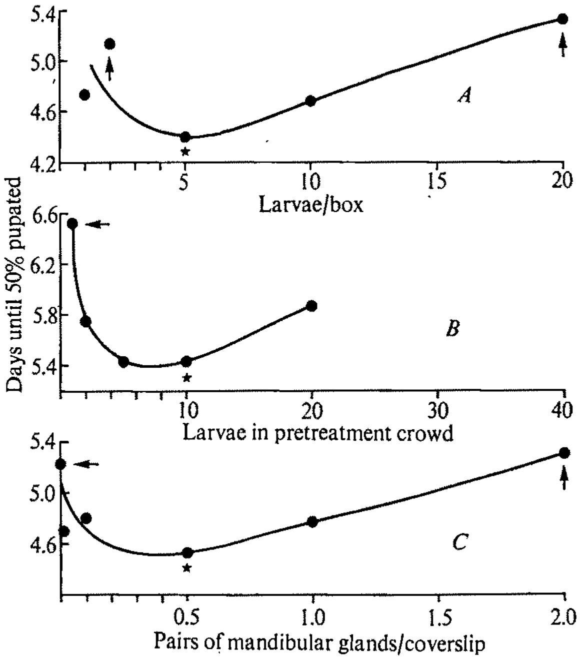


Fig. 2 Results of experiment 3. *A*, Effect of crowding on the timing of pupation. *B*, Effect of coverslips, previously exposed to crowds of larvae, on the timing of pupation of isolated larvae. *C*, Effect of coverslips, treated with ether extract of mandibular glands, on the timing of pupation of isolated larvae. The values on the ordinate are derived from cumulative plots, smoothed by a moving average of three, of the number of new pupae against time. The points marked with a star differ significantly from those marked with an arrow on the same graph ($P<0.0075$ in *A*; $P<0.05$ in *B*; $P<0.025$ in *C*; one-tailed Mann–Whitney U test).

by something deposited ~~on the~~ substratum by crowded larvae. Each larva in this experiment was kept alone in a standard box, on the floor of which was a 22×50 mm coverslip previously exposed for 1 h in a similar box containing one, two, five, ten or twenty larvae (the pre-treatment crowd). Evidently the size of the pre-treatment crowd influenced the timing of pupation of the isolated larvae subsequently exposed to the coverslips, in such a way that pre-treatment crowds of moderate size resulted in earlier pupation than did larger or smaller pre-treatment crowds. The coverslips that had been exposed to these crowds of larvae carried deposits of silk and frass as well as mandibular gland secretion. Experiment 3*C* provides evidence that it was the mandibular gland secretion that transmitted the crowding stimulus from the pre-treatment crowd to the isolated larvae.

In experiment 3*C* the larvae were again kept isolated in standard boxes. On the floor of each box was a 22×50 mm coverslip on which had been deposited a drop of ether in which had been ground mandibular glands dissected from last-instar larvae. On the coverslips of the controls drops of pure solvent were deposited. In this experiment (Fig. 2*C*) changes in the number of pairs of mandibular glands per coverslip affected the timing of pupation as changes in the degree of crowding did: the larvae pupated sooner in the presence of moderate quantities of mandibular glands than they did in the presence of larger or smaller quantities. In one of the three replicates of experiment 3*C*, each pupa was weighed on its first day after pupation, and the mean weights of the new pupae were calculated for each day. Fig. 3 shows that individuals that pupated later formed lighter pupae than did those pupating earlier.

Experiments 1 and 2 show that the amount of mandibular gland secretion deposited on the substratum is a measure of the density of crowding, and experiment 3 shows that in the presence of appropriate quantities of mandibular gland secretion, isolated larvae responded as though they were crowded. The response mediated by the secretion of the mandibular glands may help to regulate the density towards an "optimal" value, equivalent to about five larvae per box in the conditions of these experiments. Larvae at densities close to this value spin up and pupate relatively quickly, whereas those at higher or lower densities wander for a longer time, thus increasing their chances of reaching an area where the population density is "optimal" before spinning up.

The "overcrowding" effects, expressed by the right hand limbs of the curves in Fig. 2, will regulate the density of the population in three ways: by controlling the spatial distribution of individuals within their habitat, encouraging emigration from crowded situations; by lengthening the generation time of individuals in crowded populations; and by reducing the fecundity of the females crowded as larvae. Fecundity is reduced insofar as wandering larvae spin silk and lose weight as they go, so that those pupating later are, on average, lighter than those pupating earlier (Fig. 3); and lighter flour moths lay fewer eggs than do heavier flour moths[4]. In the presence of the ichneumonid parasite *Venturia canescens* (Grav.) another density-regulating effect comes into play. The mandibular gland secretion of *A. kuehniella* stimulates oviposition movements in the parasite (discussed later), and a larger proportion of the host caterpillars are parasitized in a crowded population than in an uncrowded one[6].

The secretion of the mandibular glands of *A. kuehniella* falls within the definition of a pheromone given by Karlson and Butenandt[7]. And since it functions in the regulation of population density, controlling the dispersion of individuals, the duration of larval development and the fecundity of the moths, it can be described as an "epideictic"[8] pheromone.

Venturia canescens, an ichneumonid parasite of *A. kuehniella*, responds to a component of the mandibular gland secretion of its host by making a series of ovipositional movements. When a female of *V. canescens* oviposits in a larva of *A. kuehniella* she taps her antennae against the host, unsheaths her ovipositor so that the tip points forwards and downwards, and then makes jabbing movements which, if they penetrate the host's cuticle, may result in the release of an egg. In the absence of a suitable host this series of actions is interrupted at some point before the release of the egg. In my experiments between thirty and fifty adult females of *V. canescens* were confined in a dish with a small rectangle of filter paper to which the materials under test had been applied as two spots placed symmetrically about 2.5 cm apart. For 10 min I recorded the number of "jabbing sessions" directed at each spot. A "jabbing session" involved unsheathing the ovipositor, making one or more jabbing movements, and returning the ovipositor to its resting position. For each of the ten trials in experiment 4*A*, five last-instar larvae of *A. kuehniella* were separated into (i) the heads with the mandibular glands and the silk glands attached, and (ii) the rest of the bodies. The two spots presented to the parasites consisted of breis made from these two portions of the larvae. Ninety-two per cent of the parasites' jabbing sessions were directed at the spots containing the heads, mandibular glands and silk glands (Table 1). In each of the ten trials in experiment 4*B* one crushed pair of mandibular glands was tested against the crushed pair of silk glands from the same last-instar larva. (The head, contaminated with the products of both glands, was discarded.) The mandibular glands elicited 95% of the jabbing sessions (Table 1).

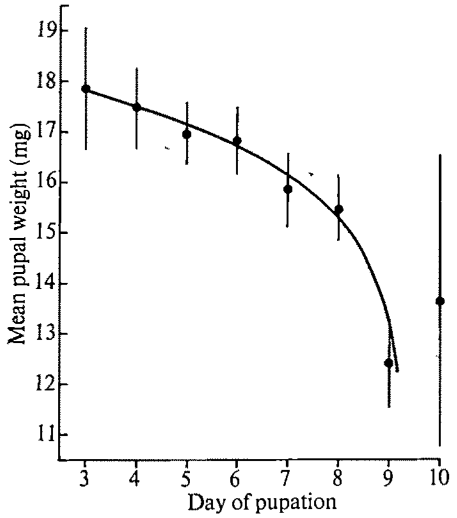


Fig. 3 Mean weights of pupae formed on successive days of experiment 3*C*. Each vertical line represents twice the standard error.

Table 1 Results of Experiment 4

	Material presented to parasites	No. of jabbing sessions
A	Head + glands of *A. kuehniella* larvae	784 (92%)
	Rest of bodies of *A. kuehniella* larvae	69 (8%)
B	Mandibular glands of *A. kuehniella* larva	530 (95%)
	Silk glands of *A. kuehniella* larva	27 (5%)

These two experiments indicate that the contents of the mandibular glands elicit jabbing by the parasites. If a coverslip is exposed to a crowd of host larvae, droplets of mandibular gland secretion are deposited on it (experiment 2). Experiment 4*C* shows that coverslips treated in this way elicit jabbing by the parasites, and that the number of jabbing sessions directed at a coverslip depends on the number of host larvae to which that coverslip was previously exposed. In each of the ten trials of experiment 4*C*, 22 mm square coverslips were put on the floor of four standard boxes containing, respectively, one, five, ten and twenty wandering last-instar larvae

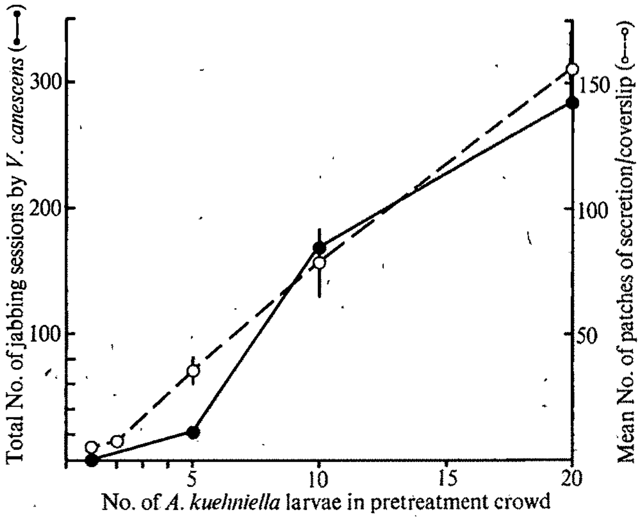


Fig. 4 ●, Effect of pre-treatment of 22 mm square coverslips by exposure for 1 h to larvae of *A. kuehniella* in eliciting jabbing by *V. canescens* (experiment 4*C*). ○, Mean numbers of patches of secretion deposited on 50×22 mm coverslips by larvae of *A. kuehniella* during one hour's pre-treatment (experiment 2). Vertical lines represent twice the standard error.

of *A. kuehniella*. After 1 h at 25° C the four coverslips were removed, arranged in a square and presented simultaneously to between thirty and fifty adult females of *V. canescens* trapped under an inverted glass dish. The number of jabbing sessions directed at each coverslip was recorded during 10 min. The summed results of the ten trials are shown in Fig. 4.

These experiments show that the parasite *V. canescens* responds quantitatively to the secretion of its hosts' mandibular glands, which carries an intraspecific message announcing crowding; the secretion contains material which is, from the parasites' point of view, a kairomone[9]. This case parallels the situation in which the predatory beetle *Thanasimus dubius* (F.) responds to a supposed aggregation pheromone of its prey, the bark beetle *Dendroctonus frontalis* Zimm.[10]. Similar responses of hymenopteran parasites to the mandibular gland secretions of their lepidopteran hosts may occur in other species; *Cardiochiles nigriceps* Viereck, a hymenopteran parasite of *Heliothis virescens* (F.), shows a searching response in the presence of the secretion of the larval mandibular glands of its hosts[11].

The finding of an epideictic pheromone in the secretion of the mandibular glands of *A. kuehniella* may be applicable in the control of lepidopteran pests, especially if the mandibular glands of other species prove to have a similar function[12]. Work is in progress on the chemical characterization of the components of the secretion, making use of the parasites' ovipositional response as a bioassay for the kairomone.

I thank Dr Philip S. Corbet, Professor J. Green, Dr P. A. Lawrence and Professor V. C. Wynne-Edwards for helpful comments.

SARAH A. CORBET

Westfield College (University of London),
London NW3

Received February 1; revised March 30, 1971.

[1] Hassell, M. P., and Huffaker, C. B., *Researches Popul. Ecol. Kyoto Univ.*, **11**, 186 (1969).
[2] Flanders, S. E., *Hilgardia*, **39**, 367 (1968).
[3] Smith, S. D., *J. Exp. Zool.*, **170**, 193 (1969).
[4] Ullyett, G. C., and Merwe, J. S. v.d., *J. Entomol. Soc. South Afric.*, **10**, 46 (1947).
[5] Wroniszewska, A., *J. Insect Physiol.*, **12**, 509 (1966).
[6] Hassell, M. P., *J. Anim. Ecol.*, **40**, 333 (1971).
[7] Karlson, P., and Butenandt, A., *Ann. Rev. Entomol.*, **4**, 39 (1959).
[8] Wynne-Edwards, V. C., *Animal Dispersion in Relation to Social Behaviour* (Oliver and Boyd, Edinburgh and London, 1962).
[9] Brown, jun., W. L., Eisner, T., and Whittaker, R. H., *Bioscience*, **20**, 21 (1970).
[10] Vité, J. P., and Williamson, D. L., *J. Insect Physiol.*, **16**, 233 (1970).
[11] Vinson, S. B., *Ann. Entomol. Soc. Amer.*, **61**, 8 (1968).
[12] *ITBON in 1965*, Annual Report of Institute for Biological Field Research, Arnhem, Netherlands.

Presumptive *X* Monosomy in Black Rats from Malaya

DURING a cytogenetic study of the Malayan species of *Rattus* Fischer, black rats, *Rattus rattus diardii* (Jentink), were trapped at Kuala Lumpur, Selangor, and Batu Pahat, Johore, between November 1968 and July 1970. Ninety animals were examined cytologically and seventeen gave chromosome counts that differed from the normal diploid number of 42 (ref. 1). Fourteen had between one and three additional small metacentric chromosomes which were not distinguished from normal members of the complement. Nevertheless, they are assumed to be equivalent to the "inert" supernumerary (or *B*) chromosomes of many invertebrate and plant species. Counts of 41 chromosomes were recorded repeatedly in preparations from four of the rats trapped at Kuala Lumpur. Karyotype analysis indicated that "Robertsonian fusion" between two acrocentric chromosomes had occurred in a male specimen[2]. In the remaining three specimens (all females) an acrocentric chromosome was absent. Observations were made on preparations from bone marrow.

Apparent monosomy was a surprising observation, and so these preparations were re-evaluated. From each animal twenty well spread metaphases were selected at low magnification and counted at high magnification by the sector method (which has the advantage that whatever errors may be made in interpretation the final count recorded is not influenced by prior knowledge of an "expected" number). The results confirmed previous counts. As a further precaution the preparations were sent to another laboratory, where the diploid number was verified to be 41 in all three animals.

Only the two longest and perhaps the shortest of the 11 pairs of acrocentric autosomes can be distinguished individually. The remainder, together with the *X* chromosome, which is also acrocentric, are difficult to separate by size[3]. It is therefore significant that the three animals were all females, and the most reasonable assumption is that all were *X* monosomics. Autopsy of one revealed normal ovaries, uterus and other female reproductive organs, but this is not an inconsistent observation since *XO* female mice are both viable and fertile[4].

The alternative interpretation of autosomal monosomy is highly unlikely. In man several examples of autosomal monosomy have been reported in cultures established from spontaneously aborted foetuses. They are, however, rare in comparison with the numbers of autosomal trisomies identified (theoretically, near equality in numbers is expected in the zygote population). In the one case of a live-born infant reported with non-mosaic monosomy-*G*[5], conservation of a normal or near normal genome by translocation to another site cannot be excluded on the evidence presented. In the mouse, new evidence indicates that autosomal monosomics may develop normally up to the blastocyst stage and even implant, but all die before the tenth day of gestation (personal communication from C. E. Ford).

In the marsupial genera *Isoodon*, *Perameles*, *Echymipera* and *Peroryctes*, loss of one *X* chromosome in females or of the *Y* chromosome in males is a normal event in many somatic tissues though not in the germ cells, which retain the zygotic *XX* and *XY* constitution respectively[6,7]. A single field mouse, *Akodon azarae*, had also been reported to possess an *XO* sex chromosome complement at least in the somatic tissues[8].

Since bone marrow was the only tissue studied in the three *R. rattus* monosomics the possibility has to be borne in mind that the loss of the one chromosome may have been confined to bone marrow and perhaps other somatic tissues. If it were so it would imply a labile system of regulation of sex chromosome loss, operative in a small proportion of animals.

Whatever the explanation, *X* monosomy, an *Isoodon/Perameles*-like system or even autosomal monosomy, the observation is unexpected, leaving many questions to be answered.

I thank Dr C. E. Ford for his interest, and for verifying my chromosome counts.

YONG HOI-SEN

Division of Genetics,
School of Biological Sciences,
University of Malaya,
Kuala Lumpur

Received May 18, 1971.

[1] Yong, H. S., *Third Oxford Chromosome Conference* (1970).
[2] Yong, H. S., *Experientia*, 27, 467 (1971).
[3] Yong, H. S., *Chromosoma*, 27, 245 (1969).
[4] Cattanach, B. M., *Genet. Res. Camb.*, 3, 487 (1962).
[5] Al-Aish, M. S., de la Cruz, F., Goldsmith, L. A., Volpe, J., Mella, G., and Robinson, J. C., *New Engl. J. Med.*, 277, 777 (1967).
[6] Hayman, D. L., and Martin, P. G., *Genetics*, 52, 1201 (1965).
[7] Hayman, D. L., Martin, P. G., and Waller, P. F., *Chromosoma*, 27, 371 (1969).
[8] Bianchi, N. O., and Contreras, J. R., *Cytogenetics*, 6, 306 (1967).

Frequency of Blood Feeding in the Mosquito *Aedes aegypti*

THE probability that a female mosquito transmits a pathogen from one host to another depends, in part, on the frequency of blood feeding. *Aedes aegypti* (L.) is a vector of several arboviruses, whose feeding frequency has been measured in the laboratory[1–4]. We now present a direct field estimate of this parameter.

Subject to several assumptions[5], the chief population parameters can be estimated by marking, releasing and recapturing individual animals of a population in its natural habitat. It is essential that marked and unmarked individuals have equal probabilities of being captured[6]. An adult female mosquito, however, repeatedly passes through a sequence of behavioural phases such as feeding, resting and oviposition, and no sampling method is known that would confer an equal probability of capture on individuals in these different phases. These disadvantages were largely avoided in our study which also enabled the frequency of blood feeding to be estimated.

The catching area was in the centre of a tree-shaded scrap heap of old motor cars in a suburb of Dar es Salaam (6° 49′ S, 39° 15′ E), on the coast of Tanzania. Large piles of rainwater-filled tyres provided a habitat for a large breeding population of *A. aegypti*. Female mosquitoes which showed attempted biting behaviour were caught by a team of four men during a 5 h period from dawn onwards, on twenty-three consecutive days from June 4, 1970. During the first 11 days (except day 1) captured *A. aegypti* females were checked for marks and given an opportunity to engorge on the experimenters. Fully engorged females, and others bearing a mark, were then lightly etherized and each painted with a single date code mark on the thorax; the technique causes no apparent harm[7,8]. After marking, the females were released at the capture site and most dispersed in the manner typical after feeding. Females not so released were killed. After the eleventh day, captured mosquitoes were merely preserved for checking. All marks were made and interpreted by one of us (G. A. H. McC.).

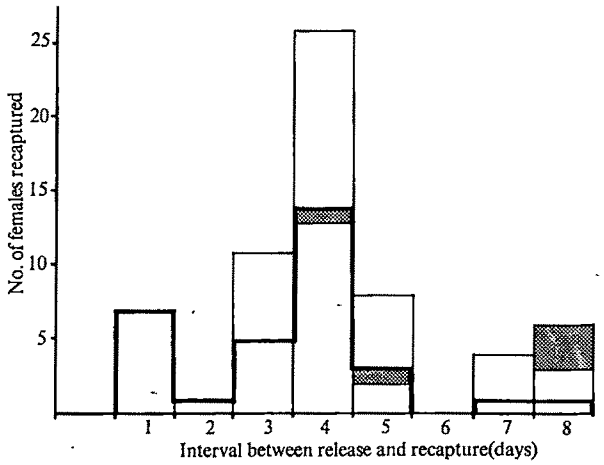


Fig. 1 The results of the mark-release and recapture of females of *Aedes aegypti* during 23 days in Buguruni, Tanzania, expressed as a frequency histogram of the intervals between release and recapture. The area below the heavy line represents females that were released after engorgement and which engorged within 6 h after recapture. The hatched portions of the columns refer to intervals between first release and second recapture for females recaptured twice.

The gross loss on capture of the 579 females caught during the first 11 days was 19.2% and included three which bore a mark from a previous day. Only 1.3% of the 474 females which engorged could not be released after handling. Of 418 singly marked females released, fifty-three were recaptured; fifty of these were released with two marks and, of these, five were recaptured. Because the difference between these recapture rates is insignificant (χ^2 (1)=0.30) data from singly and doubly marked females can be combined. The total of fifty-eight recaptures is 12.4% of the 468 females released. In an open environment this recapture rate is unexpectedly high. In a similar, though longer term, experiment in Thailand on a population of *A. aegypti*, sampled indoors, the recapture rate was only about 4% (ref. 8).

Fig. 1 indicates the frequency distribution of the numbers of females recaptured at various intervals since the date of release. For the five individuals recaptured twice, the intervals between each release and recapture as well as the overall interval are recorded separately. The fifty-eight recaptures therefore provide data for sixty-three release–recapture intervals. After deducting females dying or refusing blood and those caught after day 11, there remain thirty-three unequivocal intervals between engorgements (depicted by the heavy line in Fig. 1). It must be emphasized, however, that some females probably took additional blood meals between the known engorgements at release and recapture.

The striking 4 day modality is clearly an indication of the gonotrophic cycle at the mean air temperature of about 24° C which prevailed during the study. The average intervals between feeding recorded in the laboratory have varied from 3 to 5 days[1–4].

At least half the females in laboratory populations have been reported to feed on the second as well as on the first day of the gonotrophic cycle[2,3]. In the field, several workers[8–10] have discovered that a considerable proportion of biting females were already blood-fed, gravid or partially gravid with sufficient blood to complete ovarian development. It has been suggested[9] that double feeding, in the first gonotrophic cycle, is a response to inadequate larval nutrition. The data reported here, however, not only confirm double feeding in seven individuals recaptured 24 h after release, but, because one of these was recaptured 4 days after initial release and again on day 5, they also imply double feeding in a subsequent gonotrophic cycle.

In Fig. 1 the recaptures at 3, 5 and 7 days can be regarded as deviations from a 4 day mean, with the first day recaptures indicating double feeds in the first gonotrophic cycle only. The results could equally be interpreted, however, as representing an invariate 4 day cycle with a second feed after 1 day in each cycle. With this interpretation the intervals of 3 and 7 days are derived from individuals initially captured as "second feeders" and recaptured taking the first feed of a subsequent cycle. The 5 day intervals are derived from females caught as "first feeders" and recaptured as "second feeders" in the next cycle. The occurrence of a fairly high frequency of double feeding in *A. aegypti* implies that it can be an effective vector of viruses both at lower densities and, if the interpretation of double feeding in later gonotrophic cycles is correct, at lower survival rates than have hitherto been realized.

This study was supported jointly by a US Public Health Service research grant from the Center for Disease Control, Atlanta, Georgia, and the World Health Organization. We thank Dr W. K. Hartberg for his assistance and Mr J. W. Wright and Dr A. W. A. Brown for their interest and support.

G. A. H. McCLELLAND

Department of Entomology,
University of California,
Davis, California 95616

G. R. CONWAY

Environmental Resource Management Research Unit,
Imperial College of Science and Technology,
Silwood Park, Ascot, Berkshire

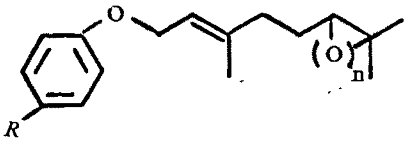
Received March 8; revised April 14, 1971.

[1] Macfie, J. W. S., *Bull. Ent. Res.*, **6**, 205 (1915).
[2] Lavoipierre, M. M. J., *Nature*, **181**, 1781 (1958).
[3] Judson, C. L., *Biol. Bull.*, **133**, 369 (1967).
[4] Gillett, J. D., *J. Insect. Phys.*, **8**, 665 (1962).
[5] Southwood, T. R. E., *Ecological Methods* (Methuen, London, 1966).
[6] Jolly, G. M., *Biometrika*, **52**, 225 (1965).
[7] Macdonald, W. W., Sebastian, A., and Maung-maung-Tun, *Ann. Trop. Med. Parasit.*, **62**, 200 (1968).
[8] Sheppard, P. M., Macdonald, W. W., Tonn, R. J., and Grab, B., *J. Anim. Ecol.*, **38**, 661 (1969).
[9] Macdonald, W. W., *Ann. Trop. Med. Parasitol.*, **50**, 399 (1956).
[10] Gould, D. J., Mount, G. A., Scanlon, J. E., Ford, H. R., and Sullivan, M. F., *J. Med. Entomol.*, **7**, 499 (1970).

Synthetic Mimics of Insect Juvenile Hormone

TERPENE derivatives other than farnesol possess excellent juvenoid activity[1]. We have examined the effects of epoxidized and unepoxidized aromatic ethers of geraniol on yellow mealworm (*Tenebrio molitor*), confused flour beetle (*Tribolium confusum*) and other insects. The compounds can be synthesized easily from commercial materials and are reasonably stable to sunlight and hydrolysis. We have investigated the relationship between their chemical structures and their biological activity in the yellow mealworm[2].

Table 1 Juvenile Hormone Activity of Certain Para-substituted Phenyl Geranyl Ether Compounds on *Tenebrio molitor* Pupae



	ED-50 (μg/pupa)*				
	Code	n=0	Code	n=1	n=0/n=1
CH_3-	I	1.0	II	0.05	20.0
C_2H_5-	III	0.1	IV	0.0025	40.0
n-C_3H_7-	V	>0.5	VI	0.1	>5.0
i-C_3H_7-	VII	0.8	VIII	0.01	80.0
CH_3O-	IX	1.5	X	1.0	1.5
C_2H_5O-	XI	5.0	XII	0.2	25.0
CH_3S-	XIII	0.2	XIV	0.03	6.7
Cecropia hormone†			XV	3.0	

* Effective dose causing abnormal development of 50% of treated pupae.

† Methyl *cis*-10-epoxy-7-ethyl-3,11-dimethyl-*trans,trans*-2,6-tridecadienoate is included for comparison, and was kindly supplied by W. S. Bowers, USDA, Beltsville, Maryland.

Candidate compounds were applied topically in 1 μl. of acetone to the venter of newly formed *Tenebrio molitor* pupea, which were then incubated at 28° C and 75% relative humidity for 7 days. Signs of abnormal development included retention of urogomphi, gin traps, and general appearance of a pupal–adult intermediate[3-5].

Test compounds were synthesized from geranyl bromide by treatment with *m*-chloroperbenzoic acid to form the epoxide, which was then reacted with phenol in 1,2-dimethoxyethane in the presence of powdered potassium hydroxide. Geranyl bromide was used directly in the ether synthesis of the unepoxidized compounds. Structures were confirmed by infrared and mass spectroscopy and by nuclear magnetic resonance.

The activities of fourteen compounds in the *Tenebrio* test are given in Table 1. Seven epoxidized and unepoxidized compounds were compared for morphogenetic activity. The epoxidized forms (VIII) always had a higher order of activity[1], ranging up to eighty times that of the unepoxidized structure (VII). Compound IV, with an ethyl substituent on the phenyl ring, had the greatest activity, and all the compounds were significantly more active than the *Cecropia* hormone (XV), IV being 1,000 times more active than XV.

Compound IV was also tested on a stored product pest, *Tribolium confusum*, to demonstrate the feasibility of pest control by hormones. Twenty adult beetles were placed in glass bottles containing 100 g of flour that had been mixed with compound IV in concentrations varying from 0.01 to 1,000 p.p.m. Untreated flour was fed to control beetles. The results are given in Table 2. Adult development of *Tribolium confusum* was completely arrested by 10 p.p.m. or more and newly formed adults died prematurely even when the compound was present at the lowest test concentration—0.01 p.p.m. Survival of the original twenty adults in the presence of the

Table 2 Effect of Compound IV on *Tribolium confusum* Populations grown in Treated Flour

Life stages present 120 days after treatment		Concentration in flour (p.p.m.) 0	0.01	0.1	1	10	100	1,000
Adults:	No. alive	600	286	180	57	20	20	19
	No. dead	6	264	120	93	0	0	1
Pupae		+++	+++	+++	+++	+++	+	0
Larvae		+++	+++	+++	+++	+++	+	+
Supernumerary larvae		0	0	0	+	+++	+++	++
Adult–pupal intermediates		0	0	0	0	+	++	0

0=none; + =few; + + =moderate; + + + =many.

highest test concentration (1,000 p.p.m.) eliminated the possibility of direct toxicity of IV to the test insects. The activity of compound IV was superior to MTDD (3,7,11-trimethyl-7,11-dichloro-2-dodecenoate) which arrested development of *T. castaneum* at 100 p.p.m. in the flour diet[6].

These results suggest that stored product pests could be controlled by compounds possessing juvenile hormone activity. They also suggest that compound IV exerts its morphogenetic action through contact and/or ingestion. Compound IV is being tested in other laboratories and already the data indicate morphogenetic and sterilant effects in a variety of insect species including mosquito, tobacco budworm, cotton boll weevil, Indian-meal moth, stable flies, livestock ticks and body lice. Rats, on the other hand, seem to be unaffected by compound IV: when dosed orally with 4,000 mg kg^{-1}, no adverse effects were observed during a 14 day observation period.

Bearing the potential of these chemicals as pest control agents in mind, tests were conducted to obtain information on the influence of environmental factors on their chemical stability. Compound IV was spread in thin films on glass plates to determine the effects of light and air on stability, and known amounts were suspended in tap water to determine hydrolytic effects. At the end of each exposure, compound IV was rinsed off the glass plates with acetone or extracted from water and appropriate aliquots were bioassayed in the *Tenebrio* test. These tests indicated that compound IV is stable to sunlight and air for at least 12 h and that it does not hydrolyse in water in 24 h. These results are encouraging since they indicate that these mimics of juvenile hormones are sufficiently stable to act in a natural environment.

We thank H. Lee, K. Tseng and F. Kamienski for technical assistance.

FERENC M. PALLOS

Stauffer Chemical Company,
Western Research Center,
Richmond, California 94804

JULIUS J. MENN
PETER E. LETCHWORTH
JOHN B. MIAULLIS

Stauffer Chemical Company,
Agricultural Research Center,
Mountain View, California 94040

Received February 1; revised April 7, 1971.

[1] Bowers, W. S., *Science*, **164**, 323 (1969).
[2] Stauffer Chemical, Belgian Patent 734,904 (1969).
[3] Bowers, W. S., and Thompson, M. J., *Science*, **142**, 1469 (1963).
[4] Bowers, W. S., *Science*, **161**, 895 (1968).
[5] Chase, A. M., *Nature*, **215**, 1516 (1967).
[6] Thomas, P. J., and Bhatnagar-Thomas, P. L., *Nature*, **219**, 949 (1968).

Natural Selection and the Evolution of Proteins

Jukes and King[1] have attacked my article[2]. They do not quote my published criticism of their arguments[3]. Many of the points are discussed therein, and so a detailed reply is hardly necessary. They do, however, raise one additional matter of importance. They maintain that a selectionist hypothesis would predict radical changes to be favoured over conservative changes. The compelling arguments against this interpretation were clearly stated by Fisher in 1930 (ref. 4). He demonstrated that selection is very unlikely to favour extreme changes.

BRYAN CLARKE

Genetics Laboratory,
School of Botany and Zoology,
University Park,
Nottingham NG7 2RD

Received June 6, 1971.

[1] Jukes, T. H., and King, J. L., *Nature*, **231**, 114 (1971).
[2] Clarke, B., *Nature*, **228**, 159 (1970).
[3] Clarke, B., *Science*, **168**, 1009 (1970).
[4] Fisher, R. A., *The Genetical Theory of Natural Selection* (Oxford University Press, London, 1930).

Deepest Photographic Evidence of an Abyssal Cephalopod

THE bathymetric distribution of marine organisms collected during oceanographic expeditions is often difficult to ascertain because retrieval methods (nets, dredges, and so on) do not always indicate the precise depth at which an organism is entrapped. The level at which organisms dwell can, however, be accurately defined by deep ocean bottom photography.

On a recent Caribbean cruise of USNS Lynch, 340 exposures of the sea floor were obtained at seventeen stations. A modified Alpine multi-exposure camera system was used consisting of two 35 mm cameras mounted on a single frame and actuated by a bottom contact switch. Both cameras utilized the Hopkins water corrected 33 mm *f*/4.5 lenses. An oblique view of the ocean floor was obtained by tilting the lower camera's viewing angle to 40° with respect to the camera frame.

Among the records was the deepest photographic observation yet made of a marine cephalopod[1], a benthic species belonging to the family Cirroteuthidae (Fig. 1). Except for the thin web which unites all arms for part of their length, much of the

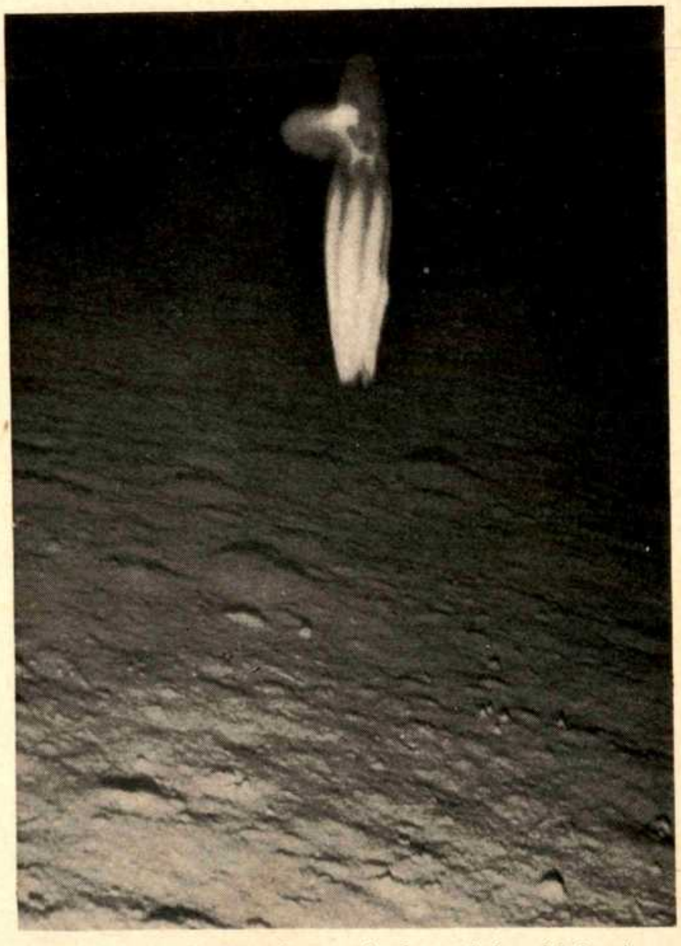


Fig. 1 Ocean bottom photograph of a cephalopod belonging to the family Cirroteuthidae. Taken 325 miles north-east of Barbados in an Abyssal Hill Province at station Lynch 33–14, latitude 17° 28.7′ N, longitude 56° 13′ W, depth 5,145 m on April 12, 1970. The specimen is approximately 1 m above the ocean floor.

external appearance of the octopod is visible. One of the obovate dorsal fins which assist the organism in balance and orientation can be seen, and also the eye, the dark spot located just above the arms. The estimated total length of the specimen is 45 cm.

The specimen may either have entered the camera's field of view from above, or more probably was startled by the camera's intrusion and is seen ascending in an escape reaction. The apparent absence of disturbed sediment would indicate that it was not lying on the sea floor. The sea floor at the camera station is fairly smooth, though numerous small lumps, depressions and oblate mounds are present. A sediment core taken simultaneously with the camera drop reveals the top 20 cm to be a brown slightly calcareous lutite. The potential temperature of the bottom water is 1.4° C (Escowitz and Amos, in preparation).

I thank G. L. Johnson and W. Ruddiman for their help and technical assistance, C. Roper at the Smithsonian Institution for identifying the specimen, and Captain J. Hobbs, the crew af the USNS Lynch and participating Navoceano personnel.

W. JAHN

Code 7400,
US Naval Oceanographic Office,
Suitland, Maryland 20390

Received April 28, 1971.

[1] Roper, C. F. E., and Brundage, W. L., *Ocean Industry Magazine*, April, 52, 54 (1970).

Skeleton of a Late Glacial Elk associated with Barbed Points from Poulton-le-Fylde, Lancashire

THE almost complete skeleton of an elk or moose (*Alces alces* L.) associated with two uniserial barbed points of bone or antler has recently been recovered from one horizon of a series of freshwater deposits revealed by the digging of foundations at High Furlong, Poulton-le-Fylde, Lancashire (NGR SD/3312 3867). An archaeological excavation subsequent to the discovery revealed *in situ* the hind parts of the skeleton and the second barbed point. Stratigraphic data were recorded, and samples were obtained for palaeoecology and dating.

The site lies near the 50′ contour, about 2 miles north-east of Blackpool. Borings were made at regular intervals along an approximately east–west line produced from the face of the cutting in which the skeleton and artefacts were found. These revealed a local depression in the till surface, approximately 40 m from east to west, in which limnic deposits of sands, clays and peats had accumulated. The basin extended in a north–south direction for at least 12 m, but building development in the area made its full extent difficult to determine.

The stratigraphy recorded close to the elk remains was as follows. (Datum was 36 cm below present ground level and 13.47 m above OD.)

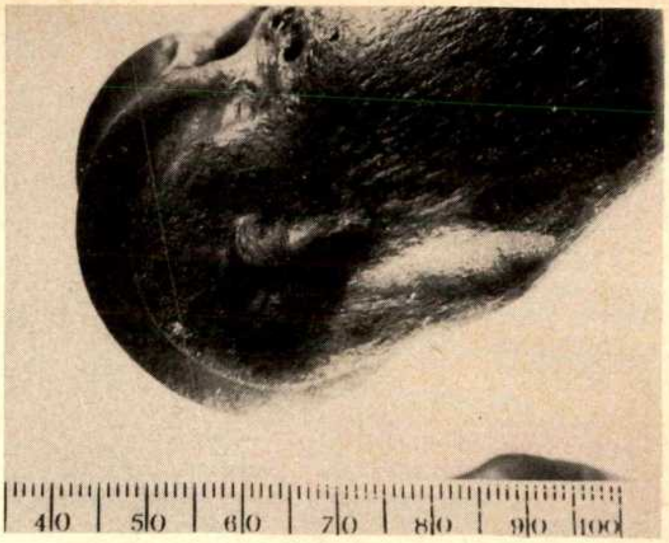


Fig. 1 Left metatarsal showing a lesion caused by one of the barbed points.

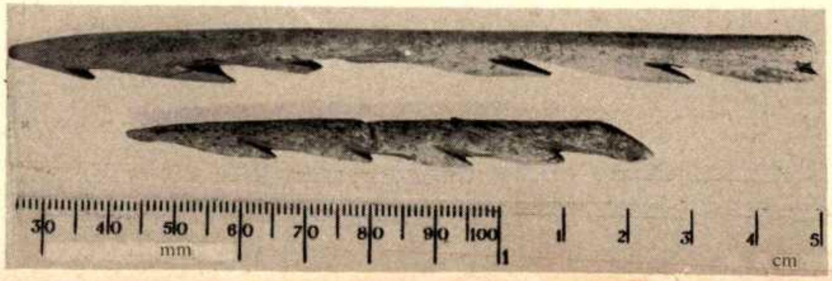
0– 12 cm	Clayey soil
12– 28 cm	Carr peat with *Betula* wood throughout, *Phragmites* leaves and *Potamogeton* fruitstones at base, and *Sphagnum* above 17 cm
28– 31 cm	Grey sandy mud with frost-shattered stones to 3 cm
31– 45 cm	Silty muddy clay
45– 55 cm	Dark grey clay with stones to 5 cm
55– 64 cm	Coarse detritus mud with *Potamogeton* and freshwater bivalve molluscs; elk bones in this horizon
64– 97 cm	Olive-grey calcareous shelly gyttja
97–104 cm	Clay with monocotyledonous fragments
104–123 cm	Red-grey thixotropic clay
123–125 cm	Sandy clay
125–127 cm	Sand with gravel to 5 cm

Nine metres west of this point, close to the centre of sedimentation, the same stratigraphic units attained a total thickness of 270 cm.

The sediments are clearly of freshwater origin. The stratigraphic sequence of clays sandwiching organic gyttja is reminiscent of the familiar "Allerød oscillation" of late Weichselian age noted at other sites in lowland Lancashire[1,2,4] and elsewhere in north-west Europe. Pollen analysis confirms that the deposits from 28 to 104 cm in the profile date from zones I, II and III of the conventional British pollen zone sequence. It is, therefore, unlikely that the age of the elk skeleton is less than 10,800 radiocarbon years BP[3].

The skeleton is that of a male elk, and is almost complete, lacking only the left maxillary teeth, part of the left maxilla, the caudal vertebrae, some carpals and parts of the ribs and

Fig. 2 The barbed points.



sternum. It bears numerous lesions (Fig. 1), and these, together with the presence of two barbed points (Fig. 2) in association with the bones, make it clear that the animal had been attacked by hunters.

The two barbed points, which are very similar, are made from either bone or antler. Both have their basal ends missing; one is 12.3 cm long and the other, found lying in two halves across the base of the left metatarsal of the elk, is 8.0 cm long. Typologically, they are more closely related to the early Mesolithic Maglemosian cultures than to the upper or epi-Palaeolithic Magdelenian or Ahrensburgian. The zone II dating of the horizon in which they were found, however, places them chronologically in the epi-Palaeolithic phase, thus providing a rare link between the cultures of the late Glacial and early Boreal periods.

The original discovery of the skeleton was made by Mr. T. Scholey and Mr. J. Audus. The archaeology is being carried out by J. S. H., and B. J. N. E.; stratigraphic evidence is being studied by B. B., who thanks Professor F. Oldfield for his assistance; and the skeleton is under study by A. J. S. A detailed report is in preparation.

B. BARNES

Poulton-le-Fylde College of Education

B. J. N. EDWARDS

Lancashire Record Office, Lancaster Road, Preston

J. S. HALLAM
A. J. STUART

Department of Geology, University of Manchester

Received March 10, 1971.

[1] Birks, H. J. B., *New Phytol.*, **64**, 270 (1965).
[2] Godwin, M., *Phil. Trans. Roy. Soc.*, B, **242**, 127 (1959).
[3] Godwin, M., Walker, D., and Willis, E. H., *Proc. Roy. Soc.*, B, **150**, 199 (1957).
[4] Oldfield, F., *New Phytol.*, **59**, 192 (1960).

Relationships of *Amphipithecus* and *Oligopithecus*

A RECENT article by Szalay[1] could be taken to be a definitive statement of the relationships of two of the earliest known specimens that can possibly be related to the origin of the catarrhine primates, the group which includes Old World monkeys, apes and men. Each of the fossils concerned—*Amphipithecus* from the late Eocene of Burma and *Oligopithecus* from the Oligocene exposures in the Fayum, Egypt—is known only from a single specimen. A different assessment of their relationships is presented here. Colbert has already shown[2,3] clearly and competently why the teeth and tooth cusps of *Amphipithecus* resemble those of catarrhines. I here outline some observations on how jaws function and why and how the jaw of *Amphipithecus* can be demonstrated to be similar to or different from those of other animals.

There has been much debate about the affinities of *Amphipithecus* as is shown by the following summary: (1) Colbert judged[2] the fragment of this animal—a jaw which contains P_3, P_4, M_1 and roots of P_2 and the canine—to be a probable member of Anthropoidea (the higher Primates) and he emphasized[3] further that this species could be excluded from significant relationship to extinct lemuroids or other lower primates. (2) Piveteau suggested[4] that *Amphipithecus* might either be a relative of South American monkeys (seemingly unaware how surprising its presence in Asia would be), or if not a platyrrhine it was perhaps not a primate at all. (3) Hürzeler found[5] the animal impossible to classify. (4) Remane assessed[6] the animal as standing between lower and higher Primates. (5) von Koenigswald thought[7] it likely to be a lemuroid, but later suggested[8] that it should be cast out of the Primates into the archaic mammalian order Condylarthra (a group of mammals, incidentally, not otherwise found in Burma or elsewhere in Asia). (6) I then repeated that if it was not an anthropoid, it might be an omomyid prosimian and I later stressed[10] again the hominoid similarity. (7) Finally, Szalay has lately argued[1] that *Amphipithecus* is an advanced lemuroid.

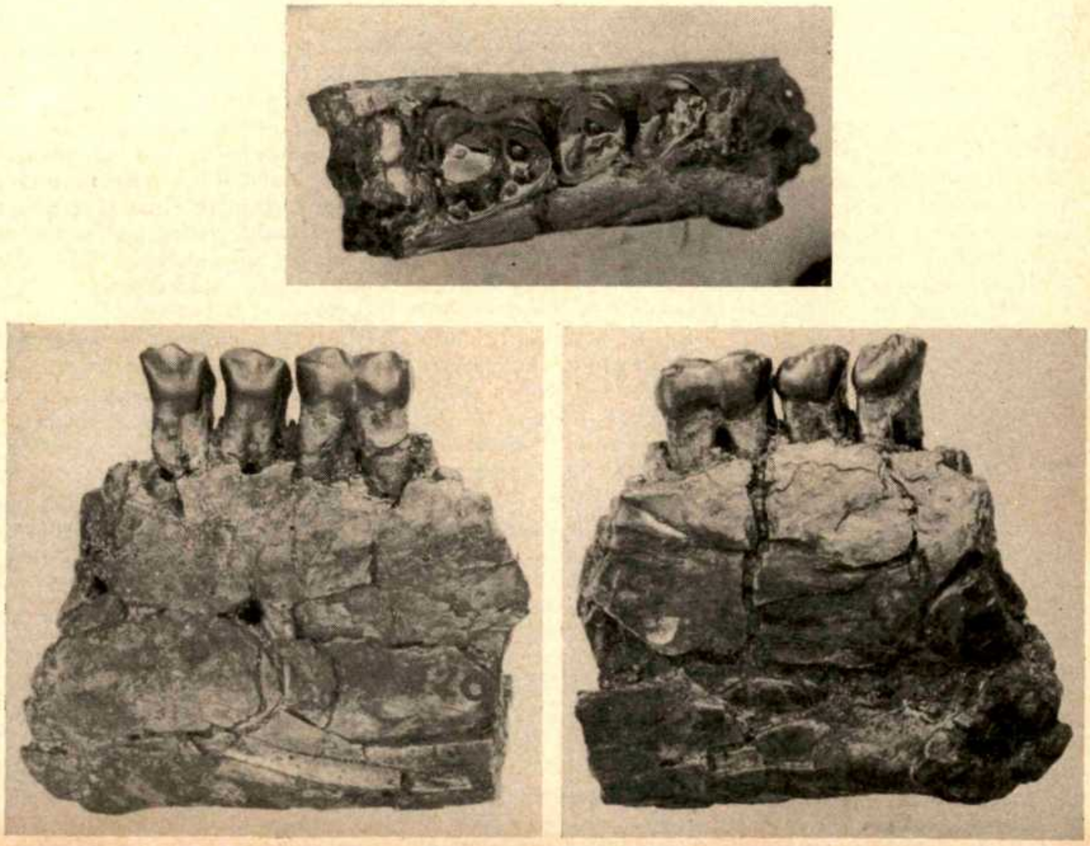


Fig. 1 Crown view (above) and lateral view of the teeth and jaw of *Amphipithecus mogaungensis* from Colbert 1935. The single known specimen is from the late Eocene of Burma.

A solution of the relationships of *Amphipithecus* can be presented in three stages. First, a basic structural and behavioural difference separates all Condylarthra from Primates and equally separates the latter from a large number of later orders presumed to have been derived from the condylarths. Two such orders are Artiodactyla and Perissodactyla but others include most, if not all, of the paenungulate orders. These, in the interpretation of Simpson[11], constitute the orders Pantodonta, Embrithopoda, Dinocerata, Hyracoidea, Sirenia, Pyrotheria and Proboscidea. All the species belonging to these orders are apparently hoofed herbivorous feeders, which cannot convey food to their mouths with their fore or hind limbs. They therefore either have trunks, as in the extant Proboscidea and the extinct Pyrotheria, or lengthened mandibular horizontal rami, usually with elongated diastemata between the cheek teeth and an anterior cropping mechanism which consists either of flared and procumbent incisors in both jaws or of a horny pad opposing the lower incisors. Generalized primates have cheek teeth which are like those of certain artiodactyls and condylarths, but because primates typically use their hands to feed, it is only in Primates and not in Condylarthra or Artiodactyla that the front teeth are not separated from the cheek teeth (the whole tooth row is crowded anteroposteriorly). For the same reason the anterior teeth of certain primates have become relatively small and vertically implanted as seems to be the case in *Amphipithecus*[2,3].

Second, *Amphipithecus* also has an extremely deep mandible compared with the crown height of the cheek teeth preserved; this depth is greater than in almost all mammals with teeth in the same size range, and exceeds even that of the early ape *Aegyptopithecus* (see Fig. 1). Such deep mandibles are characteristic only of certain primates; for example, *Gigantopithecus*[12], many colobines and some platyrrhine monkeys. Great mandibular depth correlates with symphyseal fusion. *Homo* and other hominids also show early symphyseal fusion with small, vertically implanted incisors and have a symphyseal cross-section the long axis of which is oriented almost at right angles to the long axis of the tooth row or horizontal ramus of the mandible. When food gathering shifts to hand or trunk then the importance of incisors in this function decreases. In the case of lemurs and lorises, lower canines and incisors (freed from their food gathering function) have become dental combs for grooming the fur. Elephants have lost opposing front teeth altogether, for food is conveyed to the mouth principally by the trunk. On the other hand, the prosimians generally have anteriorly shallow, separate jaw rami with an unfused anterior symphysis. The few prosimian species that do show symphyseal fusion include the large extinct Pleistocene lemurs of Madagascar, *Archaeolemur* and *Hadropithecus*. These lemurs have long been recognized as having independently converged toward Anthropoidea, and their evolution serves to support the points raised here, for their dental formulae as well as other features prove they cannot be catarrhines although they parallel them. The jaws of prosimians, living or fossil, are never particularly deep. The incisors of such prosimians as the lorises and lemurs project forward and there is little or no development of either the superior or the inferior transverse tori at the back of the symphysis connecting and buttressing the two halves of the lower jaw. This is because these latter structures apparently reach fullest development as they further reinforce the fused symphysis characteristic of higher Primates, including Catarrhini and Platyrrhini. Both these tori are quite large in *Amphipithecus* and it may be that part of the superior transverse torus actually passes the mid-line, which seems likely if, as in all other Tertiary Anthropoidea, the mandibular rami diverged posteriorly. This, together with marked toral development, gives two lines of evidence that there was symphyseal fusion. It should be stressed that there are no primates known below the grade of Anthropoidea (=higher primates), and indeed there are no other mammals which show vertically oriented symphyseal sections, comparatively deep mandibles, orthally directed, crowded, teeth and large tori.

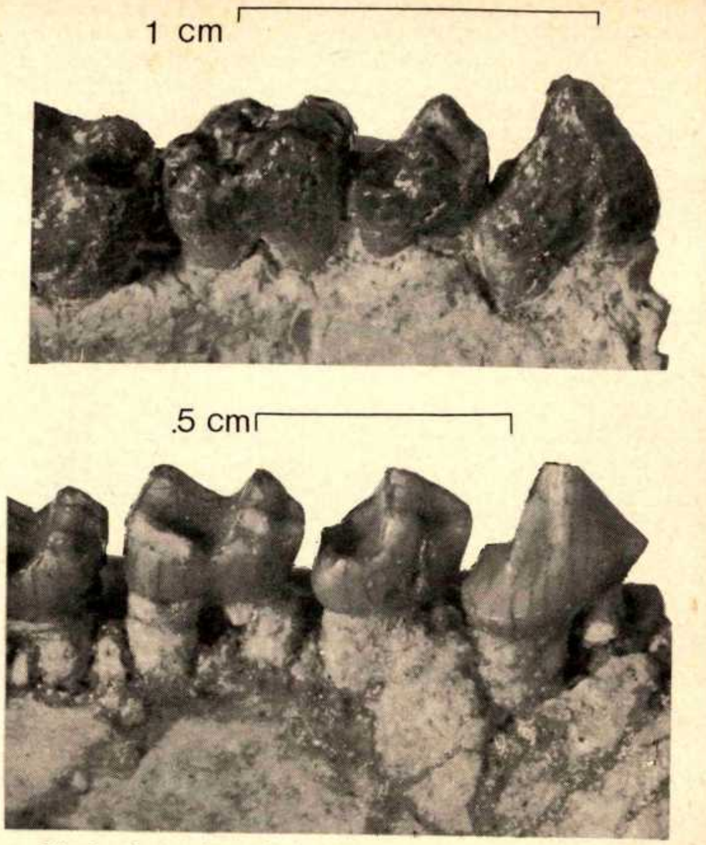


Fig. 2 Comparison of the internal aspect of the premolars and front molars of *Aegyptopithecus zeuxis* (above) and of *Oligopithecus savagei* (below). Both are from the Oligocene deposits of the Fayum badlands, Egypt.

Third, the objection that retention of three premolars is strange for a catarrhine primate has now lost any force it may have had because at least a dozen jaws of the undoubted early catarrhine primates *Apidium* and *Parapithecus* recovered recently by Yale University expeditions under my direction from the Oligocene Fayum of North Africa demonstrate that three premolars are consistently present. Both the Pondaung fauna as well as that of the Fayum contain species of the primitive anthracothere genus *Brachyodus*. This confirms a migration route of some sort between the two areas. Absence of M_1 hypoconulid, if it is indeed entirely absent in *Amphipithecus*[1,2], is not particularly strange, because this animal, even if a member of Anthropoidea, need not be ancestral to any later species retaining this structure. "Hypoconulids" can also arise as neomorphs. Modern cercopithecoids lack M_1 hypoconulids as do many early Primates. Moreover, the hypoconulid is not clearly distinct on M_1 in the archaic catarrhine *Oligopithecus*. This animal will be shown, in what follows, to be a higher primate. Further, Szalay (personal communication) now agrees that *Oligopithecus savagei* is not a prosimian but is a catarrhine.

If *Amphipithecus* is not a member of Anthropoidea then it will have to be considered as belonging to an order of mammals otherwise entirely unknown which might facetiously be called Pseudanthropoidea, or Pseudcatarrhini. *Oligopithecus* must be considered a catarrhine primate because of its complete similarity to Old World Anthropoidea: its jaw is deep, it has only two lower premolars and the anterior of these is elongate and serves as a hone against the back of the upper canine.

The P_2 or P_3 honing function of Anthropoidea is never seen in Prosimii and consequently the full development of this mechanism with highly heteromorphic P_3 and P_4 seen in *Aegyptopithecus* and *Oligopithecus* (Fig. 2) is additional evidence that these Fayum primates were definitely of a higher primate grade. Fig. 3 shows the honing facet of the unique

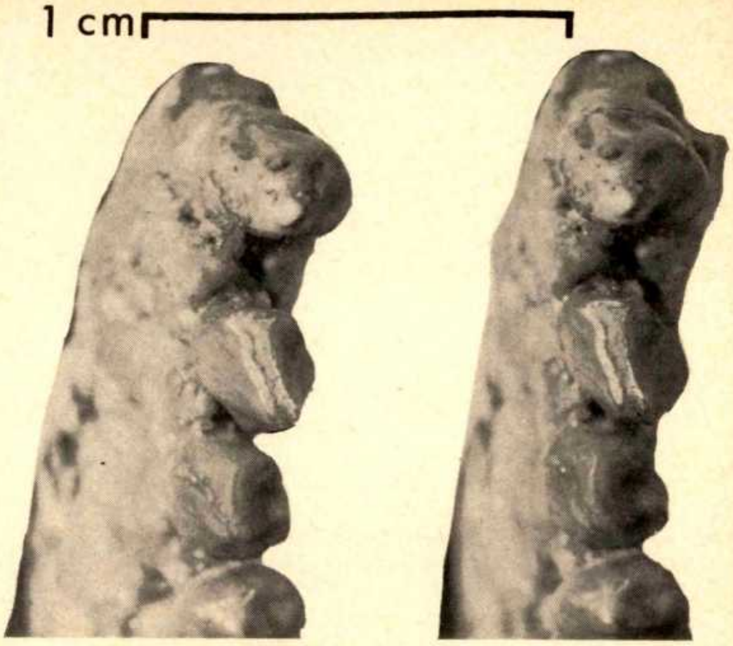


Fig. 3 Stereopair of the anterior part of the left lower jaw of *Oligopithecus savagei* showing the distinct honing facet for the back of the upper canine—a distinctly anthropoid trait.

type mandible of *Oligopithecus savagei*. Clearly *Oligopithecus* is not a prosimian. Its premolars are fully heteromorphic and unlike anything that can be found in any prosimian, living or fossil. The tooth crown surfaces of the new *Aegyptopithecus* mandible have been chemically eroded but this has not obscured its premolar resemblance to *Oligopithecus*. In both P$_3$'s the trigonid is wide open lingually, and the paraconid ridge descends anteriorly just lateral to the mid-line in the long axis of the tooth row. The metaconid ridge of P$_3$ slopes posterolingually in both and ends in the same manner in a slightly enlarged bulb, the presumed homologue of the P$_4$ metaconid. In both there is an internal basal cingulum on P$_3$ but not on P$_4$ or on teeth posterior to it, and in both this cingulum carries a slight swelling just above the space between the large posterior and somewhat smaller anterior root of this tooth. In both species the entoconid and hypoconulid of M$_2$ are closely approximated. One difference (Fig. 2) is that the M$_1$ hypoconid of *Oligopithecus* is higher and less rounded than in *Aegyptopithecus*. The P$_3$ canine hone of *Oligopithecus* (Fig. 3) is well defined and the principal direction of the grinding stroke of the canine is indicated by striations on the wear facet. No prosimian, living or fossil, shows a premolar honing facet of this sort which does, of course, reflect that the functional uses of the front teeth of prosimians are basically different from this.

One or two observations about the unique type specimen of *Oligopithecus* seem worthwhile at this point. This specimen was found by D. E. Savage and was collected by me in December 1961 in quarry E of the lower fossil wood zone of the Jebel el Quatrani Formation, Fayum, Egypt. It was found in a porous lense of gravel-sized matrix. The body of the mandible had been considerably fractured with the result that its parts had expanded and the cracks had been refilled by sand grains. Thus, the teeth, once closed together from back to front, are now separated from each other by gaps. During collection the canine and P$_3$ were unavoidably further tilted apart (see Fig. 2). It has been considered inadvisable to try to move these teeth back into closer approximation because the horizontal body of the mandible is as soft and friable as chalk. As the teeth are now splayed apart, it might be thought that *Oligopithecus* had a P$_2$, but when discovered in place there was no gap between these teeth, and the wear facet for the upper canine seen on P$_3$ (Fig. 3) completely confirms the lack of P$_2$.

In sum, the overall structure of teeth and jaws of *Amphipithecus*, *Aegyptopithecus* and *Oligopithecus* shows numerous anatomical details which only resemble living catarrhine primates. These genera, together with *Proliopithecus*, *Aeolopithecus*, *Apidium* and *Parapithecus*, are therefore the oldest undoubted evidence of Catarrhini. Although *Apidium* and *Parapithecus* of the Fayum do retain three premolars, the specifically catarrhine anatomy of the mandibles of *Oligopithecus* and *Aegyptopithecus* tends to weaken the conjecture that the Fayum primates might have been introduced to Africa by rafting across the South Atlantic Ocean from South America.

ELWYN L. SIMONS

Department of Geology and Geophysics,
Yale University,
New Haven, Connecticut 06520

Received January 19; revised April 9, 1971.

[1] Szalay, F. S., *Nature*, **227**, 355 (1970).
[2] Colbert, E. H., *Amer. Mus. Novit.*, No. 951 (1937).
[3] Colbert, E. H., *Bull. Amer. Mus. Nat. Hist.*, **74**, Art. 6 (1938).
[4] Piveteau, J., *Primates Paléontologie Humaine* (*Traité de Paléontologie*), **8** (Masson, Paris, 1957).
[5] Hürzeler, J., *Verh. Naturf. Ges. Basel*, **69** (1), 1 (1958).
[6] Remane, A., in *Menschliche Abstammungslehre* (Fischer, Stuttgart, 1965).
[7] von Koenigswald, G. H. R., *Proc. K. Ned. Akad. Wet.*, **68**, 165 (1965).
[8] von Koenigswald, G. H. R., in *Neue Ergebnisse der Primatologie* (Fischer, Stuttgart, 1967).
[9] Simons, E. L., in *International Encyclopedia of Social Sciences* (Macmillan, New York, 1968).
[10] Simons, E. L., in *Problèmes Actuel de Paléontologie* (*Colloques Intern. Cent. Nat. Rech. Scient.*, No. 163), 597 (1967).
[11] Simpson, G. G., *Bull. Amer. Mus. Nat. Hist.*, **85** (1945).
[12] Simons, E. L., and Ettel, P. C., *Sci. Amer.*, **222**, 76 (1970).

Selecting Tests in Diagnostic Keys with Unknown Responses

HERE we outline a method for constructing diagnostic keys from test results, some of which are unknown. The method is then modified to reduce the number of different tests used. Elsewhere[1] we discuss an application to yeasts, in which the primary purpose is not so much the construction of a key, but to find how few out of a set of tests is required to identify all the yeasts.

The average number (m) of steps required to identify a species can be calculated for any key. An obvious objective is to construct the key with smallest m. Other objectives include minimizing the average cost of identification and balancing

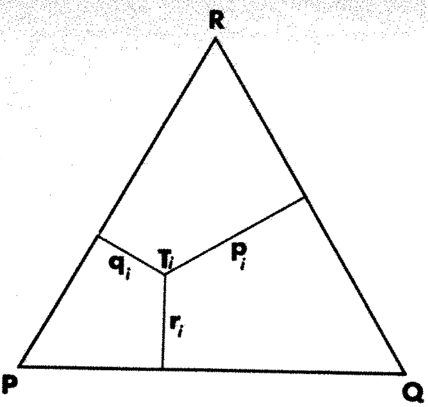


Fig. 1 Representation by areal coordinates of three proportions, $p_i(+)$, $q_i(-)$, and $r_i(?)$, for test T_i.

small m against the number of different tests used in the key. No algorithm seems to be known for minimizing m (apart from the impracticable one of enumerating all possible keys); but the empirical procedures discussed below give approximate solutions.

The results of the tests under discussion are usually presented in an all or none fashion (+ or −). The maximum number of species that can be separated by k binary tests is 2^k and, conversely, n species require at least $\log_2 n$ tests for complete separation. Tests that reduce the number of species to $<2^k$ are not fully efficient[2]. Hence, for any given set of species, in the best possible key (that with minimum m) every test divides its subjects into two groups of equal size. Because this arrangement is usually impracticable, an empirical method for constructing a key is to select tests that most nearly divide the species into equal groups. Several authors[3–6] have discussed this approach to binary tests, but we also consider unknown responses, denoted by "?". These missing values may occur because a test result has not been observed, or is difficult to interpret, or, as in the example discussed by Barnett[1], the test results are variable so that their proper handling would require a probabilistic key, with the additional complications of considering probabilities of misidentification.

When constructing the key, any species with a ? response to a given test used for branching must be included in both + and − branches and hence may appear at a number of ends of the tree. When using the key, any ? response to a test must lead to both + and − branches, so that a specimen may be given several possible identities. At each dichotomy of the key, the test to be selected is that for which the number of + responses most nearly equals the number of − responses, allowing for the ? responses. The selection criterion must depend on the proportions p_i, q_i, and r_i of +, −, and ? responses. The ith test may be represented by a point $T_i(p_i, q_i, r_i)$ in three dimensional space, but because $p_i+q_i+r_i=1$, all points T_i are inside (or on) the equilateral triangle PQR (Fig. 1) cut off by the coordinate axes on the plane $p+q+r=1$. The perpendiculars from T_i on to QR, PR and PQ have lengths $\sqrt{3/2}$ times p_i, q_i and r_i, respectively, and are usually termed areal coordinates. k tests can be represented as k points T_i $(i=1, 2, \ldots, k)$ within the same triangle. If there are no ? responses, the point T_i lies on PQ and the selected test is that nearest O, the mid-point of PQ, that is the test for which $p_i=q_i=\frac{1}{2}$ is most nearly true. When there are ? responses, the most efficient test is here taken to be that for which OT_i is shortest, $OT_i^2=(p_i-\frac{1}{2})^2+(q_i-\frac{1}{2})^2+r_i^2$. On this measure of efficiency, all those tests represented by points on any semicircle (centre O) and within triangle PQR are equally efficient; those within the semicircle are more efficient than those outside it (Fig. 2).

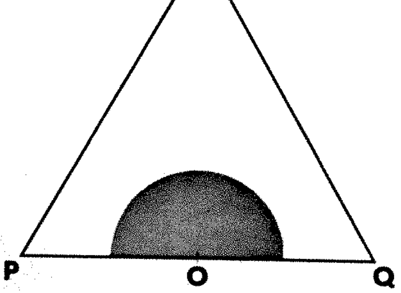


Fig. 2 All tests represented by points on a semicircle, centre O (mid-point of PQ), are equally efficient.

We considered other criteria of test efficiency; for example, the information criterion $I_i=(p_i+r_i)\log(p_i+r_i)+(q_i+r_i)\log(q_i+r_i)$ behaves almost identically to OT_i^2, especially in the region containing efficient tests. The Williams and Lambert association analysis[7] and its extension suggested by Gower[8] have not yet been tried. The criterion of OT_i^2 was chosen for its simplicity, providing an efficient algorithm when included in a computer program; it can also be extended easily to cope with multilevel responses. These virtues are important for computing when the results of many tests have to be examined at every level of the hierarchic key under construction. When several tests are nearly equally efficient, the simplest procedure is to select any one at random, and in practice to select the first encountered.

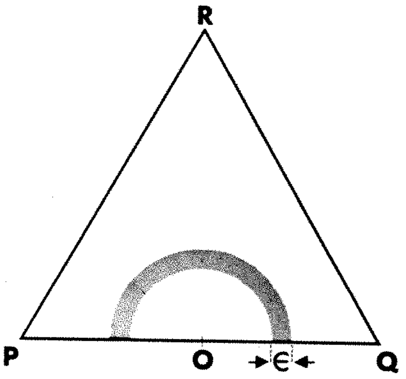


Fig. 3 To increase the chance of finding previously used tests, all points in the annulus, width ε, are taken to represent equally efficient tests.

In botanical identification a step by step method is often used; each test or observation is done consecutively, and the test use at any stage is determined by the outcome of the previous test. Keys constructed by the method described here should then be satisfactory. By contrast, in identifying microorganisms it is usual to do many tests simultaneously before referring to a key. When many hundreds of organisms have to be identified in a year, it may be important to minimize the number of tests that have to be done. A further objective is to partition the key so that the results of the first set of tests determine which tests are in the second set; in this way the number of tests for identification may be greatly decreased.

To minimize the number of tests used, the criterion described was modified to increase the chance of finding a test that occurs elsewhere on a different branch of the key. This was done by regarding as equally efficient all tests within an annular region of width given by the difference between the radii OT and $OT+\varepsilon$ (Fig. 3). Several values of ε(0, 0.05, 0.1, 0.3, 0.5) were tried for sixty-eight species of fruit yeasts; m ranged from 6.61–6.84, and the number of different tests from twenty-four to twenty-five. Here, at least, little was gained by varying ε. This was mainly because certain tests were essential for separating pairs of similar species; although these tests were not needed in the earlier levels of the key, they had to be included later. When selecting one of several tests in the ε-annulus, two strategies were tried. The selected test was either (i) that used most often before, or if none, the most efficient test, or (ii) the most efficient test of those used before, irrespective of how often used.

To obtain partitioned keys with this program, a modification of the ε-process might be used, although it was not tried. In this method, the highest level of the key at which each test has been used is recorded, and the best test used previously at the highest level is selected from the ε-annulus. The computer program constructs the key by completing the branches from left to right. Alternatively, the levels from top to bottom could be completed; this would be more suitable when trying to minimize the number of tests used in different partitions of the key, but it is less efficient computationally. This strategy, and that of building the key from bottom to top, are being investigated.

Two or more tests used simultaneously may give more help in identification than both used separately; in this context Pankhurst[4] has a simple but effective formula for + and − responses only. Following the success of applying the present work, as described in the companion paper[1], we are investigating the handling of ? responses when considering two or more tests simultaneously, and also studying the effects on the calculations of permuting the order of the tests.

J. C. GOWER

Rothamsted Experimental Station,
Harpenden, Hertfordshire

J. A. BARNETT

Food Research Institute,
Norwich NOR 70F

Received November 23, 1970; revised April 16, 1971.

[1] Barnett, J. A., *Nature New Biology* (in the press).
[2] Barnett, J. A., *Antonie van Leeuwenhoek*, **23**, 1 (1957).
[3] Gower, J. C., in discussion on Marshal, A. W., and Olkin, I., *J. Roy. Statist. Soc.*, B, **30**, 438 (1968).
[4] Pankhurst, R. J., *Computer J.*, **13**, 145 (1970).
[5] Pankhurst, R. J., *Nature*, **227**, 1269 (1970).
[6] Hall, A. W., *Taxon*, **19**, 12 (1970).
[7] Williams, W. T., and Lambert, J. M., *J. Ecol.*, **47**, 83 (1959).
[8] Gower, J. C., *Biometrics*, **23**, 623 (1967).

Electron Microscope and Optical Diffraction Studies on Isolated Cell Walls from *Chlamydomonas*

THERE are few high resolution electron microscope studies specifically concerned with the architecture of the cell walls of the green alga *Chlamydomonas*. Sager and Palade[1] state that the wall of *C. reinhardi* is a multilayered structure about 40.0 nm thick and that the structure is consistent with the cellulose wall described from light microscope observations. The description of the wall given by Walne[2,3] and by Cavalier-Smith[4] is essentially similar to that given by Sager and Palade. Barnett and Preston[5] using the freeze etching technique reported the presence of oriented microfibrils in the cell walls. The regular features were interpreted as being cellulose which was thought to be synthesized by small granules associated with the surface of the plasmalemma. In the related species *C. moewusii* the wall had a regular periodicity of 23.3 nm, and again the striations were interpreted[6] as being cellulose fibrils, but it was noted that the spacings were smaller than those observed in other structures containing cellulose fibrils[7].

The preliminary findings we wish to report concern the application of various chemical and physical methods to the isolation and characterization of the cell wall material isolated from *C. reinhardi* with particular reference to the ultrastructural features and their analysis by optical diffraction[8,9]. In addition to the wild type strain we have examined two of a series of mutant forms we have isolated[10], which show defects in various aspects of cell wall biogenesis. Cell preparations were obtained from liquid cultures and concentrated by low speed centrifugation. Pellets were placed in a Mickle shaker and disintegrated in the presence of glass beads. Fractions containing cell wall material were obtained by differential centrifugation as applied to the separation of bacterial cell walls[11]. Centrifuged pellets containing walls were monitored for purity by examination in the phase contrast microscope and by electron microscopy. The purity and concentration of a typical cell wall fraction are illustrated in Fig. 1. Examination of shadowed specimens in the electron microscope revealed typical cell walls of the type shown in Fig. 2. The striations observed at low magnification were essentially similar to those reported by Lewin *et al.*[6] but the spacing of 13.5 nm determined from Fourier transforms was considerably smaller (Fig. 2 inset).

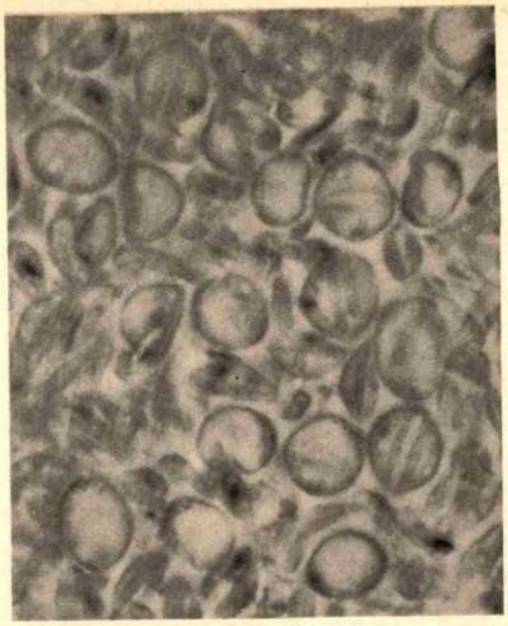


Fig. 1 Phase contrast micrograph of isolated cell wall fraction.

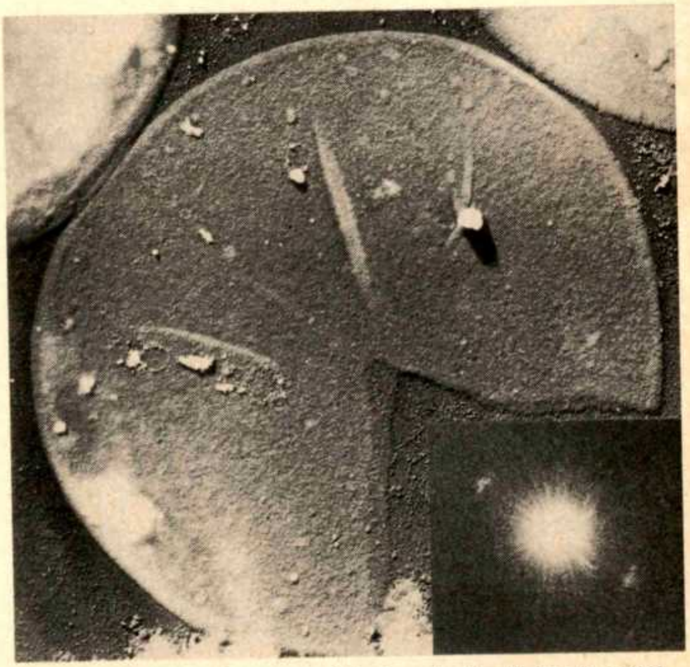


Fig. 2 Electron micrograph of shadowed cell wall. Inset: optical transform obtained from a shadowed cell wall, indicating regular periodicity of 13.5 nm.

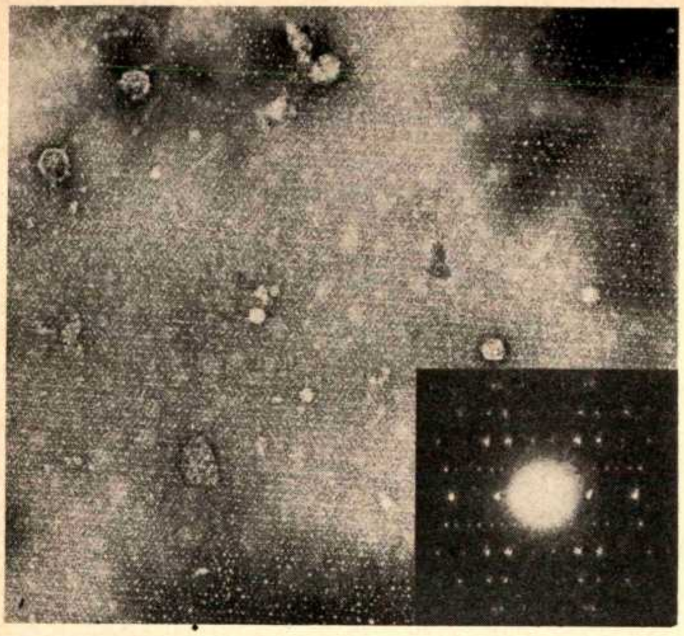


Fig. 3 Electron micrograph of negatively stained preparation of cell wall showing a regular lattice structure. Inset: optical transform from the same area.

Studies of thin sections of intact cells and cell wall fractions prepared by a variety of fixation and staining procedures showed the wall to have a structure similar to that described by the workers mentioned earlier. It was constructed of several electron dense layers arranged approximately parallel to each other. The layered region was about 19.0 nm across (after osmic glutaraldehyde fixation). In addition to the electron dense layered regions, the inner and outer surfaces of the cell wall when seen in thin sections showed different morphological features. At the inner surface a diffuse region was observed and appeared to consist of small particulate material, whereas the outer surface suggested the presence of long filamentous structures of about 3.4 nm in diameter being released from the wall.

After preparation of the wild type cell wall material in the presence of 2% ammonium molybdate or 2% potassium phosphotungstate (*p*H 6.8), and examination at high instrumental magnifications in the electron microscope, micrographs were recorded showing a well defined lattice pattern as illustrated in Fig. 3. Examinations of the micrographs in an optical diffractometer indicated that the cell wall was constructed of a complex lattice as shown in the inset of Fig. 3. The lattice structures appear to form layers in the cell wall and are positioned at preferred angles relative to each other. In the case of the pattern illustrated in Figs. 3 and 4, it is possible to interpret the structure as being two basic layers orientated at about 20°. The values relating to the layer lines from the crossed lattice are indicated in the diagram of Fig. 3 which is from the Fourier transform of the inset of Fig. 3. High resolution electron micrographs suggest that the diameter of the fibrils comprising the lattice determined from negatively stained specimens is about 3.8 nm (fifty measurements). The two mutants examined, CW2 and CW20, are defective in that the former cannot attach the wall to the plasma membrane and sheds it into the medium, and the latter, though having an attached wall, has no structural rigidity provided by its wall. Examination of the isolated walls from these mutants, however, revealed that the structural features and the spacings of the lattice were identical with those seen in the wild type wall.

Previous observations[12] relating to the composition of *C. reinhardi* have suggested that about 50% protein dry weight is present in the cell wall. Our own preliminary analysis of the amino-acid composition of the isolated cell wall fraction points to a value of about 35% protein dry weight, but a more detailed analysis is in progress and will be reported later.

We have carried out a number of experiments on the treatment of isolated cell walls of *C. reinhardi* with trypsin prepared according to the methods of Kosta and Carpenter[13]. Those treated with trypsin for 2 h at 37° C no longer retain the regular lattice structure as determined by electron microscopy. Following prolonged treatment with the enzyme they become disorganized and retain only a vague resemblance to their original shape and size. Treating the walls with 10 M KOH incubated for 2 h at 50° C also resulted in the loss of all morphological features corresponding to the original lattice and overall structure detectable by light and electron microscopy. Moreover, there was no evidence to show the presence of fibrils which would correspond to cellulose microfibrils in either the trypsin digests or after KOH extraction. In addition to treatment with trypsin and KOH extraction, several experiments were carried out in an attempt to remove the lattice structure with cellulases. Treatment with cellulase enzyme failed to remove or change the lattice material.

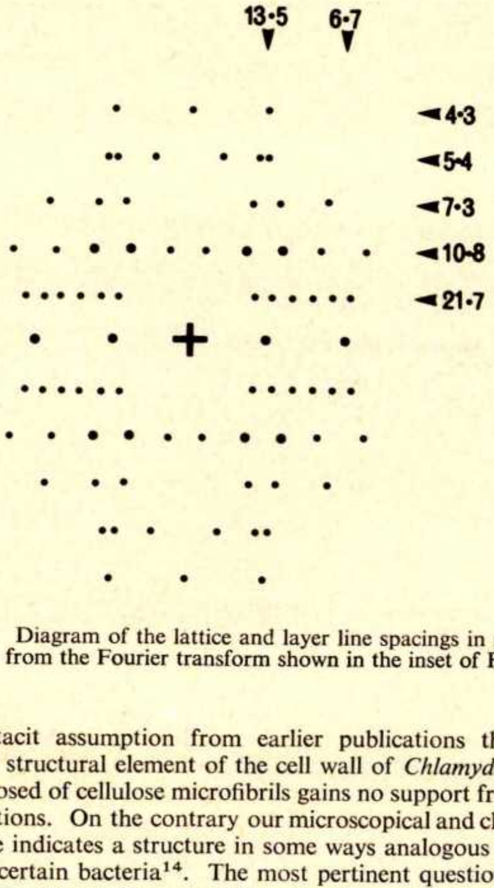


Fig. 4 Diagram of the lattice and layer line spacings in nanometres from the Fourier transform shown in the inset of Fig. 3.

The tacit assumption from earlier publications that the primary structural element of the cell wall of *Chlamydomonas* is composed of cellulose microfibrils gains no support from our observations. On the contrary our microscopical and chemical evidence indicates a structure in some ways analogous to that seen in certain bacteria[14]. The most pertinent question to be answered relates to the assembly mechanism which gives rise to the regular lattice structure depicted in Fig. 3, and in this context the eighty mutant forms we now have available which are defective in various aspects of cell wall biogenesis will be particularly useful. Our most recent studies suggest that the basic structural units for the wall are assembled from individual layers at the inner wall surface. This material, located between the plasmalemma and the cell wall, seems to be morphologically different from the slender strands which can be seen being released at the outer cell wall surface. Our tentative interpretation of these observations is that while assembly of units occurs at the inner wall surface, at the outer region of the cell wall, layers are being shed into the surrounding medium as long slender flexuous filaments with dimensions close to the structures forming the lattice pattern described above.

We thank Mr M. Rees for carrying out the preliminary amino-acid analysis, Mr G. J. Hills for assistance in optical diffractometry, and Mr S. Frey for photographic assistance.

R. W. HORNE
D. R. DAVIES
K. NORTON
M. GURNEY-SMITH

John Innes Institute,
Colney Lane,
Norwich NOR 70F

Received January 15, 1971.

[1] Sager, R., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **3**, 463 (1957).
[2] Walne, P. L., *Amer. J. Bot.*, **53**, 908 (1966).
[3] Walne, P. L., *Amer. J. Bot.*, **54**, 564 (1967).
[4] Cavalier-Smith, T., thesis, Univ. London (1967).
[5] Barnett, J. R., and Preston, R. D., *Proc. Roy. Microsc. Soc.*, **4**, 135 (1969).
[6] Lewin, R. A., Owen, M. J., and Melnick, J. L., *Exp. Cell Res.*, **2**, 708 (1951).
[7] Mühlethaler, K., *Biochim. Biophys. Acta*, **3**, 527 (1949).
[8] Horne, R. W., and Davies, D. R., *Septième Congrès Intern. de Microscopie Électronique*, Grenoble, **3**, 117 (1970).
[9] Klug, A., and De Rosier, D. J., *Nature*, **212**, 29 (1966).
[10] Davies, D. R., and Plaskitt, A., *Genet. Res.*, **17**, 33 (1971).
[11] Salton, M. R. J., *The Bacterial Cell Wall*, 42 (Elsevier, Amsterdam, 1964).
[12] Gotelli, J. B., and Cleland, R., *Amer. J. Bot.*, **55**, 907 (1968).
[13] Kosta, V., and Carpenter, F. H., *J. Biol. Chem.*, **239**, 1799 (1964).
[14] Glauert, A. M., and Thornley, M. J., *Ann. Rev. Microbiol.*, **23**, 159 (1969).

Wolffia arrhiza as a Possible Source of Inexpensive Protein

THE development of new foods is vital to the needs of the rapidly expanding population in Asia. But apart from the necessity of being competitive in market price, they must be acceptable to the human palate, a requirement that has often frustrated attempts to introduce new foods. In this light, the improvement of production and processing of a commodity which is currently marketed for direct human consumption but on a small and inefficient scale has several advantages. Such a commodity is the aquatic plant, *Wolffia arrhiza* Wimm., Lemnaceae, known to have been used as a vegetable by the Burmese, Laotians and the people of northern Thailand for many generations. The local Thai name for the plant, "khai-nam", may be literally translated as eggs of the water and suggests the oval shape of the plant (length 1.5 mm, width 1.0 mm). Khai-nam is generally regarded as a poor people's food and has attracted little attention as a potentially significant source of human food. The species also occurs in India[1], but no report has been made pertaining to its application as a food source in that country.

We have observed the native methods of cultivating *Wolffia arrhiza* and have analysed samples. Small scale cultivation by rain-fed open ponding methods is carried out by villagers living near provincial urban centres in northern Thailand. The *p*H of the water in all the ponds visited (more than 100) was between 6.5 and 7.0, and most were small (60–100 m²) and shaded by bamboo groves. No chemical fertilizers or manures were added. In controlled laboratory conditions, *Wolffia* has been found to reproduce vegetatively by budding and to have a generation time of approximately 4 days[2,3]. But natural conditions with higher light intensities and temperatures seem to be more conducive to growth. In practice, the plants are harvested at intervals of 3 to 4 days during which period the relatively small number of plants remaining from the previous harvest multiply into a thick mass of yellowish-green plants at the water's surface indicating that several generations may have arisen within 4 days. Longer time intervals between harvests invariably lead to lower yields and a product containing a significant portion of dead brownish individual plants which, being unattractive, fetch a low market price. A record of harvesting over a period of 4 months from randomly chosen ponds revealed that the average yield of fresh marketable plants (collectively containing an average of 4% dry matter) is 0.68 kg/m²/week.

In the climatic conditions of the town of Chiengmai in northern Thailand (lat. 19° N, long. 99° E), *Wolffia arrhiza* remains in its edible vegetative form between November and July. The inedible sexually reproducing form prevails between August and October. On the basis of a 9 month period of productivity the calculated annual yield is 265 tons/ha fresh weight or 10.5 tons/ha dry weight. The chemical composition and annual areal yields of *Wolffia* are listed and compared with conventional crop yields in Table 1.

Table 1 Proximate Analysis and Yields of *Wolffia arrhiza* in Comparison with Conventional Crops

Component	*Wolffia*, dry weight %	Average yield (kg/ha/year)				
		Wolffia	Soybean	Groundnut	Rice	Corn
Protein	19.8	2,080	303	229	71	179
Carbohydrate	43.6	4,589	255	164	849	1,451
Fats	5.0	533	158	397	4	87
Fibre	13.3	1,398	44	21	3	40
Ash	18.3	1,928	41	20	5	24
Water	0	0	89	51	124	238
Total	100.0	10,528	890	882	932	1,772

The protein nitrogen was determined by the Kjeldahl method. Fats were extracted by the Soxhlet technique using dichloromethane. The rice was converted from paddy to dehusked rice (ratio 0.66) and groundnut was converted from unshelled to shelled groundnut (ratio 0.60). We determined the composition of *Wolffia*; those of crops were computed from US Department of Agriculture data[4]. Other data on conventional crops were obtained from the Food and Agriculture Organization[5].

In terms of annual yield, *Wolffia* produces more dry matter than the traditional crops grown in Thailand. The protein yield of *Wolffia* is several times greater than those of the local crops. So far, no attempts have been made to increase yields by improving growth conditions but studies will be undertaken to determine the effects of supplemental nutrients, controlled incident solar radiation, harvesting periodicity and mixing in batch and continuous outdoor conditions. Dependent variables will include growth rates, areal yields, proximate analyses of the product, water requirements, nutritional value, production economics and market potential. The research will be directed towards establishing optimum production conditions giving greater yields at lower costs. The naturally occurring species, *Wolffia arrhiza*, will be studied first because of its proven market and nutritional acceptability.

KRACHANG BHANTHUMNAVIN

Department of Biology,
Faculty of Science,
Mahidol University,
Bangkok 4, Thailand

MICHAEL G. MCGARRY

Division of Environmental Engineering,
Asian Institute of Technology,
Bangkok 5, Thailand

Received March 12; revised April 29, 1971.

[1] Fischer, C. E. C., in *Flora of the Presidency of Madras*, Part IX (edit. by Gamble, J. S.) (Adlard and Son, London, 1931).
[2] Sakdisuwan, S., thesis, Chulalongkorn Univ., Bangkok (1967).
[3] Vacharabhaya, M., thesis, Chulalongkorn Univ., Bangkok (1969).
[4] *Composition of Foods, Agricultural Handbook No. 8* (United States Department of Agriculture, Agricultural Research Service, Washington, 1963).
[5] *Production Yearbook*, **23** (Food and Agriculture Organization, Rome, 1969).

Effect of Purified Luteinizing Hormone Releasing Factor on Normal and Hypogonadotrophic Anosmic Men

THE occurrence of familial and nonfamilial cases of hypogonadotrophic hypogonadism and hyposmia or anosmia (Kallman's syndrome) has been well documented[1,2]. Because the disease has been found only in humans, little is known about the site of the gonadotrophic lesion. Pathological examination of the pituitary glands of these patients has not revealed any abnormality[3]. No estimates of pituitary gonadotrophin content in patients with hypogonadotrophic hypogonadism are available, although patients who have normal amounts of circulating follicle stimulating hormone but who lack luteinizing hormone (LH)—the so-called "fertile eunuch syndrome"—have been described. In contradistinction to the Kallman syndrome, these (potentially) fertile patients have a normal sense of smell and testicular development with varying degrees of spermatogenesis[4]. Injections of gonadotrophic substances cause an appropriate gonadal response in patients with Kallman's syndrome[5] and the lesion is considered to be central in origin[6]. Attempts to stimulate the release of gonadotrophin in these patients by the use of the antioestrogen clomiphene citrate have been unsuccessful[7].

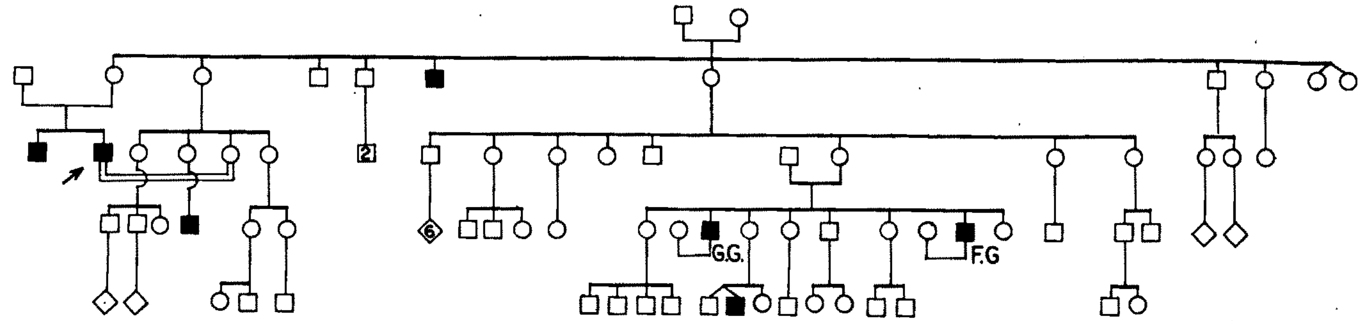


Fig. 1 Pedigree of the hypogonadotrophic subjects. Closed squares denote males with hypogonadotrophic hypogonadism and anosmia.

We have used purified luteinizing hormone releasing factor (LRF) to study these individuals. Two brothers aged 28 and 30 with hypogonadotrophic hypogonadism and anosmia are part of a kinship demonstrating this syndrome (Fig. 1). F. G. (age 28) had taken methyl testosterone (25 mg twice a day) with both subjective and objective signs of androgenization until 2 weeks before receiving LRF. G. G. (age 30) had taken 10 mg of methyl testosterone daily without any response. G. G. was pretreated with clomiphene citrate (300 mg daily for 5 days followed by 5 days of no treatment) before receiving LRF. Both patients were given 300 median eminence extract equivalents (MEE) of purified ovine LRF in physiological saline solution (stage three[8] prepared by Dr H. Gregory) by intravenous drip over a period of 3 and 6 min respectively without untoward effects after an initial episode of flushing. A normal male control (M. B., age 32) was given 100 MEE of ovine LRF by intravenous drip over a period of 7 min. All three subjects had an intravenous physiological saline drip running for 30 min before injection of LRF. The subjects were unaware of the time that the LRF infusion began. The results of this treatment were measured by radioimmunoassay of plasma LH[9] and are shown in Fig. 2. As expected, in the control male there was an abrupt increase in plasma LH with a characteristic time course including a peak 35 min after infusion began. The two hypogonadotrophic brothers had very low levels of plasma LH which at no time during treatment reached the lower limits of normal for males although they had received an LRF dose three times that given the normal male. Yet both had a rise of plasma LH from control values (1.6±0.1 mIU/ml. (F. G.); 1.8±0.15 mIU/ml. (G. G.)) to peaks at 2.7 and 3.5 mIU/ml. respectively 10 min after infusion began. Although the responses were small, they are within the sensitivity of the method and we believe that they represent a real response. At no time during the preceding 6 months did repeated control levels of plasma LH exceed 2.0 mIU/ml. in the two experimental subjects or in two other affected members of this kinship who had not received testosterone. There was no immunological LH activity in the LRF preparation when it was tested in assays which measure sheep or human LH. The possibility of a non-specific response is ruled out by the lack of response to the control infusion and the characteristic timing of the responses to the LRF infusion[10]. The lack of an effect of stress on LH levels in the male has been reported[9]. All three subjects showed what may have been a slight secondary peak in plasma LH 2 h after infusion with LRF. Dosing with clomiphene citrate (300 mg/day) did not have a measurable effect on plasma LH in subject G. G. Nor can we see any potentiating or other effect of the clomiphene pretreatment of the response of G. G. compared with F. G.

The minimal responses in affected patients could reflect a lesion in the pituitary gland or be the result of chronic depriva-

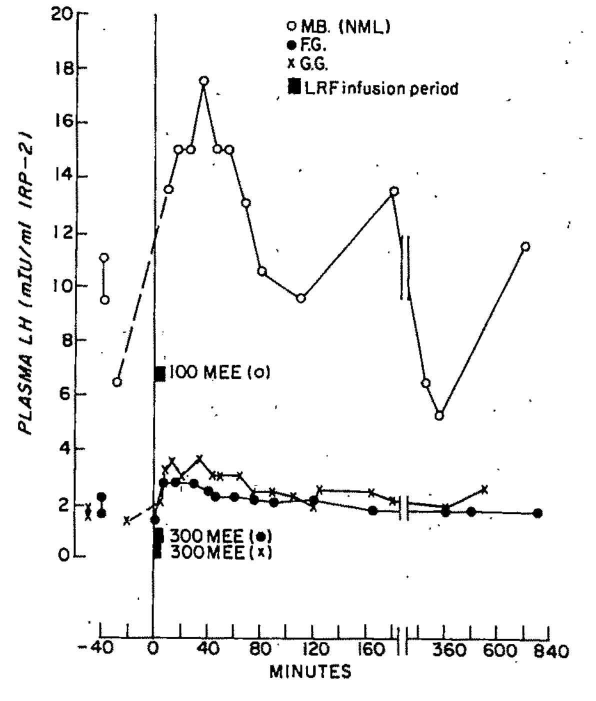


Fig. 2 Plasma LH during control periods and after LRF infusion. ○, M. B. (NML); ●, F. G.; ×, G. G.; ■, LRF infusion period.

tion of releasing factors secondary to a central nervous system disorder. In this regard studies utilizing more prolonged dosage or measurement of pituitary gonadotrophic content in these patients will be of interest.

We thank Dr C. Alvin Paulsen for reagents and Mr M. C. Sood for technical assistance. Part of this work was supported by a grant from the Medical Research Council and a special research fellowship, National Institute of Child Health and Human Development, US Public Health Service (to F. N.).

F. Naftolin*
G. W. Harris

MRC Neuroendocrinology Unit,
Department of Human Anatomy,
University of Oxford

M. Bobrow

MRC Population Genetics Unit,
Headington, Oxford

Received May 5, 1971.

---

* Present address: Department of Obstetrics and Gynecology, School of Medicine, University of California, San Diego, PO Box 109, La Jolla, California 92037.

[1] Kallman, F., Schonfeld, W. A., and Barrera, S. E., *Amer. J. Ment. Defic.*, **48**, 203 (1944).
[2] Nowakowski, H., and Lenz, W., *Recent Prog. Hormone Res.*, **17**, 53 (1961).
[3] Gautier, G., *Acta Neuroveg.*, **21**, 345 (1960).
[4] Faiman, C., Hoffman, D. L., Ryan, R. J., and Albert, A., *Mayo Clin. Proc.*, **46**, 661 (1968).
[5] Paulsen, C. A., in *Textbook of Endocrinology*, fourth ed. (edit. by Williams, R. H.), 405 (Saunders, Philadelphia, 1968).
[6] Bardin, C. W., Ross, G. T., Rifkind, A. B., Cargille, C. M., and Lipsett, M. B., *J. Clin. Invest.*, **48**, 2046 (1969).
[7] Bardin, C. W., Ross, G. T., and Lipsett, M. B., *J. Clin. Endocrinol.*, **27**, 1558 (1967).
[8] Gregory, H., Walpole, A. L., Charlton, H. M., Harris, G. W., and Reed, M., in *Pharmacology of Hormonal Polypeptides and Proteins* (edit. by Martini, L., and Paoletti, R.), 123 (Plenum Press, New York, 1968).
[9] Naftolin, F., Espeland, D., Tremann, J. A., Dillard, E. A., and Paulsen, C. A., in *Gonadotropins* (edit. by Rosemberg, E.), 373 (Geron-X, Los Altos, California, 1968).
[10] Bayman, I. W., Harris, G. W., and Naftolin, F., *J. Physiol.*, **210**, 3P (1970).

Haemoglobin Type and Fertility of Merino Ewes grazing Oestrogenic (Yarloop Clover) Pastures

Clover infertility disease in sheep is attributed to the high concentrations of oestrogenic substances found in certain legumes such as subterranean clover, variety Yarloop[1]. Reduction in flock fertility can be as high as 75% with additional losses of lambing ewes due to dystocia[2,3]. Where large areas of these clovers are grown, as in western and southern Australia, the economic loss is considerable. Significant progress has been made in selecting clover varieties which have a low oestrogen content[4] but replacement of established oestrogenic species has had limited success and the cost of renovation is often prohibitively high. We wish to report some observations which suggest that it may be possible to select and breed animals likely to be resistant to clover infertility disease using existing flocks classified on the basis of their haemoglobin (Hb) type.

The reproductive performance of fifty South Australian Merino ewes (Koonoona strain) grazing Yarloop dominant pastures was compared with that of fifty similar ewes grazing neighbouring rye grass pastures essentially free of Yarloop clover. Both groups were mated for the first time at 1.5 yr of age in February 1969 and again in February 1970. Mating and lambing performance was recorded and surviving ewes were Hb typed[5] in November 1970.

The data from 1969 and 1970 showed similar trends and are presented as combined totals in Table 1. The small number of HbAA type ewes were combined with the HbAB types to allow statistical comparison (χ^2) of fertility between Hb types within pasture types. Fertility of ewes grazing Yarloop (53.0%) was significantly ($P<0.001$) lower than that of ewes on grass pasture (83.1%). But on the Yarloop pasture, the HbBB types were significantly ($P<0.01$) less fertile than HbAA+AB types suggesting that the HbA gene is associated with a fertility advantage on oestrogenic pastures which is not apparent on non-oestrogenic pastures.

Table 1 Fertility and Haemoglobin Type of Ewes grazing Yarloop versus Grass Pastures for 1969 and 1970

	Hb type				
	AA	AB	BB	Total	χ^2 value of difference between Hb types (AA+AB)−(BB)
Yarloop pasture					
No. of ewes mated 1969	4	23	17		
1970	4	22	13		
Total	8	45	30	83	
No. of fertile matings	8	26	10	44	
% of fertile matings	100.0	57.8	33.3	53.0	7.55†
Grass pasture					
No. of ewes mated 1969	6	20	21		
1970	4	17	21		
Total	10	37	42	89	
No. of fertile matings	10	30	34	74	
% of fertile matings	100.0	81.1	80.9	83.1	0.32 (n.s.)
χ^2 values of fertility difference between pastures within Hb types	5.22*		15.35‡	18.17‡	

n.s., $P>0.05$; * $P<0.05$; † $P<0.01$; ‡ $P<0.001$.

The possibility of an association between Hb type and reproductive performance in sheep was first reported by Evans and Turner[6], who investigated Peppin Merino flocks in the Cunnamulla district and Deniliquin district of eastern Australia and found that ewes with Hb type BB and HbAB were slightly more fertile than HbAA ewes. In contrast, Obst[7,8] has found in experiments at Parndana Research Station, Kangaroo Island, using south Australian Merino (Bungaree strain) sheep that the HbAA ewes produced more lambs and that these lambs survived better than lambs from ewes of the HbAB or HbBB type.

In the light of this evidence, differences between the reports could be resolved by taking into account the previously unsuspected susceptibility of HbBB ewes to clover disease. Clover disease is a continuing problem on Kangaroo Island and it is therefore significant that Obst and Evans[8] reported that the superior reproductive performance of the HbAA ewes was best expressed during wet lambing seasons when Yarloop clover tends to dominate in mixed pastures at Parndana. The nature of the relationship between Hb type and resistance or susceptibility to clover disease remains to be determined. Some ewes are resistant to clover disease, for even in severe outbreaks they maintain good reproductive performance in spite of the measured high oestrogen content of the pasture[9].

It has been suggested that the oestrogenic activity of the plant isoflavones is due to their metabolic derivatives[10,11] and individual differences in these transformations might be expected. Differences could exist in the ability of individual animals to metabolize and excrete active substances. Alternatively, the Hb type itself may confer some yet unknown physiological advantage. HbA is known, for example, to have a greater affinity for oxygen than HbB with a different oxygen dissociation curve[12]. Conceivably, this may have implications for gamete, embryo or foetal survival.

Haemoglobin type in sheep is controlled by a pair of alleles at a single locus[5] and therefore the HbAA gene frequency of any flock can be readily increased by mating with HbAA type rams. If the relationship of Hb type to clover disease susceptibility is confirmed in Merino and other breeds by more extensive trials, now in progress, Hb type selection could provide a practical basis for low cost management of this disease.

J. M. Obst
R. F. Seamark

Department of Animal Physiology and
Department of Obstetrics and Gynaecology,
University of Adelaide

C. J. McGowan

Department of Agriculture,
Parndana, Kangaroo Island,
South Australia

Received March 10; revised May 10, 1971.

[1] Moule, G. R., Braden, A. W. H., and Lamond, D. R., *Anim. Breed. Abst.*, **31**, 139 (1963).
[2] Bennetts, H. W., Underwood, E. J., and Shier, F. L., *Austral. Vet. J.*, **22**, 2 (1946).
[3] Maxwell, J. A. L., *Austral. Vet. J.*, **46**, 533 (1970).
[4] Gladstones, J. S., *Austral. J. Agric. Res.*, **18**, 713 (1967).
[5] Evans, J. V., King, J. W. B., Cohen, B. L., Harris, H., and Warren, F. L., *Nature*, **178**, 849 (1956).
[6] Evans, J. V., and Turner, H. N., *Nature*, **207**, 1396 (1965).
[7] Obst, J. M., thesis, Univ. New England, Armidale, Australia (1968).
[8] Obst, J. M., and Evans, J. V., *Proc. Austral. Soc. Anim. Prod.*, **8**, 149 (1970).
[9] Beck, A. B., *Austral. J. Agric. Res.*, **15**, 223 (1964).
[10] Braden, A. W. H., Hart, N. K., and Lamberton, J. A., *Austral. J. Agric. Res.*, **18**, 335 (1967).
[11] Shutt, D. A., and Braden, A. W. H., *Austral. J. Agric. Res.*, **19**, 545 (1968).
[12] Dawson, T. J., and Evans, J. V., *Amer. J. Physiol.*, **209**, 593 (1965).

Effects of *l*-Δ^1-Tetrahydrocannabinol on Temporally Spaced Responding and Discriminated Sidman Avoidance Behaviour in Rats

Several investigators have attempted to find a safe but reliable means of determining whether a new drug will produce hallucinations in man[1-4]. Smythies *et al.* have found that rats show a characteristic "hallucinogenic profile" on various standard behavioural tasks. We ran rats on two of these tasks—responding on a differential reinforcement of low rate (DRL) schedule and discriminated Sidman avoidance—after administration of *l*-Δ^1-tetrahydrocannabinol (THC), a known hallucinogen for man[5-7]. We hoped to find out whether the rats would display the hallucinogenic behavioural profile; and by using the same dose range for each task, to find the dose-effect relationship.

The THC came from a single sample and was stored dry in a sealed container under nitrogen. Analysis by gas liquid chromatography (3% OV-17 on Gas Chrom Q, glass column 5×1/8 feet at 250° C) yielded 95% pure THC after 1 year of storage. The vehicle consisted of 50 µl. of double-distilled ethanol plus 950 µl. of 1% 'Tween 80' in steroid suspending medium. The THC was added to the vehicle immediately before use.

A rat on a DRL schedule receives reinforcement (usually food or water) only if it pauses for a predetermined length of time (say 10 s) since its last response. Responses which occur before the end of the interval reinstate the entire interval and postpone reinforcement, so that the schedule promotes long interresponse times. Smythies *et al.*[4,8] have reported that when rats have been trained on DRL 15 (s) their lever-pressing behaviour is susceptible to disruption by the administration of hallucinogenic drugs. Specifically, a relatively small dose of mescaline HCl (12.5 mg/kg) has "... an all or none inhibitory effect on positively reinforced behaviour. At the onset of action the animal stops pressing the lever quite suddenly and restarts abruptly sometime later"[4]. To the best of our knowledge, there have been no reports of other drugs producing this particular pattern of behaviour. Sample cumulative records obtained from our rats under 12.5 mg/kg mescaline on DRL 10 are shown in Fig. 1, and agree with those of Smythies *et al.* in spite of minor differences in procedure. The cumulative records clearly show a cycle of normal responding, complete cessation, and sudden return to accurate temporal responding. Our object was to find out if THC would produce the typical hallucinogenic (mescaline) profile.

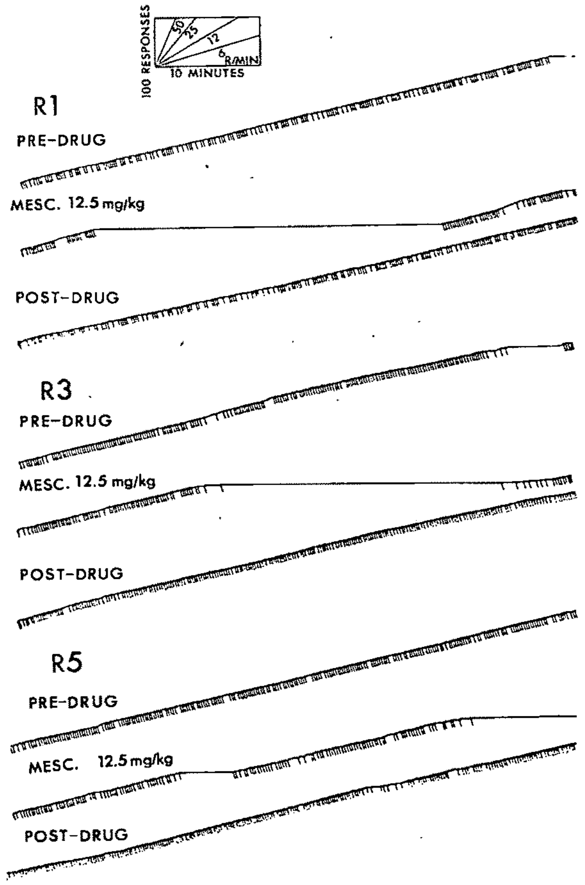


Fig. 1 Cumulative response records obtained from rats R1, R3, and R5 under 12.5 mg/kg of mescaline. Slope of line is a direct function of rate of responding. Reinforcements are indicated by downward pips. Records obtained on the day before drug (pre-drug) and the day following drug (post-drug) appear above and below the drug day.

Seven male hooded rats, about 200 days old at the beginning of the experiment, were maintained at 80% of their free-feeding body weight. A standard two-bar Grason–Stadler operant conditioning chamber with one bar removed served as the apparatus. Reinforcement consisted of 45 mg Noyes food pellets.

The principal experiment lasted 8 weeks. Animals were run daily for 60 min, 5 days a week. The DRL 10 schedule was in effect throughout. Week 1 was used to collect baseline data (that is, no vehicle or drug); during week 2 vehicle-only injections were administered on the second, third and fourth days of the 5-day week; and weeks 3–8 were drug weeks. On drug weeks each of six rats received one of six doses, each in a different order (latin-square design). The seventh animal also received all six doses and was run as a spare. During drug weeks, the appropriate dose of THC was injected on the third day of the week with the vehicle being injected on the second (pre-drug) and fourth (post-drug) days. Sessions began immediately after injection. Doses were: 1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 mg/kg intraperitoneally. Total volume of each injection was 1 ml. An additional 6.0 mg/kg dose was administered to all subjects during the week after the completion of the latin-square.

Because the results were extremely variable, all the cumulative records were rated qualitatively according to the following scheme: (M)—mescaline-like (Smythies's DRL hallucinogenic profile: that is, more or less normal rate of responding with one or more fairly long pauses between runs of responding); (A)—amphetamine-like (substantial rise in response rate with decrease in reinforcements); (B)—below baseline rate (decreased responding, but sustained throughout the session); (C)—cessation (complete cessation of responding or cessation for more than half the session); (N)—no appreciable change from pre and post-drug days. The reliability of our classifications was checked by an independent rater not involved in the experiment. Only two of forty-nine total ratings were found to be discrepant. Not only was there wide variation in the quantitative effect at each dose, but the pattern of responding, as explained later, varied markedly from one drug test to another.

Examples of response patterns taken from various rats given different doses during this experiment are shown in Fig. 2. The important point is that, although behaviour was disrupted in twenty-two of the forty-nine cases, the Smythies's hallucinogenic profile was evident in only four records (one rat at 3.0 mg/kg, another at 4.5 mg/kg and another at both 6.0 and 9.0 mg/kg). Even with these four records the correspondence with Smythies's data was not impressive: response rate between pauses was increased as shown in R3's drug record (Fig. 2). Of the remaining eighteen records in which disruption was apparent, two were classified as Bs, two as As and fourteen as Cs.

It might be argued that the 1.5 to 9.0 mg/kg dose range used in the present study was simply too narrow, and that we might have produced more of the so-called hallucinogenic profiles had we used a different range. This does not seem likely in view of the fact that one of the four most mescaline-like profiles was obtained with a dose of 3.0 mg/kg and another was found at 4.5 mg/kg. Also, some behavioural change was evident during many of the THC test sessions. In a preliminary study we found that doses of 12.0 mg/kg produced complete cessation of bar pressing on a variable interval schedule; in this study there was a tendency for overall level of responding to decrease with increase in size of dose.

To find the effect of the drug on total response rate, an analysis of variance was performed on the ratio of the total drug-day responses over the sum of the drug-day responses and the pre-drug-day responses. A score of 0.5 would indicate no effect of the drug; a larger score, that the drug increased the response rate; a smaller one, that it decreased response rate. Mean ratio scores in order of increasing dose from the six rats in the latin-square were 0.44, 0.41, 0.40, 0.50, 0.31 and 0.25. The second 6.0 mg/kg dose yielded a mean score of 0.49. Ratios based on post-drug-day scores were similar, indicating that there were no drug carryover effects. A latin-square analysis based on the response ratio scores yielded dose ($F=2.34$, d.f. = 5, 20) and animal ($F=2.40$, d.f. = 5, 20) effects approaching statistical significance (in each case, $0.05<P<0.10$). The fairly high F value across animals is probably because two rats were relatively unaffected by the drug. The drug effect can apparently be attributed to decreased performance at the two highest doses, an effect also found with the spare animal. There was no suggestion of a reliable effect across time ($F=0.74$), so an analysis of variance was completed with the ratio data from all seven rats. Mean ratio scores in order of increasing dose were 0.44, 0.42, 0.42, 0.51, 0.27 and 0.23. (The second 6.0 mg/kg dose yielded a score of 0.50.) The analysis revealed a significant dose effect ($F=3.93$, d.f. = 5, 20, $P<0.01$). Analysis of the linear and quadratic trends showed reliable effects ($P<0.005$, $P<0.05$, respectively) but these effects together accounted for only 73% of the variance. The high performance at 6.0 mg/kg probably accounts for the rest.

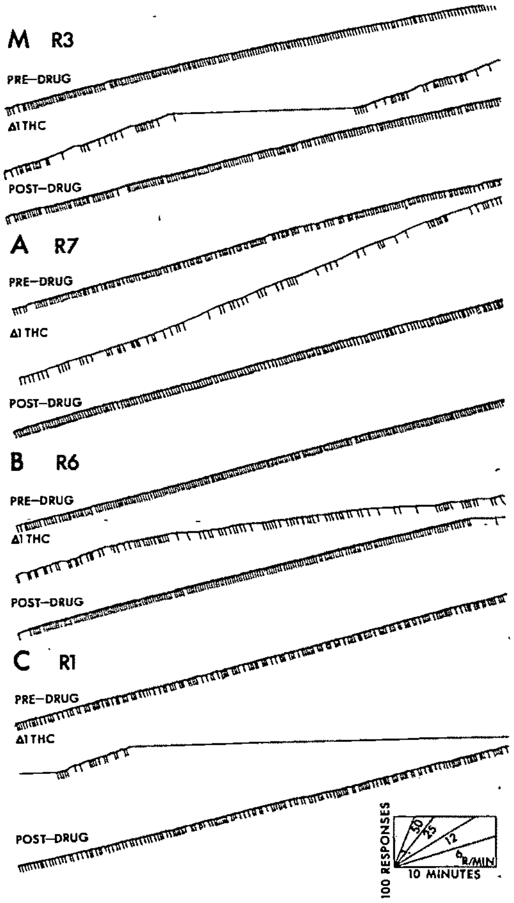


Fig. 2 Sample cumulative records portraying various response patterns under THC. Doses were 3.0, 6.0, 7.5 and 3.0 mg/kg for R3, R7, R6 and R1 respectively. See text for explanation of labels M, A, B, and C.

We decided to see if we could produce hallucinogenic profiles with mescaline. Accordingly, 2 weeks after the end of the principal experiment, all seven rats were given three 12.5 mg/kg intraperitoneal injections of mescaline spaced one week apart. The procedural details were identical to those already described except that saline was the vehicle. In sixteen of the twenty-one possible cases we produced Smythies-type profiles (Fig. 1).

Three weeks after the mescaline experiment, we administered a single 1.0 mg/kg intraperitoneal dose of α-amphetamine to five subjects. Except for one which did not respond at all, all rats had shorter IRTs[9]—the classic amphetamine effect on temporal discrimination.

The second procedure used was discriminated Sidman avoidance[10,11]. This schedule is described by referring to three time intervals. If the subject fails to respond in the response–signal interval a warning signal comes into effect and this marks the start of the signal–shock interval. If the animal fails to respond in the signal–shock interval a shock pulse is delivered at the end of that interval and the subject enters the shock–shock interval. During the shock–shock interval the signal remains on and shocks recur regularly. A response in the shock–shock interval terminates the signal and the response–signal interval is reinstated. All responses reinstate the response–signal interval to zero. Thus an animal may postpone signal onset by responding during the response–signal interval (premature responding); it may respond during the signal–shock interval (efficient responding) or, finally, it may respond in the shock–shock interval (late responding). After extensive training on this schedule, a subject concentrates its responses in the interval between signal onset and shock onset (efficient responding). The rat rarely delays pressing the bar until after shock onset (late responding) and only infrequently does it press before signal onset even though such responses are functional in postponing the onset of the signal and shock (premature responding). This discrimination can be disrupted by certain drugs: amphetamine increases premature responding and decreases late responding[11]; chlorpromazine increases late responding and decreases premature pressing[11]; and, according to Smythies *et al.*[12], hallucinogens such as mescaline, LSD-25 and N,N-dimethyltryptamine increase both premature and late responding.

The subjects were seventeen hooded male rats about 200 days old maintained at 300 g with water *ad libitum*. Twelve rats served in the actual experiment, five were predesignated as spares on the basis of their relatively poor (below 85%) discriminated avoidance performance before the start of the experiment. The apparatus consisted of three small operant-conditioning chambers each equipped with a lever, loudspeaker and white light bulb. A clicker together with the light was the signal. Scrambled shock could be delivered to the grid floor of the chamber from a Grason–Stadler shock generator set at an intensity of 1.0 mA. Shock pulse duration was 0.5 s. Response–signal, signal–shock and shock–shock intervals were set at 32, 8 and 4 s respectively.

All seventeen rats had received 130 h of training before the experiment and were avoiding more than 75% of the shocks. During the study each rat was run for two 5-day weeks with a 9-day rest between each experimental week. Half of the rats were run on weeks 1 and 3, half on weeks 2 and 4. The following routine was observed during the experimental weeks; rats were given a 30 min "warm-up" period on each day after which they were removed from the apparatus, and either handled or injected and then returned to the operant chamber. The duration of each session was 165 min, excluding the warm-up period. On day 1 of each experimental week the animals were handled, on day 2 they were injected with vehicle, on day 3 they were injected with vehicle or vehicle plus drug, on day 4 they were injected with vehicle, on day 5 they were handled. Doses of THC were 0.75, 1.5, 3.0, 4.5, 6.0 and 9.0 mg/kg THC. Two subjects were tested at each level. One of the spare rats received 0.75, two 4.5 and two 9.0 mg/kg.

There was a significant decrease in discriminated avoidance performance when drug day scores were compared with pre-drug ($t=2.66$, d.f.$=11$, $P<0.05$) and with post-drug ($t=3.32$, d.f.$=11$, $P<0.01$) scores. There were no significant vehicle effects.

Modified Bovet–Gatti profiles for the twelve rats in the experiment proper are shown in Fig. 3. In the majority of cases the profiles conform to those reported for other hallucinogens[12], that is, an increase in both premature and late responding, with a resulting decrease in efficient responses. Similar data were obtained from the spare rats. Examination of cumulative response records showed that, judging from the number of shocks received early in the session, onset of drug action was rapid. As the session progressed, premature responding increased and the subjects often failed to press in the presence of the signal. In most cases these effects persisted throughout the fairly long (165 min) session.

The THC effect was produced in both subjects tested with 1.5 mg/kg. This result, which we have repeatedly confirmed in subsequent experiments, shows that the discriminated Sidman avoidance schedule is sensitive to relatively low doses of THC. Even at 0.75 mg/kg the behaviour was disrupted in one of the two subjects.

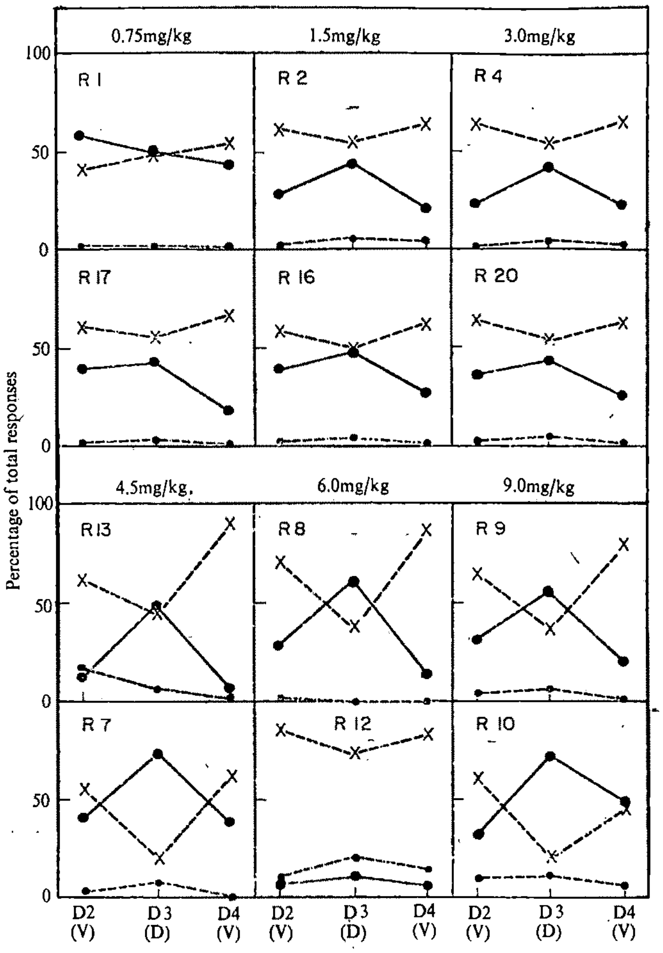


Fig. 3 Modified Bovet–Gatti profiles for twelve rats. Percentage of total responses made in premature interval (●——●), efficient interval (×- - -×), and late interval (●- - -●). Vehicle only (V) was injected on pre-drug (D2) and post-drug (D4) days; vehicle plus drug (D) on day 3 (D3).

An experiment of this design cannot be expected to reveal definite dose–response relationships. But preliminary analysis of the data from a more detailed (latin-square) study (described by M. D. W., C. D. W., and B. S. H. at the International Congress of Neuropsychopharmacology, Prague, 1970), although it confirmed the points made here, has failed to reveal any relationship between dose (within a 1.5 to 9.0 mg/kg range), and any of several measures of avoidance performance.

If a drug screening procedure is to be useful in the study of the effects of THC or other hallucinogenic drugs, it must be capable of reflecting subtle changes in behaviour at low dose levels and the particular pattern of change must be reasonably stable from animal to animal and from one drug test to the next. On the basis of the results of the first experiment, it seems that the DRL schedule does not meet either of these requirements when rats are used as subjects. With the discriminated Sidman avoidance schedule used in experiment 2, rats did show definite drug effects at low doses, and the drug effects were consistent with the main aspects of the Smythies-type avoidance profile for hallucinogens (increased premature and late responding). There was also an overall decrease in response rate only in the DRL situation. This underlines the importance of task variables and, so far, with rats, the discriminated Sidman avoidance baseline has proved to be more sensitive to THC than the DRL effects reported here. In conclusion, our findings run counter to Appel's[13] statement that positively reinforced behaviour yields more reliable psychomimetic effects at lower doses than does aversively conditioned behaviour.

Parts of this work were supported by research grants to G. C. W. from the National Research Council of Canada and the Ontario Mental Health Foundation. M. D. W. was supported by a Medical Research Council of Canada fellowship. Dr Raphael Mechoulam, School of Pharmacy, Jerusalem, Israel, provided THC. The steroid suspending vehicle was provided by the National Institutes of Health, Bethesda, Maryland. We thank Dr H. K. Kalant for helpful comments, Dr J. A. Carpenter for advice on statistics, and Michael Kocal for technical assistance.

C. D. WEBSTER

Addiction Research Foundation

M. D. WILLINSKY

University of Toronto

BARBARA S. HERRING

Addiction Research Foundation,
344 Bloor Street West,
Toronto 4

G. C. WALTERS

University of Toronto

Received July 28; revised December 14, 1970.

[1] Witt, P. N., *Die Wirkung von Substanzen auf den Netzbau der Spinne als Biologischer Test* (Springer, Berlin, 1956).
[2] Abramson, H. A., Gettner, H. H., Hewitt, M. P., and Dean, G., *J. Psychol.*, **52**, 445 (1961).
[3] Schneider, C. W., and Chenoweth, M. B., *Nature*, **225**, 1262 (1970).
[4] Smythies, J. R., Johnston, V. S., and Bradley, R. J., *Brit. J. Psychiat.*, **115**, 55 (1969).
[5] Mechoulam, R., and Gaoni, Y., *Fortschr. Chem. Organ. Naturstoffe* (edit. by Zeehmeister, L.), **25**, 175 (1967).
[6] Isbell, H., Gorodetzsky, C. W., Jasinski, D., Claussen, U., and Korte, F., *Psychopharmacologia*, **11**, 184 (1967).
[7] Hollister, L., Richards, R., and Gillespie, H., *Clin. Pharmacol. Exp. Ther.*, **9**, 6 (1968).
[8] Smythies, J. R., Johnston, V. S., and Bradley, R. J., *Nature*, **216**, 196 (1697).
[9] Zimmerman, J., and Schuster, C. R., *J. Exp. Anal. Behav.*, **5**, 497 (1962).
[10] Sidman, M., *J. Comp. Physiol. Psychol.*, **48**, 444 (1955).
[11] Bovet, D., and Gatti, G. L., in *Pharmacology of Conditioning, Learning and Retention*, 75 (New York, 1965).
[12] Smythies, J. R., Johnston, V. S., Bradley, R. J., Bennington, F., Morin, R. D., and Clark, L. C., *Psychopharmacologia*, **10**, 379 (1967).
[13] Appel, J. B., *Psychopharmacology: A Review of Progress 1957–67*, (edit. by Efran, D. H.), 1211 (Public Health Service, 1967).

Mechanism of Internal Stratification of Siliceous Sponge Spicules

BECAUSE sponges constitute an important part of many fossil records, their fine structure should be of interest to palaeontologists. Internal fine structure in spicule fossils would provide a valuable comparison with present day spicules. Furthermore, changes in the fine structure of spicule fossils may offer clues as to climatological or ecological conditions in that period.

There are two hypotheses to explain the appearance of concentric stratification of siliceous sponge spicules. Bütschli[2] contends that stratification is due to alternating layers of different refractility which he surmised is caused by a minute alveolar structure and, on the other hand, Schulze[3] maintains that the appearance of stratification represents lamellae of organic matter alternating with siliceous material. The evidence we report supports Bütschli's hypothesis of alternating layers of different refractility; but this difference seems to be caused not by minute alveoli but by periodically varying amounts of water. Schulze suggested that the concentric stratified structure is probably related to pauses in growth.

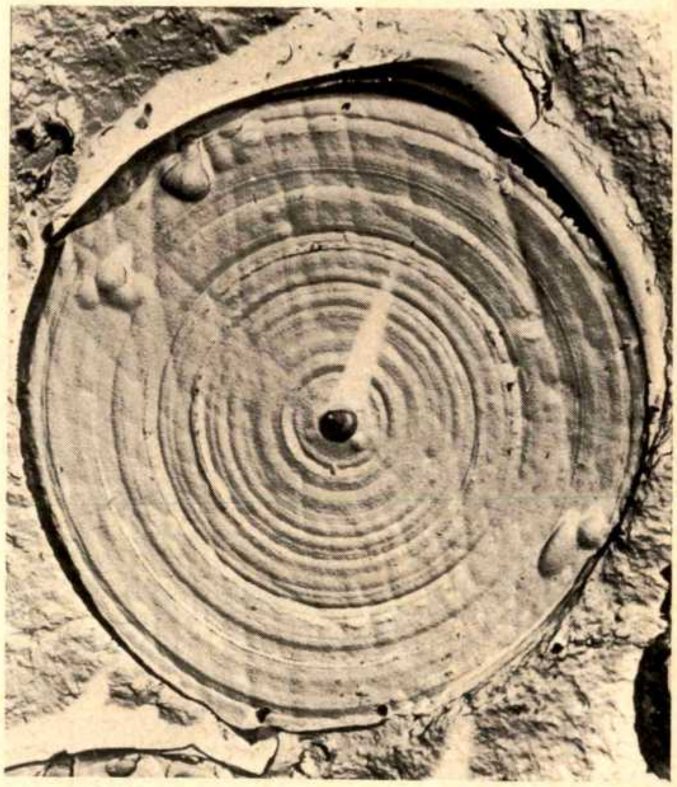


Fig. 1 Cross section of a spicule that was heated at 300° for 0.5 h then etched for 30 s in 5% HF. An extensive concentric ring structure is clearly discernible (× 8,000).

We propose that periodic pauses in the growth of a spicule permit the exposed siliceous surface to become highly hydroxylated. When growth resumes the hydroxylated surface becomes incorporated as a stratum of high etch sensitivity and low refractility giving rise to the concentric stratification revealed by electron and light microscopy[1–3]. We base this hypothesis on two new observations: first, the etch sensitivity is destroyed by heating above 300° (Fig. 1) but below 600° (Fig. 2); second, water (detected by mass spectrometry) is liberated from spicules during heating in that range (Fig. 3).

Concentric stratification is visible using light optics, but much of the detail is at or beyond the resolution of the light microscope. The following technique was therefore developed to study the fine structure of spicule sections in the electron microscope. Spicules from a siliceous sponge are embedded in epoxy resin. Sections are cut through the sample using a diamond saw so as to expose a maximum number of spicule transverse sections. The sawed surfaces are polished using cerium oxide and cleaned in an ultrasonic cleaner with distilled water. The exposed spicule surfaces are then etched in an aqueous 5% hydrofluoric acid (HF) solution for 30 s. Cellulose acetate replicating tape is used to prepare negative replicas of the etched surfaces. These are then shadowed at an angle ($\tan^{-1}$ 0.33) with platinum and carbon coated in a vacuum evaporator. Plastic is dissolved away with acetone in a vapour condensation washer and the replicas examined in a Siemens Elmiskop I.

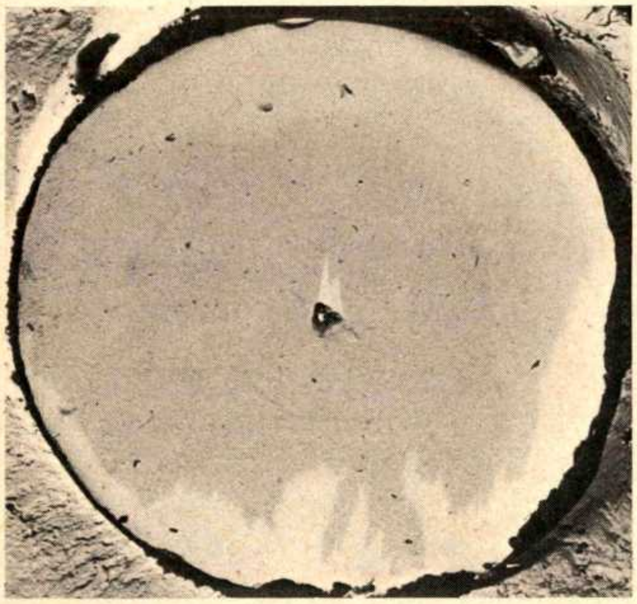


Fig. 2 Cross section of a spicule that was heated at 600° for 0.5 h. The ring structure has been obliterated (× 8,000).

Spicules are formed by specialized scleroblast cells. Growth is believed to be initiated around an organic filament and proceeds by apposition of constituent material[6,7]. Large siliceous spicules contain an axial filament which has been shown to contain protein[8]. Chemical analyses of *Acarnus* spicules indicate that there is enough organic matter present to account for the axial filament[1]. The siliceous material immediately surrounding the axial filament is more susceptible to HF attack than the bulk of the spicule. This causes the formation of a conical etch pit which in negative relief appears as a conical spire (Fig. 1). Even though the axial filament is resistant to HF attack, it is not usually preserved using this replica technique.

Fig. 1 shows a transverse section of the major macrosclere (a style ≃20 μm × 340 μm) from the siliceous sponge *Acarnus erithacus*. The replicas are negative impressions of the surface, so that any protrusions or depressions are reversed with respect to the original surface. There are several pronounced rings which probably correspond to those described by earlier investigators using light microscopy of fractured spicules[2,3]. Between these rings are many finer concentric impressions with spacings as small as 600 Å as measured in high magnification electron micrographs. Unetched spicule sections do not show any ring structure nor is any alveolar structure discernible[1].

Electron micrographs of spicules that are heated for 0.5 h at 300° show that internal structure is not appreciably altered by this treatment. Spicules heated for 0.5 h at 600°, however, no longer have a discernible ring structure (Fig. 2). Thus there seems to be a direct correlation between the loss of water vapour, or more specifically loss of inner constituent hydroxyls, and the obliteration of ring structure within the spicule.

The form of amorphous silica present in sponge spicules is probably that of silica gel[4]. Water content varies from about 6 to 13% corresponding to the formula $(SiO_2)_{2-5}H_2O$. Mass spectrometric analyses show that water vapour is evolved as spicules are heated (Fig. 3). The water peak (mass 18) rapidly rises between 300° and 400° and then drops off between 500° and 600°. Fripiat and Uytterhoeven[5], from studies involving infrared, thermogravimetric and chemical techniques, were able to distinguish among adsorbed water, surface hydroxyls and inner hydroxyls in commercially prepared silica gel. From these results they hypothesized that inner constituent hydroxyls are evolved at or slightly above 500°, whereas surface adsorbed water hydroxyls are evolved from 180° to 500°. This correlates quite well with our mass spectrometer data since the evolution of water vapour extends within about the same temperature range. Indeed it may even explain what appears to be two separate peaks in the mass spectrometer tracing. The initial peak probably represents surface adsorbed water and hydroxyls while the second peak represents inner hydroxyls.

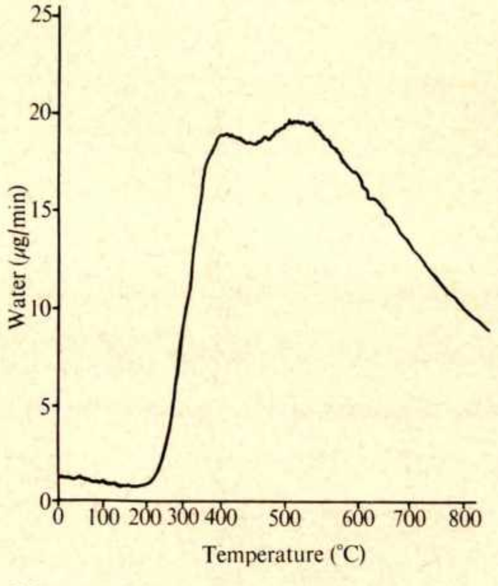


Fig. 3 Mass spectrometric tracing at mass 18 as the spicules are heated to show the evolution of water vapour.

We thank Dr J. Scala and Dr E. J. Hornyak for helpful suggestions and discussion. We also thank Dr R. K. Ware for mass spectrometer analysis.

D. W. SCHWAB

Corporate Research Laboratories,
Owens-Illinois Technical Center,
PO Box 1035,
Toledo, Ohio 43601

R. E. SHORE

Department of Biology,
University of Toledo,
Toledo, Ohio 43606

Received January 7; revised March 22, 1971.

[1] Schwab, D. W., and Shore, R. E., *Biol. Bull.*, **140**, 125 (1971).
[2] Bütschli, O., *Z. Wiss. Zool.*, **69**, 235 (1901).
[3] Schulze, F. E., *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, **4** (Fischer, Jena, 1904).
[4] Lewin, J., and Reimann, B. E. F., *Ann. Rev. Plant Physiol.*, **20**, 289 (1969).
[5] Fripiat, J. J., and Uytterhoeven, J., *J. Phys. Chem.*, **66**, 800 (1962).
[6] Minchin, E. A., *Proc. Zool. Soc.*, London, **2**, 661 (1908).
[7] Jørgensen, C. B., *Biol. Medd. Kgl. Dan. Vidensk. Selsk.*, **19**, 1 (1944).
[8] Garrone, R., *J. Micros.*, **8**, 581 (1969).

BOOK REVIEWS

Biophilosophy

Biophilosophy. By Bernhard Rensch. Translated by C. A. M. Sym. Pp. xi+377. (Columbia University: New York and London, May 1971.) £5.65.

THIS is a valuable book. The author is a distinguished German biologist who has worked in neurology and behaviour studies but is chiefly known as a member of the small international group who made important contributions to the synthetic theory of evolution, his being on *Evolution above the Species Level,* the title of an earlier work available in English (Methuen, London, 1959). People who are interested will find his authoritative, lucid, and up to date discussion of this theory most useful, in particular his "100 rules of infra-specific and trans-specific evolution". This section justifies the volume, which is well translated from the German of 1968.

The greater part of the book presents the author's matured views on more general issues. Science must endeavour to start with absolutely certain facts and these are the mental processes which each of us is aware of within himself, resulting chiefly from perception. It is therefore essential to examine critically the process of cognition by perception, and this leads the author to the problems of mind/matter (he infers a "psychophysical substratum"), theories and laws, the causal analysis of organisms, and, briefly, free-will, ethics, and religion. Biology, in my view, cannot long avoid coming closer to grips with some of these issues and I expect that many biologists, such as those studying behaviour among others, will find Rensch's analysis helpful, whether or not they go the whole way with him.

Rensch explains that his views are developments of those of Th. Ziehen (1862–1950), and he calls his own attitude "realistic" and "positivist", in special senses which he explains. He infers proto-mental properties from early evolutionary stages onwards, and he assumes not one but many basic laws in physics and biology. As a philosopher Rensch is in some respects original; as an evolutionist he is orthodox and even, for an elsewhere cautious philosopher, a trace dogmatic on points where, clarity being lacking, certainty must be also. He repeatedly asserts the standard view that "random" mutations and the recognized selective processes can together account for the entire evolutionary process. But he does not, though writing in 1968, explain what "random" means or warn the reader that this central dogma lacks precision until the term has been analysed in the light of the recently acquired knowledge of organic structure, for example in molecular biology.

Adopting the author's own high intellectual standards, one wonders if it is not possible that when morphogenesis—important for evolution theory since selection acts on phenotypes—and the internal coordination and hierarchical structuring of organisms (stressed by him) are better understood, the current formulations of evolution theory may have to be improved to make them more precise and comprehensive. No orthodoxy is aware of all its assumptions, and few remain unchanged when a later generation brings these to light. Great advances often stem from the realization that what has been taken for granted is ambiguous in a new context. Should not a senior biophilosopher bear this in mind for the sake of his young readers to whom this task of clarification may fall?

LANCELOT LAW WHYTE

Memoirs of a Master

Wanderings of a Biochemist. By Fritz Lipmann. Pp. viii+229. (Wiley-Interscience: New York and London, May 1971.) £4.25.

JUST thirty years ago, in the first volume of *Advances in Enzymology,* Fritz Lipmann presented his classic paper on the central roles of energy-rich phosphate compounds and group transfer in biology. How he arrived at these ideas and how they have dominated biochemistry ever since is the central theme of his scientific memoirs in this charming book, his "wanderings".

Lipmann was trained in medicine with a view to going into general practice, but after his final examination he began to question the morality of patients purchasing their health, and doctors selling it. For this and other reasons he drifted away from medicine and returned to his home town of Koenigsberg in eastern Germany to attend the chemistry courses given in Meerwein's department. From there Lipmann moved to Otto Meyerhof's laboratory in Berlin in 1927.

Two strong personalities dominated the Kaiser Wilhelm Institute: Otto Meyerhof, a "spiritual presence" who engaged in "unwieldy muscle energetics", and Otto Warburg. The latter, on the top floor of the institute, had a mystery about him, but his "puritanical, pragmatic approach which rejected compromises and speculation filtered through to us from the fourth floor. He became our hero: his stern insistence on letting experiments speak and keeping interpretation to a minimum has dominated our generation". For three years Lipmann dabbled in the biochemistry of phosphorylated intermediates of metabolism, which included some of the hexose phosphates and creatine phosphate, and for this he gained his PhD. Then came a brief collaboration with Lohmann, who at that time had become the master of organic phosphates, classifying them by their very different susceptibilities to acid hydrolysis. Lipmann ended his "biochemical adolescence" with Meyerhof on some important experiments which demonstrated the direct connexion between muscle contraction and the breakdown of creatine phosphate.

The next two years were spent with Albert Fischer in Berlin, and at the Rockefeller University with Levene. In New York he turned his attention to a phospho-protein present in egg yolk and from it isolated serine-phosphate.

In 1932 Lipmann returned to Fischer, now in Copenhagen, as a component of the three big L's of Copenhagen: Linderström-Lang, Lipmann and Lundsgaard. Here he was able to prove that acetyl-phosphate was an intermediate in the oxidative decarboxylation of pyruvic acid and, further, that this phosphoanhydride is used for the enzymatic phosphorylation of adenylic acid. But then the European situation deteriorated and around the middle of 1939 the Lipmanns left Copenhagen for Cornell Medical School, New York. It was here in 1941 that, influenced by the significance of phosphorylation as found by Kalckar, Lundsgaard and others, he developed his ideas on the biosynthetic importance of phosphate derivatives and group transfer.

There are three main points in the review. First, organically-bound phosphates can be divided into two main groups: those with a low energy content and those with a high energy content or "energy-rich". Second, the energy-poor compounds are generally phosphate esters and arise by transphosphorylation either from an energy-rich or energy-poor phosphate donor. By contrast, energy-rich phosphates are derived by transphosphorylation from an energy-rich donor or by other means, such as oxidative phosphorylation. Third, these phosphorylated compounds, particularly those with high energy bonds, are used for biosynthetic purposes. Lipmann drew a distinction between these two kinds of phosphate bonds by introducing the famous "wriggle" or "squiggle" or ~P. Among several suggestions, he proposed that perhaps proteins are constructed by polymerization of amino acyl-phosphates: not correct, but not far off either.

The review elicited a very negative response from physical chemists, who regarded the whole concept as dilettantish. Nevertheless, for biochemistry as a whole it was a very major step forward. For the first time a clear distinction had been drawn between the energy requirements of biosynthetic and degradative processes.

Present-day job hunters should take heart: Fritz Lipmann had given a poor talk at an important meeting and, whether or not this was the reason, at this stage of his career he had difficulty in getting a position. Eventually he was accepted in the Department of Surgery at Massachusetts General Hospital, supported by a Ciba fellowship. Fired with high energy phosphate bonds, Lipmann now set out to show that his acetylphosphate was the universal acetylating agent. But hard as he tried, acetylphosphate did not do the job either in the acetylation of aromatic amines in detoxification, or in the formation of acetylcholine. This led directly to the discovery of a new cofactor, which he called coenzyme A (A for acetylation), in 1947. For this discovery Lipmann later received the Nobel Prize for Medicine.

The general thesis of active phosphates led Lipmann and his colleagues to discover such diverse compounds as carbamyl-phosphate in the synthesis of citrulline and of "active" sulphate, an anhydride of sulphate and adenylic acid (PAPS). Since then they have turned their attention to protein synthesis, being encouraged by their neighbours Hoagland and Zamecnik. Activated amino-acids turned out to be elusive, until Paul Berg found a novel form of active acetate, acetyl-adenylate. Subsequently amino-acyl-adenylates were identified as the true intermediates. During the past several years the Lipmann group, now at the Rockefeller University, has been largely responsible for characterizing the transfer factors needed in bacterial protein synthesis and how GTP is involved in the function of the ribosome.

Lipmann's book is divided into two parts separated by a series of personal photographs. In the first half he describes, often with delectable candour, some of the great scientists who impressed him and how he came to do what he did—a sort of scientific novel which contains a sprinkling of extracts from some of his early papers. The latter part of the book contains several more recent papers, including his Harvey Lecture, delivered in 1948.

Fritz Lipmann's seventieth birthday was celebrated at the 1960 Cold Spring Harbor Symposium on Protein Synthesis. The published volume contains a short appreciation of him which starts "There is no biochemist like Fritz Lipmann. He has always been at the heart of biochemistry". And that story, which should inspire the younger generation, is delightfully told in the *Wanderings of a Biochemist*. MARK S. BRETSCHER

Man Evolves

Man's Natural History. By J. S. Weiner. (The Weidenfeld and Nicolson Natural History.) Pp. xii+255. (Weidenfeld and Nicolson: London, April 1971.) £3.50.

FOR those who seek a broad view of the biological history of man, and a general understanding of the form and causes of human diversity, there is no better book than this new addition to the Weidenfeld and Nicolson Natural History series. Professor J. S. Weiner has pioneered the modern holistic approach to the study of man; more than anyone else he is responsible for the transformation of physical anthropology from a dry and dusty academic byway, as dead in ideas as the bones with which it was largely concerned, to its present focal position in the human sciences, with relevance to almost every aspect of human existence, and engaged with some of the most exciting scientific problems of today. Weiner's views are simply and well expressed in this book. The approach is an evolutionary one, which is surely the most appropriate for an overall consideration of man's natural history: evolutionary theory provides the integrating framework for human biology as it does for the study of other organisms, and profoundly influences perspectives both in the definition of issues and in subsequent consideration of them.

Weiner, in some detail, first compares man with other higher primates, principally to identify the major trends in hominid evolution, and traces the course of human descent as it is revealed in the fossil record. Here, perforce, attention has to be focused on skeletal material, but this is considered in functional terms, and wherever possible with reference to what is known or presumed about ecology and behaviour. In recent years, studies of living primates have revealed a vastly greater diversity of form than was previously suspected, and much of this, particularly as it is manifest in locomotor systems, is of focal concern, not only in considering the affinities of man with his closest relatives, but also in attempting to discover the factors for the emergence of the hominids, with their many distinctive features. Concurrent with this increasing interest in form, function and ecology has been a spate of both laboratory and field studies of Primate behaviour. It is still not at all clear how far these studies illuminate our understanding of the origins of human sociality, but few would object to the rather broad but insightful generalizations which Weiner makes.

Following the change from an arboreal to a terrestrial existence, man's ancestors adopted a hunting mode of life. These early terrestrial hominids were confined to tropical regions and probably hunted in the heat of the day, which no doubt explains the remarkable heat tolerance capacity of man. Weiner discusses the nature of this heat tolerance, along with the many other facets of adaptability which are to be found in human physiological systems, but, as here indicated, the viewpoint is ecological. Like other human physiologists he is concerned with the nature of physiological mechanisms, but unlike so many of his colleagues he is also concerned with the effects of physiological systems as they affect and are affected by the daily lives of people.

Weiner, as a physiologist, is also rare in having a deep understanding of genetics, and he discusses at some length in this book the factors which determine the genetic structure of human populations, and which cause geographical diversity in human genetic systems. He also brings together this understanding and his physiological knowledge in interpreting the anthropological history of the various peoples of the world, and never loses sight of the importance of cultural and technological factors, both in considering adaptation to local ecological conditions and in determining the broad course of human history. After all, *Homo sapiens*, in spite of his full range of biological capacity, was still a very rare species until the discovery of technologies which allowed crop cultivation and animal domestication. Today, of course, the problems facing the species are not of rarity, but of overabundance, and in a final chapter called "Towards a World Ecology", Weiner has something to say about this which all who are concerned with the human condition would be advised to read.

G. AINSWORTH HARRISON

Good Development

Developmental Processes in Higher Vertebrates. By R. Bellairs. Pp. xiv+366. (Logos: London, March 1971.) £6.

In this book the author provides a modern account of the development of amniotes, that is, of reptiles, birds and mammals or "the higher vertebrates". As far as I know no other similarly specialized treatment is available. For mammals, in particular, there are few general works, because the earlier stages of mammalian development are usually extremely hard to investigate. Most vertebrate developmental biology concerns the Amphibia which are constantly referred to, even in this book.

The first three chapters deal with general concepts; the fourth and fifth with topics special to birds; the sixth with embryonic inductions, mostly in the chick; the seventh and eighth with topics special to the early development of mammals and reptiles respectively; the final four deal with general problems, in particular the mechanisms and control of organ growth, teratology and immunology, and some of the molecular biology of development. I find this organization logical and very satisfactory from the point of view of one trying to understand developmental processes and their control rather than to assimilate experimental minutiae. As there is very little formal structure to the study of development the author is often forced to consider several conflicting explanations of the same process without being able to come to a firm conclusion, that is, she rarely finds it possible to reject an explanation on theoretical grounds. Indeed this reinforces the view that unifying principles such as Wolpert's "positional information" are vital to the development of embryology as a science.

The book contains an extremely useful, factual summary of many aspects of amniote development; it is surprisingly up to date, and it is accurate. The only failing lies in the lack of interpretation of some of the data presented, but I feel that the role of the book is really to stimulate thought and experiment, rather than to supply answers. Thus it should be useful to both theorists and experimentalists and can be recommended to all biologists.

Anthony Robertson

Connecting Neurones

Neurons and Interneuronal Connections of the Central Visual System. By E. G. Shkol'nik-Yarros. Translated from the Russian by Basil Haigh. Translation edited by Robert W. Doty. Pp. viii+295. (Plenum: New York and London, 1971.) $22.50.

This book was originally published in Russian in 1965 and has been revised and updated by the author for the English edition. Although entitled *Neurons and Interneuronal Connections of the Central Visual System* it is principally concerned with neurones of the visual cortical areas and, to a lesser extent, of the lateral geniculate nucleus. The neurones of these structures are described in considerable detail and several species, ranging from hedgehog to man, are examined. One is fascinated, page after page, to see the drawings of neurones which come in all shapes and sizes, from the well known pyramidal neurones to their less famous brethren, "the parasol" and "semilunar" neurones.

Although the author states somewhat towards the end of the book that "every architectonic characteristic, however trivial, corresponds to a different pattern of interneuronal connections", it is clear from the beginning that he is much impressed by cytoarchitectonic differences, and describes in some detail the different arrangement and types of cells in areas 17, 18 and 19 although it is not clear whose definition of areas 18 and 19, particularly for monkey and man, is used. This limits somewhat the value of the descriptions. It seems a pity that the author should not at least refer to the studies of Lashley and Clark and of von Bonin and Bailey. These authors, to varying degrees, failed to find the cytoarchitectonic differences so confidently described by Brodman (who is mentioned). Having described the cytoarchitectonics and Golgi picture of area 17 and (the undefined) areas 18 and 19, the author gains enough confidence to predict their connexions. For he states that "most association connections originate in areas 18 and 19, which follow from the neuronal structure of these areas ... while area 17 is connected principally with subcortical structures and the periphery. This conclusion is also supported by the narrowness of layer III of area 17 in primates". Such a statement may be received with some surprise, for the fact is, of course, that area 17 has abundant association connexions in almost all species described so far.

The most interesting parts of this book are undoubtedly the sections that deal with the structure, shape, dendritic trunks and axonal branchings of the individual cells, but the rigid criteria that we now demand as proof of actual "contact" between cells are, unhappily, missing. The Golgi method, on which the description is largely based, does not and cannot, on its own, allow the identification of interneuronal contacts. The author, for example, believes that axo-axonal contacts exist in the cortex and shows a very unconvincing Golgi photomicrograph to prove his case, although axo-axonal contacts have been extremely difficult to demonstrate in the cortex by electron microscopy and must be presumed to be very rare, if indeed present at all. Many other types of contacts are described, including connexions between spines and capillaries and axonal swellings and capillaries, almost all based on light microscopy. What was reasonable evidence in Cajal's day is not sufficient evidence now.

The book abounds in speculation, frequently based on scanty anatomical evidence. All the more pity, therefore, that precious little is said of "physiological" cells among which contemporary anatomists feel much more relaxed and at home than among "anatomical" cells such as the "cup-shaped" ones. Nothing is said about "simple" and "complex" cells, for example, although in a final chapter there is much speculation on colour vision as a function of the presence and shapes of specific groups of cells in the visual pathways.

This said, however, the book is bound to have some interest to students of the cortex, if only for the descriptions of the great variety of cells that make up the cortex. In the final analysis, all these different shapes of somata must mean something, just as much as the branching patterns and course of their dendrites and axons do. The book may also help to familiarize scientists in the West with Russian literature on the subject. Much of the material referred to in the book is apparently available in Russian only and therefore presumably unknown to most workers in the West. The highly readable translation will, it is to be hoped, remedy this situation, if only slightly.

S. Zeki

Hydronautics

Hydronautics. Edited by Herman E. Sheets and Victor T. Boatwright, Jr. Pp. xvii+454. (Academic: New York and London, September 1970.) £10.25.

Hydronautics is one of those recently-coined words which mean different things to different people. If aeronautics is the art of navigating in the air, then I would have thought that hydronautics should be the art of navigating in (and possibly on) the water: its etymology is certainly this. However, this book (to quote the preface) "treats the major scientific and engineering disciplines related to ocean technology". There are seven chapters each by a different author.

The chapter on ocean resources, by J. H. Clotworthy, is readable, well informed and includes some very sensible comments on potential exploitation. It covers the whole field from petroleum and fish to pollution (a "resource in reverse") and recreation. The only serious fault with this chapter is an almost complete lack of references, which is a pity since he so often succeeds in arousing one's interest in a topic, but having such a wide field can only deal with it briefly.

In the second chapter F. N. Spiess discusses the oceanic environment as it relates to information gathering and transmission interpreted rather broadly. About two-thirds of the chapter is devoted to acoustics, which is in proportion to its importance, but there are useful sections on electromagnetic systems (including light and infrared), gravitational variations and background radioactivity. This is another well written and informative chapter and it has a good bibliography.

R. L. Wiegel's chapter on ocean dynamics is a great disappointment in that it would be more appropriate to a research review than to a book on marine technology. For example, twenty-five pages are devoted to a scholarly review of the processes of wave generation, but only eighteen lines to the wave climate, which is possibly the most important aspect of ocean dynamics in relation to marine engineering.

The chapter on underwater navigation, by F. A. Andrews, devotes most of its space to acoustic position fixing, but has good descriptions of other navigational aids such as electromagnetic logs, inertia navigation systems and doppler-acoustic navigators.

Dealing with marine vehicles and structures, O. H. Oakley discusses the design of research and survey vessels, floating platforms and submersibles. This is a considerable part of the field of naval architecture, and in sixty-four pages means that the treatment is bound to be somewhat superficial. However, it is generally a good introduction to the subject with an adequate bibliography. An important omission is the absence of any discussion of the various modes of failure of pressure hulls.

The subject matter of sea systems by J. P. Craven and P. R. Stang largely duplicates that of other parts of the book, and in particular the previous chapter. One rather feels that "systems" is a popular word at present and that a chapter with this title had to be included even though the editors had no clear idea of what they meant by it. It is written in a pretentious style and adds little to the value of the book.

I am afraid that I have to admit to finding articles on policy generally a bore, particularly when they deal with the principles of planning. It is therefore possibly not H. E. Sheets' fault that I find his chapter on policy planning unreadable.

To sum up, the value of the good chapters in the book is such that they should be read by all students of marine technology, and it is a pity that the high price will stop most individuals from buying it. M. J. TUCKER

Particulate Systems

The Dynamics of Aerocolloidal Systems. By G. M. Hidy and J. R. Brock. (International Reviews in Aerosol Physics and Chemistry, Vol. 1.) Pp. xv+379. (Pergamon: Oxford and New York, January 1971.) £10; $27.

THIS book is intended to provide a broad introduction to a new series of reviews in aerosol physics and chemistry, and to be an up to date monograph on the subject of aerosol dynamics. Aerosol scientists who have worked in a field practically devoid of specialist literature until the advent of Fuchs and of Green and Lane (Fuchs, N. A., *Mechanics of Aerosols*; Pergamon, Oxford, 1964; Green, H., and Lane, W., *Particulate Clouds, Dusts and Smokes*; Spon, London, 1964) may find themselves overcome by the amount of literature now appearing, and the prospect of the regular appearance of further books in this series may seem rather daunting.

The chapter titles promise a comprehensive coverage of the subject, but more detailed reading gives the impression that while some topics are dealt with exhaustively, others get only cursory treatment. The scope and limitations of the book are honestly set out in the introduction, where the aim is given "to explore the dynamical behaviour of idealized aerosol particles in the light of modern developments in classical mechanics". Thus there is no reference to the effect of particle shape on aerosol behaviour and no mention of the important recent work of Stöber and others on this subject. This is a pity, for however intellectually satisfying it may be to provide theoretical treatments for the behaviour of spherical particles, they bear little relation to most real life aerosols.

Again, according to the introduction, the effects of electrostatic charging on the particles are taken into account, at least in an elementary fashion. But there is no mention of the charging mechanism or charge distributions or the now classical work of Bricard.

It is inevitable, although perhaps unfair, that this book will be compared with Fuchs and Green and Lane. It adopts a very much more theoretical viewpoint than either of the other two, and although experimental work is very frequently referred to, it is generally quoted quite uncritically and sometimes rather uninformatively.

It will therefore appeal to the theoretician rather than to the experimental physicist. This is perfectly acceptable, and no doubt the series as a whole will give a balanced view of the entire subject.

The errors I found were entirely in the indexing, for example, references to Green, H. and Grad, H. are mixed up. This is unimportant, but there is little excuse for the misspelling of a particular author's name everywhere it occurs.

W. J. MEGAW

Discrete Astronomy

Relativity and the Question of Discretization. By D. G. B. Edelen and A. G. Wilson. (Springer Tracts in Natural Philosophy, Vol. 20.) Pp. xii+186. (Springer-Verlag: Berlin and New York, 1970.) 38DM; $10.50.

THE possibility that objects of astronomical interest have a discrete structure has not been seriously considered by many workers in this field, presumably because there is no great body of evidence to support this view. The authors of this book, however—one a mathematician and the other an astronomer—take the minority view and argue for the possibility that the sizes of galaxies form a discrete sequence similar to that found for angular momentum in quantum theory. This they attempt from two different viewpoints, one theoretical and the other observational.

As the title indicates, general relativity provides the framework for the development of the theory in the first section. Consideration is restricted to EO galaxies since a definition of the idea of "size" for these presents fewer problems than for the non-spherical cases, though rather than use the radius to a surface where some physical parameter is specified the authors introduce the concept of a "jump discontinuity". This is physically identified with the surface where galactic space structure gives way to free space. A length scale is derived using this concept, and on the assumption that a parameter appearing in the derivation does not vary from galaxy to galaxy a sequence of possible sizes is obtained. The assumption seems to have no physical basis and to have been made for no reason other than that a discrete set may result, so one is doubtful of the conclusions drawn.

The second part of the book deals with what observational evidence there is to support their view, and starts by examining a sample of thirty-one galaxies for evidence of discretization. It is surprising and disturbing to find that of the two slightly different methods used to obtain linear diameters using redshifts as a distance indicator, one yields significantly discrete diameters and the other does not. Also a surprise is the apparent dependence of the minimum possible diameter on redshift, and an extension of the method indicates discretization of redshifts without being convincing.

The style of writing makes this book difficult to read and tends to obscure rather than clarify the issues. This is not a volume that I feel I should recommend. R. F. CARSWELL

CORRESPONDENCE

Scientists' Salaries

Sir,—There is already an attitude among administrators that because Fulton is beginning to have an effect professional staff (scientists and the like) can expect their highest rewards by transferring from their basic class to the administrative posts which may become available. (Four of the eight recently appointed Regional Managers in the Ministry of Agriculture were from the professional ranks.) This is surely to be deplored and resisted in that the most valuable scientists and technologists often have more to contribute in their professional work than in any transfer to administrative duties and should expect parity with their administrative colleagues which will now be lost if the present pay award is not altered by arbitration.

Secondly, I must mention a particularly mean action which is being proposed and so far has not been altered by negotiation. It affects a relatively few senior members of the former National Agricultural Advisory Service who transferred from colleges and county councils in 1946 to form the then newly formed service. They were given an undertaking that their retirement age would remain at 65 and this would thus partially compensate them for the loss of pension rights which they suffered in the transfer. (The years of employment prior to 1946 are only counting as half the years in calculating their retirement pensions.)

In the arrangements for the unfortunate reduction in the size of the service, those under 60 are being offered redundancy payments but those over 60 are to retire at once and are thus likely to lose up to five years of salary and 5/80 of their pension payments. The Ministry and, I understand, the Civil Service Department are saying that the undertaking given to these officers when they transferred from their former employment is not legally binding and that they will not therefore provide any redundancy payments for those compelled to retire between the ages of 60 and 65.

(Our correspondent wishes to remain anonymous.)

Baffling Brevity

Sir,—I refer to the conclusion of a letter about mercury pollution[1]: "We thank Dr Walter Olszewski, Buffalo General Hospital, for supplying the human brains."

Usually I would fully support your strictures[2] about superfluous acknowledgments, but here I feel that your readers must be left baffled by brevity. Are we to believe that Dr Olszewski has made a noble sacrifice to the cause of environmental improvement?—in which case the acknowledgments should surely be bolstered by the George Cross at the very least? Or is it that the authors of the letter are themselves not humans but computers?—a feat of programming for which Dr Olszewski might expect a large award from some electronics firm to supplement the meagre "we thank . . ." from his own creations.

Yours faithfully,

TOM CORREYBACK

3 Church Road,
Potters Bar,
Hertfordshire

---

[1] Glomski, C. A., Brody, H., and Pillay, S. K. K., *Nature*, **232**, 201 (1971).

[2] Leading article, *Nature*, **232**, 75 (1971).

Micrometeoroid Muddle

Sir,—I was very pleased to see our work on lunary microcraters and erosion phenomena mentioned (*Nature*; **231**, 492; 1971). Unfortunately, your geomagnetism correspondent fell victim to our unclear terminology. In addition, we were somewhat misquoted.

The terminology in question is the usage of the words "cosmic particles". Unfortunately, this term is in usage by workers in the field of "cosmic dust", "micrometeoroids", etc., as well as by people interested in cosmic and galactic particle tracks. No clear definition exists. In the first case it refers to *solid particles* of minute size. Particles in the 10^{-6}–10^{-13} g range of extraterrestrial or extralunar origin were described by many workers. In a broader sense the term refers to any solid matter of small grain size in space and therefore the term "cosmic particles" comes into usage. It is synonymous with "cosmic dust", "micrometeroids", etc.

In the second case the term "cosmic particles" refers to particles on an atomic scale, consisting of high energy radiation of solar or galactic origin, which are more commonly called "cosmic rays".

Over the past 6 months we have become increasingly aware of this problem of terminology and have recently decided to abandon the term "cosmic particles" completely in our work. Instead we use "micrometeoroids" and thus hope to avoid future misunderstandings.

In essence the difference is that of absolute size and/or mass coupled with vastly different flight velocities. Upon impact on a target, the "micrometeoroids" produce a minute impact crater and the "cosmic rays", due to their increased energy per mass unit, are capable of penetrating solid matter leaving characteristic tracks, *i.e.* lattice defects, etc. Thus both particle types are capable of doing damage to solid matter and are capable of mass removal. Both types of "particle" thus are lunar erosion agents. The quoted paper deals in an indirect way with the effectiveness of the two erosion mechanisms.

Our investigations quoted refer exclusively to phenomena of micrometeoroid impact. We have no data on cosmic rays, nor did we mention their effect on cosmic erosion. At the time of writing (August 1970), no such data were available. The main conclusion at that time was that the prime agent of lunar erosion is the mass removal due to micrometeoroid impact. "Cosmic ray" erosion seemed to be insignificant. Your correspondent misunderstood our reasoning and completely reversed our main conclusion. By now (July 1971), we have a somewhat better feeling for effect of the two erosion agents, and our statements in the *Earth and Planetary Science Letters* paper are substantiated.

Yours faithfully,

FRIEDRICH HORZ

National Aeronautics and
Space Administration,
Manned Spacecraft Center,
Houston,
Texas 77058

Our Geomagnetism Correspondent writes: Notwithstanding Dr Horz's willingness to take much of the responsibility for my misunderstanding, I must apologize to Dr Horz and his colleagues for making the error. My false sense of security was perhaps bolstered by their use of the term *primary* cosmic particles, for "primary" and "secondary" are, of course, adjectives used with specific meaning in cosmic ray studies. On the other hand, it is somewhat of an exaggeration to suggest that I reversed the main conclusion. The same ambiguity in the term "cosmic particles" also led me to suppose (wrongly) that Dr Horz and his colleagues were extending the term "micrometeorites" to cover cosmic rays. Nevertheless, in my interpretation of their conclusion I was careful to distinguish between "cosmic particles" (which I mistakenly called cosmic rays) and secondary ejecta from meteorite impacts—and this was surely the critical distinction, not that between the cosmic rays which were not considered by Dr Horz in the original paper and micrometeorites. Thus I do not think I misunderstood the authors' reasoning, only their terminology. In any event, this correspondence will have been useful if it leads to the avoidance of ambiguity in the future; and I am happy to have the record set straight.

Announcements

University News

James N. Butler, Tyco Laboratories Inc., has been appointed Gordon McKay professor of applied chemistry in the Division of Engineering and Applied Physics at **Harvard University**, and **Philip B. Tomlinson** has been appointed professor of botany.

The title of professor has been conferred on **Dr F. J. Weatherall**, Department of Medicine, **University of Liverpool**.

Dr J. Pitt-Rivers, Ecole Pratique des Hautes Etudes, Paris, has been appointed to the chair of anthropology, London School of Economics, and **Professor J. G. Taylor**, University of Southampton, has been appointed to the chair of mathematics at King's College, London. The following titles have been conferred within the **University of London**: professor of sociology, on **Dr P. S. Cohen**, in respect of his post at the London School of Economics; professor of biochemistry, on **Dr J. B. Pridham**, in respect of his post at Royal Holloway College; professor of chemistry, on **Dr J. H. Ridd**, in respect of his post at University College; professor of mathematics, on **Professor Herman Bondi**, in respect of his post at King's College; professor of bacteriology and immunology, on **Professor D. Gwynne**, in respect of his post at the Lister Institute of Preventive Medicine; professor of chemistry, on **Dr M. L. Tobe**, in respect of his post at University College; professor of nuclear medicine, on **Dr E. S. Williams**, in respect of his post at the Middlesex Hospital Medical School.

Mr V. R. Tindall has been appointed to the chair of obstetrics and gynaecology, **University of Manchester**, in succession to **Professor W. I. C. Morris**.

Dr J. J. Turner, University of Cambridge, has been appointed to the chair of inorganic chemistry at the **University of Newcastle upon Tyne**, and **Dr F. E. Hytten** has been appointed to a personal professorship in human reproductive physiology at that university.

Dr D. L. Gardner has been appointed to the Musgrave chair of pathology and to the directorship of the University Institute of Pathology in the **Queen's University of Belfast.**

Dr Findlay L. Swinton, University of Strathclyde, has been appointed to the second chair in chemistry in the new **University of Ulster**, and **Mr Malcolm Skilbeck**, University of Bristol, has been appointed to the third chair in education.

Appointments

Clayton R. Iverson has been appointed director-general of the **Defence Research Establishment Suffield** at Ralston, Alta., in succession to the late **B. J. Perry.**

Dr N. B. Kreitman has been appointed director of the **MRC Unit for Epidemiological Studies in Psychiatry**, at the Royal Edinburgh Hospital, in succession to **Professor G. M. Carstairs.**

Miscellaneous

Professor George B. Kistiakowsky, Harvard University, has been awarded the Priestley medal of the **American Chemical Society.**

International Meetings

August 26–31, **Stereology**, Berne (Anatomisches Institut, Bühlstrasse 26, CH-3012, Berne, Switzerland).

September 2–3, **Biochemical Society Meeting**, Brighton (Executive Secretary, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

September 2–4, **Control of Human Ageing**, Rüschlikon-Zurich (John G. Cartwright, Gottlieb Duttweiler Institute for Economic and Social Studies, The Green Meadow Foundation, Rüschlikon-Zurich, Switzerland).

September 13–15, **Surface Chemistry of Oxides**, Uxbridge (The Assistant Secretary, The Faraday Society, 6 Gray's Inn Square, London WC1).

September 15–16, **Plastics in Medicine and Surgery**, Newcastle upon Tyne (A. F. Hall, North Eastern Section Plastics Institute, ICI Agricultural Division, Billingham, Teesside TS23 1LD).

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Experiments in Participation. By George F. Thomason. Pp. 54. (London: Institute of Personnel Management, 1971.) £1. [16

Proceedings of the Royal Irish Academy. Vol. 71, Section B, No. 7: The Electronic Structures and Stabilities of Hexacarbonylchromium and Nitrogenpentacarbonylchromium. By William J. Chambera and Noel J. Fitzpatrick. Pp. 97–108. (Dublin: Royal Irish Academy, 1971.) 16p. [16

BP Statistical Review of the World Oil Industry 1970: Reserves / Production / Consumption / Trade / Refining / Tankers/Energy. Pp. 24. (London: British Petroleum Company, Ltd, 1971.) [26

Department of Education and Science. Education Survey No. 10: Potential and Progress in a Second Culture—a Survey of the Assessment of Pupils from Overseas. Pp. 38. (London: HMSO, 1971.) 30p net. [26

The Work of Aslib for the year ended December 1970. Pp. 33. (London: Aslib, 1971.) [26

Other Countries

Harmonizing Technological Developments and Social Policy in America. (Monograph 11 in a series sponsored by the American Academy of Political and Social Science.) Edited by James C. Charlesworth and Alfred J. Eggers, Jr. Pp. viii+247. (Philadelphia: American Academy of Political and Social Science, 3937 Chestnut Street, 1970.) $2. [185

South Australia. Institute of Medical and Veterinary Science—32nd Annual Report of the Council, July 1969—June 1970. Pp. 62. (Adelaide: Institute of Medical and Veterinary Science, 1971.) [185

Australia: Commonwealth Scientific and Industrial Research Organization. Report of the Division of Dairy Research for 1970. Pp. 47. (Melbourne: CSIRO, 1971). [195

US Department of Agriculture: Agricultural Research Service. General Catalogue of the Homoptera. Index to Genera and Species with Addenda and Corrigenda to Parts 1–17. Fascicle VI: Cicadelloidea. Compiled by Virginia Wade Burnside. Pp. 269. (Washington, DC: Government Printing Office, 1971.) $1.25. [195

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature+ Nature Physical Science	**£24**	**$83**	**$90**
Nature+ Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)

(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431

American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004

All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, August 13 1971

NATURE Volume 232
AUGUST 20 1971

Waiting for Lord Rothschild

A YEAR ago, the present British government was boasting of how it would differ from its predecessor by being good at management and by conducting its affairs openly, with ample discussion with interested parties before important decisions were arrived at. Nobody will forget that in the past year, the government has had a good deal on its mind. With luck, it may scrape into the European Communities, but Northern Ireland is likely to be a more durable distraction. That said, however, it is more than a little hard to understand precisely how the government hopes to rescue its reputation in its dealings with science, scientists and higher education. Certainly there is no sign of what used to be called open government. There is precious little evidence of the good management of which the government used to boast. If the issue were merely the government's reputation, of course, there are many who might consider that a fall from grace would do very little harm. The sad truth, however, is that several important matters are now being held up by the slowness with which urgent decisions are being made.

The way in which the Dainton report on the organization of basic science in Britain has vanished into limbo (see *Nature*, **232**, 287; 1971) is only one sign of the malaise. Mrs. Thatcher, the Secretary of State for Education and Science, has been telling the House of Commons for months that she would be able to make a decision on the future of the support for universites only after the University Grants Committee has completed its review of higher education. Not until the autumn, and only then with luck, will the first fruits (if that is the word) of Lord Rothschild's review of public expenditure on research and development as a whole begin to appear. And no doubt months will go by thereafter before all the uncertainties are resolved. Is it really now the government's intention to abolish the Atomic Energy Authority? What will happen to the research councils? Are they to be more closely integrated with each other and with higher education, or to be recreated in a new no man's land in between higher education and public service proper?

While no decision has been made and all the relevant documents are kept still unpublished, it is no wonder that there is widespread anxiety about the recipes for change which the government is devising. Certainly the way in which senior government scientists have been offered no pay award this year has been interpreted by many as a sign that there are harsher rods in pickle, and no amount of exhortation about the objectivity of the Pay Research Unit will convince the people concerned that their prejudices are misplaced. Most probably, people's worst fears will turn out to be illfounded, but that is only another way of saying that the government would have done a considerable public service if it had simply chosen to publish more of the raw material on which its decisions will eventually be made. If the Select Committee on Science and Technology wishes to redeem its most recent error—the scheme for setting up a special government agency to keep an eye on the growth of the British population (see *Nature*, **231**, 140; 1971)—it could do worse than go after Mr Heath's government for these inexcusable omissions and delays.

There are also policy decisions to be made, the simplest of which is one that might have been a gargantuan task as recently as a year ago—the refashioning of the machinery for administering what the government spends on basic research. This is what the Dainton report is all about. The questions with which it no doubt deals go deeper than how most efficiently to deal with the spending of £115 million a year (the present rate of expenditure) on university research and supporting facilities for university research in Britain and abroad, together with the miscellaneous collection of research projects more directly aimed at practical problems which are supported on the shoulders of the Agricultural, Medical and Natural Environment Research Councils.

In the first place, there is the question of how the research councils should be related to the other sources of university funds. For the past five years, it has been supposed that the object of the research councils should be to encourage in the pattern of university research a diversity which the necessarily egalitarian procedures of the University Grants Committee cannot provide. The policy has been succesful even though there is a chance that pure reason by itself might have worked wonders in some areas of research. Yet it is at least worth asking the question, in present circumstances, whether the research councils would not be more effective than at present if they were less directly concerned with the bread and butter of higher education, especially the support of postgraduate students, and more concerned to be highly discriminating grant-givers.

Next comes the now more familiar question of what should be the relationship of the research councils to each other. Is it really necessary to have four research councils working in the natural sciences, with another in the social sciences as well? Understandably, the people who operate the present machinery are always eager to describe its virtues, yet it remains a fact that important fields of research—whole organism biology, for example—have frequently fallen between the fingers of the two or three councils chiefly concerned. There is also the embarrassing conflict of philosophy between councils which consider that their chief obligation is to the universities and those who consider that they must "get things done", usually by the creation of intramural laboratories.

Over all these questions lies the crusty shadow of what is called the Haldane principle, the doctrine laid down by Lord Haldane during the First World War that there should be a kind of tension between those carrying out research within the government and those government departments likely to make the greatest use of the research. This is the spirit in which, first of all, the National Physical Laboratory was established as a kind of custodian of the then government's scientific conscience. At the time, the Haldane principle may have been a necessary simplification. After all, when research was still a novelty, there may have been good practical reasons for not spreading it too thinly. Now, however, the Haldane principle has outlived its universal influences. Good government departments—those concerned with transport and agriculture, for example, are ready enough to regard research as a necessary part of the machinery of government. Others, less imaginative, shelter behind the Haldane principle, which has become to them an excuse for doing comparatively little. It is no accident that the Department of Health and Social Security and the Medical Research Council between them carry out much less research of the kind likely to yield practical benefits in the operation of the health service than circumstances require. In short, this by itself is a sufficient proof that the time has come to abandon Haldane. Whatever Dainton may have to say on the relationship of the research councils to the universities and to each other, the chances are high that he will have recommended a shading of this ancient doctrine.

The government's relationship with industry is a continually vexed question. Over the years, the government has accumulated a whole string of anomalous policies. The research associations, the darlings of the early fifties, are now considered pariahs. The National Physical Laboratory, intended in an earlier age to stimulate industrial innovation, has settled down to quite a different task. The recommendations of the Trend committee in the early sixties, echoed by Mr Anthony Wedgwood Benn in his scheme for reorganizing his research and development establishments, that there should be independent research and development authorities for different fields of industrial activity, is also now a dead letter. In circumstances like these, it must be exceedingly tempting for somebody like Lord Rothschild to seek an entirely novel formula for helping to stimulate industrial innovation by the sensible use of public money.

By good chance, Lord Rothschild should know that there are many ways in which the job could be done. One of the most important is to find some way of helping to make it seem to commercial enterprises that spending money on research and development is in the long run profitable. How is this to be accomplished? One necessary change of direction is that companies should be persuaded not merely that the manufacture of a successful typewriter, for example, may be more important than the manufacture of an unsuccessful aircraft engine but that research and development have as important a contribution to make to the former as to the latter. Exhortation might help, but the precedents for this are few. Taxation (or the amelioration of it) might also help. Government purchasing, by selectivity and even its willingness to go abroad, could have an important influence on the zeal of British enterprises. In the long run, however, example is what matters most.

Should not Lord Rothschild in his Jovian view of British science examine one field from which his predecessors have usually been excluded, either by design or cowardice—the nationalized industries? At first sight, at least, too many people are doing far too little research in the nationalized industries, even though these are publicly owned. The cost of this neglect is well typified by the scandal of the broken down generating sets in the United Kingdom two years ago. To bring about a better climate for research and development in this important field would be valuable in itself, an important example to other industries and also a way of using a stock of talent which seems to have become an embarrassment to the politicians.

Higher education is also being quickly muddled, not so much through malevolence as indifference. Two weeks ago, Mrs Thatcher announced in the House of Commons her decision that the polytechnics would be given extra funds to help with their development, a step which in itself is wise and even humane, for the polytechnics have a long way to go to whatever goals they and Mrs Thatcher, in their several ways, consider to be justifiable. The trouble is that nobody is quite sure what strategy the government has for the development of the polytechnics and for their relationship with the universities on the one hand, and the colleges of education on the other. Is it, as it might be, that pushy polytechnics will in future be encouraged to usurp the position of the weaker universities in the academic pecking order? Is it, alternatively, that Mrs Thatcher was trying her best to make a virtue of necessity in much the way Mr Anthony Crosland was forced to make the best of a *fait accompli* when he first argued the case for what is called the binary system of education in 1964? Here again, perhaps, Mrs Thatcher is being compelled to wait for the report of yet another committee—Lord James's inquiry into the education of teachers. The trouble is that by continuing to spend public money on higher education without doing the government's essential job in the formation of policy, Mrs Thatcher is creating a set of circumstances from which nothing but disappointment can spring.

This is one field in which the Rothschild inquiry could help to unlock the log-jam in which important decisions seem to be imprisoned. It is only fair to say, however, that his influence will be diminished if he cannot find some way of simplifying and making intelligible the machinery at present used for making the decisions that seem to elude him. Over the years, the decision-making apparatus has grown by accident. The research councils are said now to know more clearly where they stand than under the previous government, but there is a fuzzy line between them and the University Grants Committee alongside them and the Council for Scientific Policy above them. But in any case, the council is merely advisory, as was Sir Solly Zuckerman's old Advisory Committee on Science and Technology, a body apparently distinguished by the irregularity with which it met. Scientific advisers abound—Sir Alan Cottrell in the Cabinet Office has replaced Sir Solly Zuckerman, who nevertheless has stayed on as an occasional consultant. The Ministry of Defence has its own apparatus for devising policy on research and nobody quite knows how all of them are subservient to Lord Rothschild's office, the nearest thing in Britain to the Office of Management and Budget in the United States. The consequences of this complicated and ill-defined structure are apparent in complaints like this. Although it is by now apparent that the British government habitually loses a great deal of decisiveness by its failure to be open with its constituents, it is also true that in the management of its policy on research and development, the machinery that exists is a hindrance, not a help. If ever the tablets on which Lord Rothschild is supposed to be working are brought down from the mountain, this is the first thing to be written on them.

Ducks and Drakes

IT is to be hoped that only pre-Keynesian bankers will be dismayed at the discovery that it has been necessary to restrict the convertibility of the United States dollar. Elsewhere in the world, there is plenty of recent experience to show that the most upright and high-minded people can survive the shame even of devaluation, a much more conspicuous way of passing over the claims of creditors than the package announced earlier this week in Washington.

To be sure, there are two other components of recent memory that will make those outside the United States uneasy. First of all, draconian as Mr Nixon's proposals may appear, they resemble all too closely the kinds of stratagem that British Chancellors of the Exchequer have been forced to use in the past few years in the attempt to avoid full-scale devaluation, so that even those who wish Mr Nixon well may be tempted to hedge their bets by converting whatever dollars the system will now allow. The second vivid memory is the histrionic quality of what is called the crisis through which it is now said the world is passing. If reason has any place in monetary affairs at all, events of the past week are most of all remarkable not because it has been necessary to hedge about the relationship between the dollar and other currencies but because the United States has been able to keep on buying gold at one ounce for $35 for more than a quarter of a century. How can it be that a numerical relationship established in 1946 at a remote village in New Hampshire should have persisted so long?

The first thing to be said is that the relationship between gold and the dollar has been in itself artificial. President Franklin D. Roosevelt is said to have left the gold standard in the early thirties, but nobody really knows what that means. Each decade there seems to be another crisis of conscience in the United States, and each time there seems to be a reaffirmation of the view that gold and the dollar must (at some price) be at one. The affection of the United States for gold is, of course, a powerful stimulus of the gold-mining industry but in reality the objective is not so much to provide an absolute monetary standard but rather to relate the prices paid in dollar bills for commodities imported or exported to a quantity of material which can be reckoned to have a fixed value on a time scale which is longer than the interval during which commercial transactions between nations are settled. In other words, the function of the relationship between the dollar and the price of gold has been to provide a yardstick by means of which currencies might be converted. Its disadvantage has been that stocks of gold, sometimes many times the nation's Gross National Product in value, have also been given their face value.

What can scientific research and development contribute to a solution to this problem ? The most obvious device would be relating the value of the dollar not to gold but to some naturally radioactive material, preferably one with a lifetime comparable with the lifetime of a human generation (so that hoarders would not feel too cheated) but not so great that jackdaw nations could dominate the international monetary scene to a degree not matched by their current economic performance. (In some quarters, it will be a shock to know that strontium-90 appears to have a suitable lifetime.)

These, however, are devices which share with the monetization of gold the defect of being symbolic. Why not instead seek means by which currencies are converted into each other at rates determined by the current economic performance of the countries concerned but smoothed out over an interval of time (say three months) so that commercial transactions would not be seriously impeded ? By now, the economic textbooks are thickly peppered with proposals of this kind, some of them known by the unedifying name of the crawling peg. It is almost beyond belief that in a world which chooses to rack its conscience regularly on the issue of whether the profession of science is sufficiently responsive to social needs, economists and bankers should be allowed such licence as they assume in the handling of the international exchanges. At this point, few people may be able to guess how much gold to swap for dollars three months from now, but it is a near-certainty that by then each ostrich will once more have buried his head firmly and, he hopes, permanently in the sand again.

100 Years Ago

CLIFTON COLLEGE SCHOOL OF NATURAL SCIENCE

The accompanying is one of these fortnightly papers :—

MAGNETISM

1. Soft iron can never be permanently magnetised, yet a piece of soft iron in contact with a magnet becomes a magnet. Why ?
2. What do you understand by *coercive force*, and magnetic *saturation ?*
3. How is magnetism influenced by heat ?
4. Mention the substances which are attracted by a magnet in addition to iron.
5. State one or more of the methods by which steel bars may be magnetised.
6. What is the *declination* or *variation* of the magnetic needle, and the present extent of it?

CHEMISTRY

a. For First and Second Sets, Modern Side only.

1. Mention the oxygen compounds of phosphorus, and the action of water upon them.
2. Give an account of arsenicum and its chief characteristics.
3. What are the constituents and characteristics of arseniuretted hydrogen ?
4. What is "white arsenic," and how may it be prepared ?
5. You are given a liquid suspected to contain arsenic ; by what means would you examine it ?

b. For all other Forms.

1. Ammonia gas, and hydrochloric acid gas, are brought into contact : what is the resulting compound, and to what may it be compared ?
2. What is *ammonium ?* Describe the formation and appearance of ammonium amalgam.
3. What do you know of chloride of nitrogen ?
4. What is nitric acid, and by what means may it be procured ?
5. State the action of nitric acid upon metals,—copper, tin, antimony,—and the general tendency of the acid.

From Nature, 4, 329, *August 24*, 1871

OLD WORLD

CENSUS RETURNS

Malthus Fulfilled

PRELIMINARY returns now published suggest that the population of the United Kingdom is growing less quickly than was expected as recently as 1968 (*Census 1971 England and Wales. Preliminary Report*. HMSO, £0.60). The first figures show that in April this year, the population of the United Kingdom was 55.35 million, an increase of 2.64 million or 4.9 per cent since the previous census in 1961. If this preliminary figure is to be relied on, it suggests that there has been no net growth of the population of the United Kingdom since 1968 when, in evidence to the House of Commons Select Committee on Science and Technology, the Government Actuary in the Registrar-General's Office quoted forward projections of the population with 1968 as base year with an actual population of 55.4 million. At that point, it was estimated that the population of the United Kingdom would grow more or less monotonically by about 350,000 a year until 1980. If the growth seems not to have materialized, it will invalidate either the forward projection of the British population or the method used for counting it. For what the figures are worth, the 1971 census suggests that the population of England has risen by 5.4 per cent, that of Wales by 3.0 per cent and that of Scotland by a mere 0.9 per cent. It is something of a surprise that over the decade, Northern Ireland should have the highest growth rate at 6.8 per cent.

There are few surprises in the report, although there are shifts of emphasis from 1961. The fastest growing region in the ten years was the belt from the North Midlands through Leicestershire and Peterborough to the Solent, which coincides with a slackening growth rate in the home counties. A number of local authorities have roughly doubled their population in the past ten years, several in the belt from the Midlands to the Solent.

Coinciding with the drop in the south, West Suffolk and Huntingdonshire become the fastest growing counties with increases from 128,918 to 164,201, and 159,175 to 202,337 respectively. Essex has the biggest single population leap—now 1,353,564, a gain of some 250,000—mostly overspill from London.

The figures also indicate a draining away of population from the centres of large cities—Liverpool shows a loss of 20.4 per cent over the ten years and Manchester 19.9 per cent. Although Greater London has the highest net loss of 613,429, as a percentage it is only 7.9 per cent. All seven of the largest cities in England had population drops. In the conurbations, however, the drop is less marked—on Merseyside for instance it is only some 8.8 per cent—which suggests that people are moving into the suburbs and surrounding towns of large cities.

The population in the extreme North of Britain decreased, with Lancashire, Cumberland and Northumberland all returning figures lower than in 1961, and Durham showing the smallest percentage growth of any county.

Wales still claims the most thinly populated areas of either country, with Montgomeryshire, Merionethshire and Radnorshire not only the least populated counties but also those with the lowest density of population per hectacre. The population in the ex-coal valley of the Rhondda fell while the population in the Welsh urban areas fluctuated—Cardiff and Merthyr Tydfil fell whereas Bangor and Swansea grew in numbers.

SCIENTISTS' PAY

Tribunal Tribulations

THE Institution of Professional Civil Servants and the Civil Service Department presented their respective cases to the Civil Service Arbitration Tribunal on Thursday August 12. This was the first time that the two sides had come together since negotiations were broken off on July 2.

The IPCS entered the tribunal riding high on the success of their protest meeting at the Central Hall, Westminster, two days earlier when more than 3,000 scientists from all corners of Britain came to support the institution's case. The Civil Service Department has remained unmoved by all the protest meetings that have been going on since early in July and its offer and the arguments that substantiate it have remained unchanged.

The substance of the government's offer to more than 17,000 scientists, was given in detail in *Nature*, **232**, 434; 1971, together with the IPCS's counter claim. The government offered no increase to the higher grades—Principal Scientific Officers, Senior Scientific Officers, Chief Experimental Officers, Senior Experimental Officers and Experimental Officers—and only a small increase to the other scientific grades well below that demanded by the institution. The attitude of the IPCS to this offer was shown in their submittal to the tribunal which states, "members are bitterly resentful of an 'offer' which gives no increase to many, and little to others at a time when the cost of living has risen substantially, and when other groups of employees in the public and private sectors have received substantial increases".

Mr William McCall, General Secretary of the Institution of Professional Civil Servants.

The Civil Service based its offer on the result of an independent pay research inquiry into the salaries of scientists both inside and outside the Scientific Civil Service, whereas the IPCS would have liked greater emphasis placed on internal relativities within the Civil Service. The government made its position clear in its submission. "The Official Side does not accept that it is necessary, or that it would be appropriate, to resort to horizontal relativities as a primary or even a major consideration in determining the pay of the scientific grades on this occasion

—Photograph by Mark Gerson, FIIP, ARPS.

Rt Hon. the Earl Jellicoe, Minister in charge of the Civil Service Department.

". . . we are satisfied that the comparisons established by the (Pay Research) Unit are fair and accurate, and that they provide solid evidence on which to base the pay of the scientific grades".

The IPCS countered this by saying that "Pay Research provides an inadequate and unreliable basis for the determination of pay of scientists in the Civil Service because (1) of the quite different circumstances relating to the aims, functions, organization and staffing policies between research and development in government and in industry ; and (2) the pervasive influence of the government (by far the largest single employer of scientists) on the pay of scientists in industry vitiates fair comparisons. The institution therefore considers that the pay of scientists should continue to be based solely on internal relativities."

These statements emphasize the extent of the differences between the parties. The institution pressed for the use of internal relativities to determine salaries and advanced several arguments that purported to show that pay research has several shortcomings when used in the present context. These arguments were chiefly based on the different career patterns of the government scientist and the scientist in industry. Among other factors, this results in significant differences in the ages of people doing similar jobs; for example, the equivalent of the Principal Scientific Officer in industry is nine years younger than his counterpart. The Civil Service tribunal announced their decision on the pay of government scientists on Wednesday. The overall result is a compromise between the increases asked for by the IPCS and that offered by the Civil Service Department. There will be an increase of 5% to all those classes not offered anything in the original offer. Mr. McCall described the award as deplorable.

BOX GIRDERS

Yarra Disaster

THE report of the Royal Commission into the lower Yarra river bridge disaster, published in Australia last week, has apportioned the blame for the disaster among the consulting engineers, the contractors and the bridge authority. The direct result is that the joint consulting engineers Freeman, Fox and Partners of London, and Maunsell and Partners of Melbourne, have been dismissed. To complete the bridge, the Lower Yarra Crossing Authority has instead appointed a team of engineers under its direct control. Dr W. A. Fairhurst, of W. A. Fairhurst and Partners of Glasgow, will head the team, and a company spokesman said on Tuesday that Dr Fairhurst will be involved as an individual and that the company will not be directly involved.

The commission's report sets out the series of events that led to the tragedy on October 15, 1970, when 35 people lost their lives. A gap of several inches was found between sections 10 and 11 of the west side of the bridge when they were fitted together, and the report states that difficulty in getting the two half girders to the correct relative position "without doubt was due to the fact that the camber curves on the two halves were different not only in amplitude but in the shape of the curve, so that even when connexions had been made at a number of diaphragms it was still necessary to use large forces to make the remaining parts fit."

The large force in this case was applied by means of 80 tons of concrete blocks raised onto the bridge. On September 9, 1970, a buckle was observed and the report says "there can be no doubt that the act of adding the kenteledge"—that is the concrete blocks —"was the precipitating cause of the buckle. . . . The buckle was a clear indication that partial failure of the structure had occurred. The margin of safety against complete collapse must then have been small".

The collapse occurred after an attempt to straighten the buckle on October 15. Previously a buckle had appeared in sections 8/9 on the east side of the bridge, and this had been successfully dealt with in a similar way to the ill-conceived sequence of events that took place on sections 10/11 on the west side. The objective was to remove a series of bolts and so alter the distribution of stress in the buckled structure. The commissioners sum up what followed by saying ". . . a dramatic change took place. First, the vicious buckle, which up to that stage had been limited to the inner and upper panel, spread into the adjacent two upper panels. This was accompanied by the buckling failure of the upper part of the inner web plate." The report goes on to say "from that time onwards the north half span had inadequate strength to sustain its own weight, and only survived because it was able to bear down on to the south half through the interconnected transverse diaphragms." The collapse of the bridge occurred 50 minutes later which the commissioners feel was a surprisingly long time in the circumstances, and they suggested that ". . . a small increase in safety margin might have made all the difference".

The plans and calculations of Freeman, Fox and Partners come under close scrutiny in the report, which says that "the calculations contain a great many errors of arithmetic and of engineering principle and these have gone unchallenged until this commission". The commissioners also stated that "they could not agree with Freeman, Fox and Partners' witnesses that "the design, as it now is, is unquestionably sound". Perhaps the most damning criticism is the following quotation from the report: "We reject . . . claims of non-culpability by Freeman, Fox and Partners, and find it hard to believe that those who made such claims can themselves believe them to be true". The report goes on to say ". . . a basic cause of the tragedy . . . was the design inadequacies which led to the safety margins being much too low and certainly lower than the specified values".

Freeman, Fox and Partners in their London office are silent about the report, although Sir Ralph Freeman, one of the senior partners, has been reported in a speech from Melbourne as saying that he categorically denies that the design of the west gate caused or contributed to the collapse. Sir Ralph also stated that he felt that none of the serious allegations made against his firm was well founded.

Indeed the Royal Commission's report and conclusions are the work of three men—Sir Hubert Shirley-Smith, Mr Justice Barber and Professor Bull—and there will be many differing views and opinions on the facts. It is, however, gratifying that a full report has been published. Although a bridge with a similar design collapsed at Milford Haven in June 1970, the whys and wherefores have yet to be made public.

The aspect of this disaster and report which will be of most concern to engineers in Britain is whether it will have any effect on the ability of British firms to bid for contracts abroad. A spokesman at the Institution of Civil Engineers said this week that he did not believe that the report would have any adverse effect. Only time will tell whether this is so.

ATOMIC ENERGY

Aldermaston March

THE Executive Committee of the British Society for Social Responsibility in Science has come out against the decision to transfer the Atomic Weapons Research Establishment at Aldermaston from the UK Atomic Energy Authority to the Ministry of Defence. In a statement issued earlier this week, the committee says that it is opposed to any steps to increase the size of the British defence establishment. It also argues that there are potential dangers in giving a single minister responsibility for the manufacture of nuclear weapons and for planning the strategy for using them. The statement goes on to say that under the new arrangements, it will be hard to encourage the recent tendency to divert the energies of the Aldermaston establishment to civil work, and that the publication of results not necessarily of military importance will now be impeded. It singles out for special mention the seismologists at Aldermaston who may be less valuable members of the British scientific community after being transferred to the Ministry of Defence. The committee complains that Mr John Davies, the Minister at the Department of Trade and Industry, has been "guided solely by administrative convenience" in accepting the recommendations of the Rayner committee that Aldermaston should be transferred to the Ministry of Defence, and asks him to change his mind before it is too late.

The Atomic Energy Authority confirmed earlier this week that the seismic unit at Blacknest, Aldermaston, is indeed to be transferred to the Ministry of Defence. Some of those connected with the work of the unit are confident that the transfer will entail no impediment to the free dissemination of the results of the work not directly of military significance. If there is anxiety at Aldermaston, it consists more of a fear that the Civil Service proper will be a less stimulating environment for creative work than the Atomic Energy Authority has provided. A more sombre speculation now coming to the surface is that the decision about Aldermaston, following as it does on the separation of those parts of the Atomic Energy Authority responsible for the manufacture of fuel elements and nuclear fuel, and also radio-isotopes, has left the government with very little option but to abolish the Atomic Energy Authority as such and to set up the Atomic Energy Research Establishment at Harwell as a self-contained concern as free to go its own way as any government establishment can hope to be.

FOUNDATIONS

Drawing in of Horns

THE Smith Kline and French Foundation, one of the smallest and newest of the sources of scientific funds in Britain, has been forced by rising costs and encouraged by recent successes to modify its policy on grant-giving. In the eighth Annual Report, now published, the foundation says that it will in future increase the maximum for an urgent grant from £500 to £700, even if this means that there will be less to spend on more deliberate applications of somewhat larger sums. The foundation has been spending close on £25,000 a year for the past eight years, and specializes in comparatively small equipment grants, of which eighty-one were awarded last year.

The foundation, whose trustees are Professor F. G. Young, Professor D. A. K. Black, Dr W. A. M. Duncan, and Professor Sir Ashley Miles, was set up in 1961 to dispose of funds donated by Smith Kline and French Ltd, principally for research with a medical bias. The grants, which do not exceed £2,500, are made to individuals working in universities, hospitals or technical colleges, and are most often spent on items of equipment. Ninety per cent of the grants in 1970 were to new applicants, who are given priority over applicants who have received grants before.

The foundation aims to provide for research which could not expect help from public funds, which is presumably the reason for the virtual absence of the two great medical fields of cancer and immunology from the list of research projects assisted in 1970. Five of twenty-eight recipients are working on various cardiovascular problems, but otherwise the range of fields covered is extremely heterogeneous, extending over the role of dopamine in locomotor control, endocrine function in sleep and the biosynthesis of mycobactins.

CONSERVATION

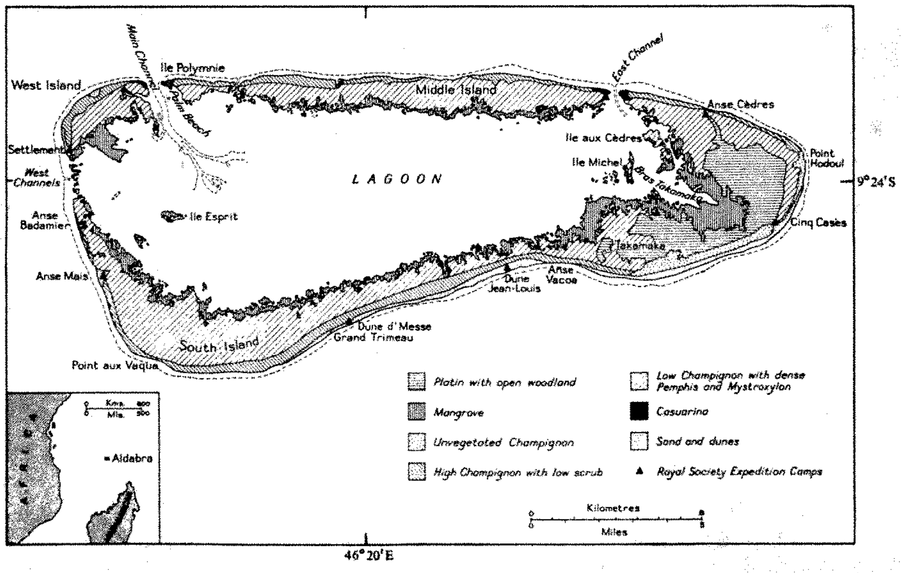
Royal Society at Aldabra

A SAFE future for the Aldabra atoll seems to be assured at last. The Royal Society has bought a fourteen year lease of this unique group of coral islands in the Indian Ocean and will pay only a nominal rent to the Commissioner of the British Indian Ocean Territory.

The Aldabra atoll is an oval ring of inhospitable coral divided into four principal islands surrounding a shallow lagoon; it is 34 km long and 14 km wide and lies northwest of Madagascar. The future of the atoll was hotly debated in 1967 when the British government was considering a proposal that it should be developed for defence purposes. The Royal Society, the United States National Academy of Sciences and the Smithsonian Institution all expressed grave concern at the time that such activities would seriously threaten almost the last natural home of the giant land tortoise (*Aldabrachelys gigantea*) and the flightless rail (*Dryolimnas cuvieri*) among other species.

At the end of 1967, the British Government abandoned a plan to build a military airfield and the Royal Society went ahead with its long term research programme which is directed by a committee under Professor T. S. Westoll of the University of Newcastle upon Tyne. The first step was the construction of a research station which was completed in June this year at a cost of £50,000. It includes a laboratory building and accommodation for a permanent staff of four, wives included, and for six visiting scientists. Although the station was principally funded by the Royal Society's grant from the British Government, United States National Academy of Sciences contributed $12,000 for the visiting scientists' accommodation and the World Wildlife Fund donated £2,900 for the purchase of a motor launch. Two-thirds of the £7,500 which the society has paid a Seychelles company for the lease was given by Mr Peter Cadbury but the day to day running of the research activities on the atoll is to be paid for out of its covenant grant. It has been costing about £30,000 a year.

Although the present lease expires in 1985, with the option of renewal for a



The Aldabra atoll.

further twenty years, a spokesman for the Royal Society emphasized this week that the society's interests are chiefly in the scientific research which can be carried out on Aldabra and that after the period of time considered necessary for the completion of the research programme, the society would hand over the lease to a conservation body.

The principal aim of the research is to understand as completely as possible the structure and function of an elevated limestone island ecosystem. Much of the groundwork was carried out during the expedition to Aldabra which the Royal Society mounted in 1967 when there was an imminent danger of extensive changes to the ecosystem. More than sixty scientists have visited the atoll since 1967 and many of these have been supported by the Natural Environment Research Council; the Royal Society, however, maintains its central planning and coordinating role in Aldabra research.

UNITED NATIONS

Doom-Watcher

SUGGESTIONS that the United Nations should play a more vigorous part in dealing with natural disasters have now resulted in a resolution of the Economic and Social Council of the United Nations, adopted at the meeting in Geneva at the end of July, which spells out a few practical steps that might be taken. The resolution will be put forward at the next General Assembly in New York. Its chief proposal is the appointment of a man whose job would be the coordination of disaster relief, at least so far as the various organizations of the United Nations are concerned. The idea is that the disaster relief coordinator should have a small independent office at the United Nations, and that he should be equipped not merely to spend money contributed for disaster relief by governments and other organizations, but also to provide technical assistance and, where appropriate, research on the prevention or at least the prediction of natural disasters of various kinds.

Although the new proposal should go a long way to provide a more easily identifiable centre within the United Nations at which disaster relief could be organized, it falls short of the more ambitious suggestions which have been made in the past that the United Nations should, for example, be equipped with some of the materials which have been shown to be valuable in recent disasters—helicopters chief among them. Instead, the Economic and Social Council has suggested that potential donor governments should let the disaster relief coordinator know in advance what kinds of facilities and services they might be able to provide.

ARCHAEOLOGY

Looting for Museums

from our Archaeology Correspondent

IN the past few weeks, museums and collectors have been alarmed by the discovery of several clever fakes, but in the long run science and scholarship may be more seriously damaged by the increasing flow on to the art market of genuine antiquities derived from illicit excavations. In *American Antiquity* (36, iii ; 1971), Robert Adams, Director of the Oriental Institute in Chicago, writes a strongly worded comment on this situation, stressing the increasing scale of looting and the irreparable loss to archaeology: "If we do nothing, we condone these growing outrages by our silence and jeopardize the international progress of our field".

Gun-running, indeed, is not as profitable these days as antiquity-running. It is true that in many nations of the world (although not yet in Britain), archaeological excavation is illegal unless authorized by the national antiquities service and archaeological finds are automatically the property of the state. Moreover in many Near Eastern countries, as well as in Central America, antiquities may only be exported under an export licence. Unfortunately, however, most of the pieces which now command so high a price in the art markets of the world—the Mexican sculptures, the Greek vases, the Iranian bronzes—have been illicitly excavated and illegally exported.

This is, of course, of serious concern, since the importance of such finds is not simply their intrinsic worth or even their beauty but the information which the circumstances of their discovery can yield about man's past. The archaeological interest of a hoard of gold coins in an earthenware pot, for instance, may be the opportunity it offers for dating the pot and hence of building up a ceramic chronology which in turn allows monuments and other finds to be dated: the gold itself may be secondary. All this and much more is lost in clandestine digging, where the associations are never reliably recorded and all the information obtainable by the use of scientific aids in archaeology goes unremarked.

It is widely and increasingly felt that much of the responsibility for the increasing loss of archaeological knowledge through illicit digging must be borne by those museums and private collectors who pay vast sums for unprovenanced antiquities, with no questions asked. In many cases these august institutions are, in effect, knowingly receiving stolen goods illegally exported from their country of origin. Indeed it is not unknown for Classical statues and vases, found in Greece or Italy, to be deliberately smashed by the diggers so that the pieces may be more conveniently exported than would the complete object, and subsequently reassembled for sale in Switzerland.

The problem has been discussed recently in the context of the "Boston Affair"—not the strangler but the Museum of Fine Arts. The museum, already under a cloud for smuggling into the United States a painting allegedly by Raphael, has also been attacked for obtaining an impressive hoard of prehistoric gold jewellery, obviously from the eastern Mediterranean. The treasure, bought by an anonymous benefactor, is itself regarded by some as at best a collection of unassociated finds put together by an astute dealer and, at worst, a bunch of fakes.

The acquisition rather unexpectedly produced a strong swell of protest in the archaeological world, reported in *Antiquity* (**44**, 88, 171 and 257 ; 1970) in editorials by Glyn Daniel. In a sense, the museum was only doing what many others have done. It is ironical, therefore, that the "Pennsylvania Declaration", a somewhat sanctimoniously worded document from the University of Pennsylvania Museum renouncing the intention of buying unprovenanced antiquity (*Antiquity*, **44**, 171 ; 1970), should come from an institution which only recently bought a comparable treasure in very similar circumstances. Indeed, that hoard of gold jewellery from the east Mediterranean seems more probably genuine than the Boston hoard, and it was recently given authoritative publication in an admirable and scholarly article by Dr George Bass (*American Journal of Archaeology*, **74**, 335 ; 1970). Despite its holier-than-thou attitude, the Philadelphia Museum is right. International legislation is needed and, above all, a real public awareness in the purchaser countries that to buy important unprovenanced antiquities is directly to support looting and the destruction of the past.

Every museum and many private collectors have this sort of skeleton in their cupboards—the Elgin Marbles and the Codex Sinaiticus are British examples. Yet the urgent problem is not so much one of restitution as of stopping the looting. That is why it is right to pillory the unhappy authorities of the Boston Museum.

INDUSTRIAL ARCHAEOLOGY

Through the Past Darkly

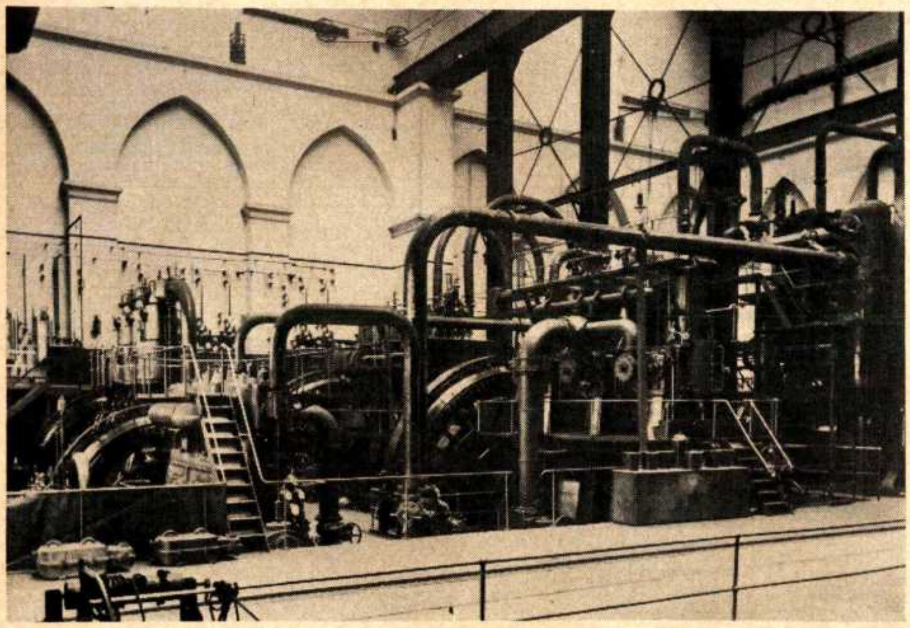
ELECTRICITY'S past may be preserved in a museum. The museum subcommittee of the Electricity Council meets next week and may recommend that a museum should be established. But it is still a matter for debate. Since December last year, Mr J. P. Tanner, formerly manager of the Northern District of the London Electricity Board, has been asking the area boards for details of equipment in their possession that might be of historical interest. The response has been mixed, but most of the boards have appointed somebody to keep in touch with Mr Tanner and the information he wants is beginning to come in.

It is not yet clear if there will be enough equipment worth preserving, but if there is—as seems likely—a museum is not the automatic answer. Would there be sufficient public interest in such a venture to make it worthwhile? Where would it be? And how would it be financed? All these questions need to be answered.

The site problem could be solved by using an old power station. There are several in London, for example, that are being phased out, but clearly only one complete generating plant and assorted smaller items could be displayed in the space available, and to allow for more than one complete plant would require a very large site. And if the museum were to be open to the public, it would have to be easily accessible. But the cost of a large site near the centre of a city could be excessive. An alternative method would be the continuation of Mr Tanner's work of compilation and documentation in considerably greater depth, with detailed descriptions of early power stations and equipment put on record, along with accurate models. Films could be made about old generating plants that are still in use before they are phased out and dismantled.

But the basic question to be resolved is for whom this preservation of gleams from the past is intended—the general public or the specialist. A detailed compact record, with models and film, available to only a limited number of people at any one time, would presumably be a more economical operation than establishing a large and costly museum for a possibly apathetic public. The cost of either project would be high, although the museum would probably prove to be the more expensive of the two, and government aid would almost certainly be needed to make either scheme viable.

At present Mr Tanner is based at the Science Museum where Mr Brian Bowers, keeper of the electrical section is advising him. They are not alone in their work for since March 1965 members of the Institute of Electrical Engineers History of Technology Group have invited interested members to send in record cards of existing historical pieces to the group who collate the information and offer it to museums. Until a full assessment of the equipment available has been made, and a decision taken as to the precise purpose of preservation, the best method of preserving the past cannot be decided.



These Ferranti Alternators (10,000 volt, 1 megawatt), with a 35 foot flywheel ring, and with armature and crankshaft weighing 225 tons, were installed in Deptford Power Station for the London Electricity Supply Corporation at the turn of the century by Ferranti.

Miscellaneous

BRITISH production of both plastic materials for packaging and aerosol cans is expected to increase by about 15 per cent during 1971 compared with an increase of 20 per cent in 1970, according to a review carried out by Pira, the research association for the paper and board, printing and packaging industries. But the market for glass containers is expected to go up only by 2.5 per cent to 6,400 million items. Sales of packaging materials in 1970 were worth more than £900 million of which £180 million was accounted for by tinplate containers and £120 million by plastic materials. Plastic prices reached their minimum in 1969 and in some cases (notably PVC polystyrene) have since begun to rise. All packaging prices increased during 1970.

THE mirror blank for the 3.6 metre telescope of the Meudon Observatory has now been delivered to the French National Institute of Astronomy and Geophysics. After two years of grinding and polishing, the mirror will form the main part of the largest telescope in Europe, probably in the Pyrenees. Although the new telescope will be a little smaller than the Anglo-Australian telescope (144-inch compared with 155-inch) and that to be installed at Cerro Tololo by the Kitt Peak Observatory (158-inch), the French mirror is, like those of these other modern instruments, made from "Cer-Vit", the material with a low coefficient of thermal expansion that has brought telescopes of 300 inches or more within the range of practical propositions.

THE Council for Scientific Policy will be joined for the new term beginning in September by Dr S. L. Bragg, Vice-Chancellor elect of Brunel University, lately a research director at Rolls-Royce. The new recruits also include Dr J. G. Collingwood, director of research at Unilever Limited, and Professor Marie Jahoda, professor of social psychology at the University of Sussex. Professor Jahoda should help to introduce the Council for Scientific Policy to psychoanalytical ways of looking at problems, but it may be of more immediate value that in private life she is married to Mr Austen Albu, MP, long a stalwart of the Labour Party's science policy. After all, as everybody knows, the council will need all the political aid it can muster to help it through the months ahead, when Lord Rothschild's pronouncements may be expected to make up in daring for the way in which the promise of them has helped to justify prevarication in Whitehall.

NSF and NASA get their Money

by our Washington Correspondent

CONGRESS, on the eve of its summer adjournment, has finally approved appropriations for the National Science Foundation and the National Aeronautics and Space Administration. Both agencies are to receive budgets for 1972 which are almost identical to the appropriations asked for by the Administration. The budgets were contained in a bill allotting $18,300 million to the Department of Housing and Urban Development, which was passed by the House on July 29, the Senate on August 3 and signed by President Nixon last week. The bill, which is the fruit of much horse-trading in a conference committee appointed to resolve differences amounting to $224.5 million in the HUD appropriations originally passed by the House and Senate, allocates $622 million to the NSF and $3,298 million to NASA for fiscal year 1972 (which started on July 1). The Administration had requested $623.1 million and $3,301 million respectively for these agencies.

The National Science Foundation can breathe a sigh of relief that the cut of $38 million in its budget recommended by the House Appropriations Committee and approved by the House on June 30 (see *Nature*, **232**, 9 ; 1971), has been more than restored by the conference committee. The House Appropriations Committee, under the chairmanship of Edward P. Boland, suggested a budget of $585 million for the NSF, but the parallel Senate Appropriations Committee set the foundation's budget at $648 million. Predictably, the conference committee opted for a final appropriation midway between the two.

In its passage through the Congressional mill, the NSF's budget request has been under strong attack both from the Science and Astronautics Committee in the House and from the appropriations committees, its chief complaint being the NSF's new programme of "research applied to national needs" (RANN). The Science and Astronautics Committee stripped $30.6 million from the $81 million earmarked for RANN, and redistributed it to the support of institutions and science education. This move was later followed by the House Appropriations Committee, which acidly stated that "the committee will expect the Foundation to utilize the funds included for science education support and institutional support for science as recommended, instead of the diversion of additional funds to basic or applied research", and cut even further the amounts requested for RANN.

The appropriations bill signed last week gives the foundation some $109 million more than it received in fiscal year 1971, most of the increase being $40 million earmarked for picking up basic research projects dropped by other agencies. This item is close to the amount requested by the Administration in its budget application, and its main effect will be to carry on projects that would otherwise have to be abandoned under the terms of the Mansfield amendment which effectively requires the Department of Defense to give up basic research not related to weapon system programmes. Other items included in the appropriations are $99.3 million for science education support, $28.8 million for institutional support of science and $26.8 million for summer institutes and other training programmes for secondary school science and mathematics teachers.

In this year's deliberations over the NSF's budget, the usual Congressional mechanism got itself badly out of step, for the appropriations were passed by both the House and the Senate before the funds had been authorized by Congress. What should happen is that the House Committee on Science and Astronautics and the Senate Committee on Labor and Public Welfare between them set upper limits for the foundation's budget and the appropriations committees then set the final budget levels. This year, however, Congress finally agreed to the authorizations the day after the appropriations were sent to the White House for approval, and the House found itself in the peculiar position of authorizing upper limits for expenditure that had already been appropriated.

The chief reason for this state of affairs was that the House Authorizations Committee set an upper limit of $622 million for the NSF, while the Senate Committee wanted to authorize $706.5 million. The matter was then referred to a conference committee which eventually agreed to set a limit of $655 million. The Senate authorization committee, which was under the chairmanship of Edward M. Kennedy, also recommended that the NSF should receive $907 million for fiscal year 1973, but this was deleted by the conference committee, and in its place was substituted a request that the NSF should provide the Senate Committee on Labor and Public Welfare and the House Committee on Science and Astronautics with a two-year budget projection for fiscal 1973 and 1974.

Although Congress has looked with some disfavour on the NSF's RANN programme, the foundation is pressing ahead with plans to take a closer look at industrial research and in particular at the possibilities for industry/university collaboration in research projects. It has recently appointed a high-level committee under the chairmanship of Mr Jack E. Goldman, vice-president of Xerox Inc, to consider industry/university relations. Initiation of this committee, however, goes back further than the start of the RANN programme, because when Dr William D. McElroy took over the directorship of the NSF in 1969, he proclaimed that more could be done. Dr McElroy began in fact to recruit members for the committee in April, when he sent letters to individuals pointing out that he believes that there is a gulf between the universities and industry which can be bridged with the help of leadership and stimulation from the NSF and inviting them to serve on the committee. Exactly how the committee intends to bridge the gap is as yet unclear, but in view of the harsh words of the House Appropriations Committee on the NSF's stake in applied research, it will clearly have to tread carefully.

Like the appropriations for the NSF, the final appropriations for NASA agreed by Congress fall short of the Administration's request by only a small amount, for the HUD budget that was agreed to last week contained just over $3,298 million for the agency compared with the $3,300 million requested. But in this case the House of Representatives managed to agree on authorizations for the agency two days before it passed the appropriations, and in the Senate, a full six days elapsed between authorization and appropriation. Again, the conference committee which settled the difference between the House and Senate versions of the NASA budget came up with few surprises, for it settled on a budget for the agency almost midway between the House and Senate appropriations, adding $26.5 million to the House bill, but removing $22.6 million from the Senate bill.

There has been some encouragement for the NASA programme for the development of a nuclear powered rocket (the Nerva programme), and $39 million is specifically earmarked for this project. The Administration's budget had set aside only $17 million for Nerva, but even the final appropriation falls short by $11 million of NASA's original budget request. It seems that NASA originally asked for $50 million for Nerva for the financial year now begun. But when told by the Office of Management and Budget to reduce its overall budget request, the agency pared some of the costs of the Nerva programme. The Office of Management and Budget then seized the opportunity to remove all the funding for the project, and eventually agreed under strong pressure from NASA to restore $17 million to keep the project ticking over.

Although the appropriations are not usually broken down into separate figures for each project—only a global sum for research and development, construction and management is given—it seems clear that the conference committee has done well by this shuttle programme. The agency has, to some extent, staked its future on the shuttle, allowing the budget for Nerva and the "grand tour" to be reduced to preserve intact the funding for the shuttle programme, and the Administration's budget request contained $100 million for the programme for fiscal 1972. In spite of a virulent attack on the programme by forces in the senate led by Walter F. Mondale and in the house by Mrs Bella Abzug, Congress authorized $115 million for the shuttle. Also in favour with congress is the Skylab programme, which received an extra $15 million from the authorization committee.

Of the total research and development expenditure authorized by Congress, funding for manned flights is expected to consume some 50.7 per cent—the lowest proportion since 1963—due to the near-completion of the Apollo programme.

NASA's budget for fiscal 1972 represents a decrease of $14.6 million from last year's funding—a drop which was described by Mr Richard Fulton during the house debate on the final appropriations as financially constraining, and likely to lead to cancellation of several potentially rewarding projects. But this did not appeal to Mrs Abzug, who has been seeking for some time to plunge her knife into the NASA budget. She told the house: "There is sad irony in noting that in the same bill providing a total of $3,206,324,000—for that department (HUD), there is also provided a larger amount—$3,271,500,000—for the National Aeronautics and Space Administration. Few more abject demonstrations of our misplaced priorities could be provided".

HIGHER EDUCATION

Straws in the Wind

The Senate went off on holiday on August 6 after doing as much as could be expected of it to win friends among university presidents and faculty. The Senate, like the House of Representatives is looking forward to July 1, 1972, when the legislation now in force and which regulates federal assistance for colleges and universities will expire.

If in due course the Senate is able to reach a satisfactory compromise with the House of Representatives, at present more concerned to help institutions than students, and if, at the same time, it proves acceptable to the Administration, many of whose proposals on higher education have been incorporated into the Senate bill, there is at least a chance that college presidents in the mid-seventies will be able to spend more time on education and less on fund-raising than has been their custom in the recent past. It is, therefore, of some importance that the Administration has let it be known that the $18,000 million that would be spent under the Senate bill in the three years 1972–75 is an "unrealistic" figure and that Senator Warren G. Magnuson, chairman of the Appropriations Committee concerned, has let it be known that the federal budget would not be able to accommodate such a scale of spending "in present circumstances". In fact, what the Senate proposes is a scale of expenditure roughly three times that at present advocated by the Administration, which has just been awarded a total of $1,340 million in the budget for the current year for higher education.

The novel features of the Senate bill are that students would be paid a small stipend—$1,400 a year—and that the institutions which they attend would also receive financial support directly from the federal government. There are also provisions in the bill for supplementary grants to poor students, an echo of the proposal put forward by the Administration. The schemes for student loans at present in force or in preparation would be continued, as would schemes for seeking out particularly students especially likely to profit from higher education. It is also planned that there should be a honeypot of $150 million from which universities and colleges which find themselves in a plight similar to that of the Lockheed Aircraft Corporation could be helped out. The Senate has also agreed with the Administration that there should be set up within the Department of Health, Education and Welfare two new agencies to be known as the National Foundation for Post-Secondary Education and the National Institute of Education. It is just possible that academic enthusiasm for the Senate's action will be moderated, at least in part, by a requirement that students receiving federal support should promise not to use the money for purposes which are not strictly educational. A gallant attempt by Senator Birch Bayh to write into the bill a requirement that women should not be discriminated against in higher education was ruled out of order towards the end of the Senate debate.

On student support, the bill lays down that the basic grant per student shall be either $1,400 or one half the actual cost of attendance at a university, whichever is the smaller. It is also proposed that the amount of the grant should be reduced by the contribution expected to be made by the student's family, calculated on a sliding scale which has yet to be determined but which takes into account such things as the family income, the number of dependents (particularly those in higher education), the capital assets of the student and his family and such unusual expenses as may be occasioned by medical catastrophes. In general, grants will be valid for no longer than four years, but there are exceptions to cater for five-year courses, for supplementary courses and for circumstances in which the amount of money at the disposal of the Department of Health, Education and Welfare is insufficient to meet the demand. The last provision of the Senate bill is held by its supporters to be a device for making sure that the Administration will not be able to skimp on higher education without damaging the cause of poor students to which it professes to be most deeply attached, but the effectiveness of this strategy, attributed to Senator Claiborne Pell, remains to be seen. It is estimated that the total cost of the basic grant programme, will amount to about $1,000 million a year.

There are also schemes in the new bill for helping poor and other deserving students. These supplementary grants will be administered by institutions. The Senate bill proposes that something like $200 million a year will be spent in this way, usually in grants not exceeding $1,000 (except when a student has performed well in the previous academic year). Supplemental grants of this kind will continue for four years or for the duration of an undergraduate course, whichever is the normal practice of the institution concerned. It will be for universities and colleges to select eligible students, but the federal funds available will be shared out among states in proportion to the numbers of full-time students in higher education. The Senate also suggests that $50 million a year should be transferred to states prepared to operate a student incentive grant programme.

NEWS AND VIEWS

New Ways with Volcanoes?

THE article by Dr David R. Waldbaum on page 534 of this issue seems well designed to put a cat among the geological pigeons. One way and another, many of the processes considered to take place within the crust of the Earth entail a marked change of pressure. This is most obvious when volcanic material reaches the surface from a substantial depth beneath it, but it is now clear that transformations of the same kind must occur when material is added to tectonic plates at mid-oceanic ridges and, conversely, when the edges of the plates are re-incorporated into the Earth's crust at subduction trenches. The question that Waldbaum seeks to answer is whether conventional thermodynamics can be used to predict changes of temperature likely to accompany these pressure changes.

It is only fair to say that the issue is exceedingly complicated, not least of all because of the difficulty of defining with sufficient clarity the conditions under which these thermodynamic changes take place. The nub of Waldbaum's conclusion is that there must be many circumstances in which a decrease of the pressure to which some part of the Earth's crust is subjected will be accompanied by an increase of temperature. Such an effect would of course provide a neat explanation of the ways in which igneous material moving upwards towards the surface of the Earth remains molten or at least plastic.

How does all this come about? Waldbaum falls back, quite properly, on a discussion of the application to the materials of the Earth's crust of the Joule–Kelvin effect, best known for its importance in refrigeration processes, when cooling is brought about by the adiabatic throttled expansion of a gas. Liquid CO_2 at ambient temperature can, for example, be converted directly to dry ice at −80° C by this means. For every gas, however, there is at a given pressure an inversion temperature above which throttled expansion results in a temperature increase. (The inversion temperature decreases with increasing pressure.) As Waldbaum points out, the extrusion of a solid substance under similar circumstances will result in a rise in its temperature. In physical terms, the work of extrusion is converted into heat either by direct viscous dissipation or by dissipation as kinetic energy. Since the internal energy of a solid is not strongly dependent on pressure, the heat produced during extrusion must raise its temperature.

It is important to know how closely this chain of argument applies to the processes which go on in the Earth's crust. On the face of things, at least, the pressures within the mantle are great enough to satisfy the interests of most thermodynamicists. Given that the so-called adiabatic gradient for the materials of which the Earth's crust is made may amount to as much as 1 K for each kilobar, it is obviously also possible to think of circumstances in which solid materials are made molten simply by changes of pressure. The chances are, however, that there need to be refinements of the simple picture that suggests itself—the possibility that solids under high pressure at great depths in the crust or mantle could be induced to fly upwards as liquids whenever decreases of pressure took place. Moreover, much depends on whether phenomena such as convection in the mantle, described, for example, by Turcotte and Oxburgh (*J. Geophys. Res.*, **74**, 1458; 1969), can be properly regarded as adiabatic processes. Waldbaum says that even if friction, viscosity and turbulence are ignored in these discussions, the circumstances are such that the ratio $(\partial T/\partial P)_H$ is greater than the ratio $(\partial T/\partial P)_S$, so that the temperature must increase. Where most discussion of Waldbaum's article will be concentrated is on the supposition that these processes are strictly adiabatic or isenthalpic in the sense of the Joule–Kelvin equation.

Only a close definition of what precisely is meant by constant enthalpy—the old-fashioned heat content—is sufficient to resolve the difficulty. Does the definition of the enthalpy of a material include kinetic and gravitational energy, or are these quantities excluded? If the Joule–Kelvin equation should be applied only when changes of gravitational and kinetic energy can either be ignored or compensated for, there is obviously a possibility that the estimates of Joule–Kelvin heating which Waldbaum has made are substantially too great. Obviously it would be valuable to see a closer discussion, along the lines already provided by Mackenzie (*J. Geophys. Res.*, **72**, 6261; 1967) among others of the departures from hydrostatic equilibrium of the pressure differences within convecting regions of the crust and mantle of the Earth. Another difficult, requiring further examination is that there may be many circumstances in which it may be invalid to assume that convection is an adiabatic process. Certainly those parts of the Earth's crust where gravity measurements suggest that convection is rapid are also places at which the heat conduction anomalies are most marked.

For practical purposes, what Waldbaum has predicted is that there will be circumstances in which the process of viscous dissipation within a convecting fluid could result in an increase of temperature in the rising parts of the fluid. This is the hare that thermodynamicists are invited to chase. More immediately, however, it does seem clear that Joule–Kelvin heating of a solid may be important in processes of rapid change as in the impact of meteorites or the effects of seismicity beneath the surface. The process also may be important in the formation and intrusion of kimberlites as well as in violent volcanic eruption. How much more widely applicable may be Joule–Kelvin heating in tectonic processes remains to be seen.

EARTHQUAKES

Control by Explosion

from our Geomagnetism Correspondent

BECAUSE earthquake prediction has proved so intractable a problem, the emphasis within earthquake research has moved strongly towards the possibility of control and modification—a development which was as unexpected as it is exciting. In common with many great scientific discoveries, the idea that major earthquakes might be modified rather than allowed to take their sometimes disastrous course arose "accidentally", though with human help. From the earthquakes which apparently followed the filling of the Hoover Dam during the late 1930s and the disposal of waste chemical and biological weapon materials into a Denver well during the early 1960s there developed the hope that the controlled injection of fluids into regions of tectonic stress might lead to the more gradual release of strain energy. A second possible control mechanism later became apparent when it became clear—chiefly from activities in Nevada—that underground nuclear explosions often lead to the release of energy not derived directly from the explosions themselves. Each of these examples, however, well illustrates the two-sided nature of the strain release phenomenon. The hope that fluid injection and explosions may one day be used to ensure a gradual strain release must be balanced by the fear that less well-regulated experiments could lead to a man-made disaster.

As far as underground nuclear explosions are concerned the best evidence for the interaction between the explosive source and the surrounding rock is the frequent production of horizontally polarized SH and Love waves. Analysis of the radiation patterns of these waves suggests that relaxation of stress leading to the radiation of strain energy takes place around the explosion cavity. The amount of strain energy released and the radius of the relaxation zone have not yet been determined accurately, although from the explosion studies carried out so far it is clear that the energy released in hard rock can be less than the equivalent seismic energy of the explosion itself. The only known, but important, exception was explosion Hardhat (in granite).

Toksöz and Kehrer (*Science*, **173**, 230; 1971) have now taken previous studies further by analysing the surface wave radiation patterns produced by two underground nuclear explosions at the Nevada Test Site, Pile Driver and Greeley, both of which generated Love waves and released considerable strain energy. Pile Driver (58 kton) was detonated in granite and Greeley (825 kton) in zeolitized tuff. Comparison of the results with similar data from other explosions clearly shows that explosions in harder rock (for example, granite) usually release more tectonic strain energy than explosions in soft media such as tuff, alluvium and salt. In the latter cases low rigidities prevent the accumulation of shear strain energy owing to plastic deformation under stress. This is particularly true of salt in which no Love waves are generated at all.

It does not follow, however, that all explosions in a given medium radiate proportionally the same amount of strain energy. Thus in granite, for example, the ratio of the surface wave energy for strain release to the surface wave energy of the explosion itself in the 15 to 22 s range (E_t/E_e) was 13.65 for Pile Driver, 12.00 for Hardhat and only 1.05 for Shoal (western Nevada). It would seem that the magnitude of the pre-existing tectonic stress in the rock is also important—as might only be expected, the E_t/E_e ratio is greater for greater ambient stresses. But tectonic stress and the particular medium seem to be the only two principal parameters involved. Thus Pile Driver and Hardhat, whose sites were only half a kilometre apart, each released strain energy proportional to its own explosive energy. Toksöz and Kehrer conclude, furthermore, that it is the regional tectonic situation which controls the radiation pattern and that the strain energy release arises from close to the explosion and not from earthquakes triggered 10 or more kilometres away. Adjustments at these distances take place gradually as evidenced by the aftershock activity produced by some large explosions.

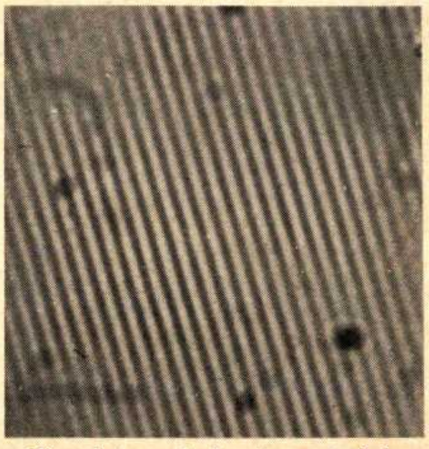
As Toksöz and Kehrer point out, the fact that Pile Driver, Hardhat and, to a lesser extent, Greeley, had high E_t/E_e ratios means that under certain circumstances explosions may be used to release strain energy far in excess of the energy of the explosion itself. The prospects for earthquake modification through artificial strain release are thus encouraging. And because the strain is released from close to the explosion point it would seem that in a practical situation the point of stress reduction could be defined and controlled quite closely. By the same token, however, it would not be possible to deactivate regional stress fields by a single explosion. Nor is it yet known how long it would take for a destressed area to

Lasers, Gratings and Microcircuits

IN next Monday's *Nature Physical Science*, Dr V. I. Little and his colleagues at the Royal Holloway College, London, report another elegant method for producing finely ruled gratings with a laser. Their original method made use of the selective etching of gold from a glass substrate at the antinodes of the electric field associated with the standing light wave in a ruby laser cavity, but they have now discovered the opposite effect—that the condensation of gold vapour onto a glass plate can be selectively inhibited with a periodicity characteristic of the laser standing wave.

During a 50 ns giant pulse from a Q-switched laser, gold is evaporated from a film inside the laser cavity and some of the gold atoms approach a glass collector plate placed at an angle to the axis of the laser and about 0.1 mm from the gold film. Provided that the gold reaches the plate before the laser pulse decays, Little *et al.* find that it only deposited at the nodes of the standing wave and that the pattern generated is similar to that produced by the etching method. The next step in the work will be to devise a system for producing these "modulated" films in a more controlled manner using a continuous standing wave from an argon ion laser.

In the longer term all these techniques could have important consequences for the manufacture of microcircuits and, as well as making modulated gold films, Little's group have been able to deposit dielectric films in a similar way. This opens up the interesting possibility of producing a series of very small capacitors or waveguides, spaced 2.5 μm or so apart, by depositing a dielectric layer between two conducting layers. If the deposition process could be carried out in two laser beams, aligned in such a way that two crossed gratings are formed, then the matrix of rectangular capacitors so produced could be the basis of a cheap and compact electronic memory of storage device.



Microphotograph showing part of the gold pattern.

reaccumulate its original stress. Pile Driver, detonated within half a kilometre of Hardhat but four years later, radiated proportionally the same amount of strain energy as Hardhat. This could mean that the original stress had built up again within four years. The results are inconclusive, however. The yield of Pile Driver was about ten times greater than that of Hardhat and so the volume within which strain was released was correspondingly larger.

But although these results are, in a sense, encouraging, they also have their disturbing aspects. Because the proportional energy release (but not the absolute energy release) is independent of the shot yield, but is instead controlled by the rock properties, a large explosion could release large quantities of tectonic strain energy. A one-megaton explosion with an E_t/E_e ratio similar to those of Pile Driver and Hardhat, for example, would release energy equivalent to a magnitude 6.3 earthquake. A ten-megaton explosion would, under these circumstances, be equivalent to a magnitude 7.2 earthquake. For explosions of these yields hard rock and high tectonic stress are clearly a hazardous combination.

VISNA VIRUS

Oncogenicity Detected

from our Cell Biology Correspondent

At the end of last year and early this year four reports appeared stating that reverse transcriptase, capable of using endogenous viral RNA as well as added synthetic RNAs and RNA.DNA hybrids as templates for DNA synthesis, was present in visna virus and simian foamy virus particles. Until then this enzyme activity had, among viruses, only been detected in the virions of the RNA tumour viruses, where its presence could be explained in terms of Temin's provirus hypothesis of transformation by these viruses.

Reverse transcriptase in two viruses which had never previously been suspected of being oncogenic frayed the edges of an otherwise tidy situation; the sweeping generalization that only RNA tumour viruses contain reverse transcriptase, because only they among the RNA viruses need to integrate their genome into that of their hosts, could no longer be made. But was that really the case? Neither visna virus, which causes a slowly progressing neurological disease of sheep, nor simian foamy viruses, which have not been associated with any known monkey disease, had ever been tested for oncogenicity. So Takemoto and Stone set about remedying that; they are now able to report (*J. Virol.*, **7**, 770; 1971) that visna virus and an antigenically related virus, progressive pneumonia virus (PPV) of sheep, which causes a slowly developing pneumonia and also contains reverse transcriptase as Takemoto and Stone have found, are able to transform mouse cells.

Some three to four weeks after infection with either visna or PPV, cultures of mouse cells (line AL/N and Balb/c 3T3) develop foci of spindle shaped cells from which Stone and Takemoto have established stable transformed lines, which grow rapidly, are not subject to contact inhibition and are tumourgenic for susceptible mice. The medium in which these transformants grew lacked detectable virus, but by cocultivating the transformants with normal sheep testis cells both visna virus and PPV could be rescued; moreover, the rescued virus is identical to virus isolated directly from sheep cells and can be used to transform fresh cultures of murine cells.

To obviate the obvious criticism that some other known oncogenic virus was responsible for the transformation after cells were exposed to these two sheep viruses, Takemoto and Stone tested for antigens of murine sarcoma leukaemia viruses and for T antigens of oncogenic DNA viruses; all the transformed lines gave negative results. Clearly, visna virus and PPV, which have many of the morphological characteristics of RNA

The Mechanism of Phloem Transport

Transport of sugars in plants, from sites of synthesis or food storage to those of utilization, takes place in the phloem through a system of fine tubes—the sieve tubes—which are interrupted at frequent intervals by a series of perforated baffles, the sieve plates. The mechanism by which large quantities of sugar solution are transported at rates which are sometimes in excess of 100 cm per hour is one of the principal unsolved problems of plant physiology.

More than a century has passed since it was suggested by the German botanist, Hartig, that the motivating force for phloem transport is a hydrostatic pressure gradient developed between the source of carbohydrate and its sink. This "pressure flow" hypothesis satisfactorily accounts for many of the features of phloem transport and is still favoured by most plant physiologists today. It has been calculated that the hydrostatic pressure gradients likely to exist are sufficient to induce flow at the rates observed—at least in plants with wide sieve tubes—as long as the pores in the sieve plates are not obstructed.

Much attention has been paid recently to the state of the pores in functioning sieve tubes, but the evidence from electron microscopic studies is contradictory. Sometimes the pores appear open, and in other cases they are clearly blocked. Much seems to depend on the way the material is prepared for microscopic examination, and to confuse the matter further the same observations are interpreted differently by different investigators. Proponents of the pressure–flow hypothesis contend that the pores are normally open but can readily become blocked during preparation of the tissue. Other investigators maintain that the pores in functioning sieve tubes are filled with fibrous membranous structures which somehow assist in transport and that these materials are sometimes displaced leading to the appearance of open pores in sections.

In an attempt to resolve this dilemma, Jarvis and Thaine, of the Grassland Research Institute, Hurley, have been experimenting with new methods of preparing phloem for microscopic observation and they report their observations in next Wednesday's *Nature New Biology*. As an alternative to chemical fixation, the tissue is frozen rapidly while still attached to the plant and sectioned in a cryostat for examination in the light microscope. In material prepared in this way strands with a distinct boundary layer enclosing groups of parallel sub-structures were seen in sieve tubes. The diameter of a strand was similar to that of the sieve pores into which some strands appear to pass. These strands are believed to be the "transcellular" strands reported earlier by Thaine and looked on by some other investigators as artefacts.

There has been a suggestion that the Thaine strands are identical to the slime or P-protein fibres which regularly occur in sieve tubes and seem to be involved in blocking sieve pores in response to wounding. Jarvis and Thaine put forward the idea that slime strands may be constituents of the larger transcellular strands which have been partly degraded.

If, as Thaine believes, the transcellular strands are tubular structures passing through the sieve pores, it is likely that they are the conduits in which longitudinal transport of sugar takes place. Thaine has suggested that the solution is propelled through these tubes by rhythmic contraction and relaxation of protein fibres in the walls. A theoretical analysis of such a peristaltic mechanism has recently been made by Ailkman and Anderson (*Ann. Bot.*, **35**, 761; 1971) and they concluded that the model is a feasible one. The observations of Jarvis and Thaine provide further evidence for the physical reality of transcellular strands on which the hypothesis is based.

tumour viruses and an RNA genome which in size and response to denaturation is remarkably similar to those of the RNA tumour viruses (Lin and Thormar, *J. Virol.*, **7**, 582; 1971), should be grouped with the oncornaviruses. Moreover, Stone and Takemoto's findings, which are fully in keeping with Temin's hypothesis, should spur on the screening of other RNA viruses initially for reverse transcriptase and then oncogenicity if they have the enzyme.

But why do visna virus and PPV contain a reverse transcriptase when they do not apparently cause tumours in their natural host, the sheep? Both are so called slow viruses which chronically infect sheep for many months and even years; both cause very slowly progressing diseases (the parallels with tumour viruses and cancers are obvious enough), and it may well be that the maintenance of slow virus infections depends on the production of a DNA provirus, as does tumour virus infection. As Takemoto and Stone point out, the concept of a DNA provirus should not be overlooked in studies of any slowly progressing disease which has been suspected of having a viral aetiology.

JUPITER

Great Red Spot

from a Correspondent

JUPITER'S Great Red Spot is an unsolved problem of long-standing in astronomy. Nobody to date has produced a satisfactory explanation for the features of this elliptical blemish, although high resolution observations and subtle theorizing are now reducing the number of possibilities.

Observers at the New Mexico State Observatory have photographed Jupiter with a 61-cm telescope on every possible opportunity since 1963, and they can fix the position of the red spot to within 0.2°. A summary of the 1970 observing season prepared by E. J. Reese (*Icarus*, **14**, 343; 1971) gives a good impression of the motions of the surface markings. Last year the red spot was very dark and well defined, and it had mean lateral dimensions of 27,800 km by 13,800 km. There is now considerable information on the detailed motion of the spot, particularly its drift in longitude. In Reese's data the 3-month oscillation discovered five years ago shows up strikingly, with an amplitude of around 1°, and Reese comments that the motion is sufficiently regular for the extreme points of this harmonic motion to be predicted with an uncertainty of only 4 days. Moreover, on longer time scales, the spot probably has a 9-year oscillatory motion, and there is a systematic long term wandering which is aperiodic.

High quality observations such as these are winnowing down the possible models of the red spot, and Carl Sagan, of Cornell University, has recently tackled the problem using propositional calculus (*Comments Astrophys. Space Sci.*, **3**, 65; 1971). His method is to set down a few well established facts with which an acceptable theory must be consistent; these include such properties as the red colouration of the spot, its uniqueness, its periodic longitude excursions and its long term stability. These features are then assembled into a "truth table" or check list for interrogating the theories.

The floating raft hypothesis advanced in 1881 has had a good run; basically it says the red spot is something floating in something else, and is careful not to be too precise. The Jovian raft founders, however, because it cannot really explain the oscillations, and Sagan's table prognosis is decidedly negative. Another class of theories treats the red spot as a meteorological phenomenon. Foremost of these theories is Raymond Hide's Taylor column hypothesis: when a homogeneous fluid flows over an obstacle a relatively stagnant tube of fluid is formed; the red spot is a visible manifestation of a Taylor column caused by a topographical feature on the planet. Sagan's analysis, however, gives this idea a bruising, and he questions how the uniqueness, colour and motion of the red spot can be fitted into such a model.

A novel approach to the problem of the red spot is advocated by W. B. Street, H. I. Ringermacher and G. Veronis (*Icarus*, **3**, 319; 1971). They fuse the attractive parts of the raft and column hypotheses to produce a Cartesian diver model which gives a good account of the mobility of the red spot. In its simplest form the Cartesian diver is an inverted float, containing a small amount of trapped gas, in a jar of fluid. The diver can be made neutrally buoyant at a fixed level by exerting a pressure on the fluid. The Jovian diver is visualized as a light phase of solid hydrogen floating in a dense hydrogen–helium fluid; it lies at depths of several thousand kilometres, in the megabar pressure regime, and its base is heated by the planetary interior. Consequently the diver rises to a higher level, where the lower pressure causes some of the hydrogen to melt. As the solid erodes the bubble of heat is released, and so the Cartesian diver can descend to a lower level and grow again.

Although the argument put forward by Street *et al.* is highly speculative the physical processes behind it are reasonable and they are consistent with the observed changes in the size and colour of the spot over the past century. The value of the hypothesis comes in considering the oscillations, because the equations of motion for a diver in a rotating ocean suggest that the longitudinal motion of the red spot consists of several oscillatory components of different amplitude and frequency. These predictions are in precise agreement with periods derived by Reese *et al.*

Nitric Acid in the Stratosphere

RECENT developments in infrared and submillimetre techniques have led to the identification of several previously unknown molecules in the Earth's atmosphere and stratosphere. But in order to make quantitative determinations of the concentration and distribution of such species it has also been necessary to undertake new laboratory investigations of the molecular vibrational and rotational spectra. The necessary use of long optical paths in infrared observations carries with it the great advantage over chemical probe techniques that local contamination by the observation system can be minimized. Recently Murcray *et al.* (*J. Opt. Soc. Amer.*, **59**, 1131; 1969) detected nitric acid in the stratosphere by studying the near infrared absorption of solar radiation using a balloon at a height of 30 km. The spectra were recorded near sunset to obtain a long path length through the stratosphere, and the minimum altitude observed was about 20 km.

These authors have suggested that the nitric acid is associated with the ozone layer in the upper atmosphere but it is also important to know whether nitric acid is a characteristic constituent of the stratosphere or whether it is accumulating because of pollution in the lower atmosphere. New measurements by Drs Harries, Burroughs and Duxbury (National Physical Laboratory and Bristol University) are reported in the forthcoming issue of *Nature Physical Science*, and these confirm that the nitric acid concentration does indeed decrease with decreasing height as does the concentration of ozone. The new observations are of the submillimetre emission spectrum of the stratosphere observed from a Comet aircraft flying along a controlled path at a height of 12.5 km. At this height there are several important advantages in the emission technique compared with the solar radiation method.

The emission spectrum between 18 and 34 cm^{-1} shows the well known atmospheric emission lines of H_2O, O_2 and O_3 and several unidentified lines. A parallel laboratory study has, however, shown that, although nitric acid has a strong characteristic rotational spectrum in this region, the unidentified lines cannot be attributed to HNO_3.

BARN OWLS

Catching Mice

from our Animal Behaviour Correspondent

BARN owls (*Tyto alba*) can successfully catch mice in total darkness; they rely on the noise the mouse makes as it rustles through leaves to guide them to their prey. R. S. Payne (*J. Exp. Biol.*, **54**, 535; 1971) has demonstrated that the owls can do this even in the absence of other clues as to mouse's whereabouts, such as its smell or the heat given off by its body, and his experiments have shown that the owls are remarkably accurate in both the horizontal and vertical localization of a sound source.

He induced owls to fly towards a loudspeaker that was emitting mouse-like rustles and gauged their accuracy by measuring the distance between the centre of the loudspeaker and the centre of the imprint which the owls' talons made on impact with plasticine surrounding the loudspeaker. This method revealed that the owls seemed to need frequencies above 8.5 kHz, because when these frequencies were removed from the sound source, the birds were much less accurate.

Regardless of its position when it first hears a mouse rustle, an owl always turns to face the mouse and then must have one further sound before it can strike successfully. Payne suggests that this is because the owl localizes a complex sound source by trying to make all frequencies as loud as possible in both ears and would then automatically come to face the source of the sound. To investigate this, Payne placed a microphone in the head of a dead owl at the site of the (now removed) ear-drum and played sounds of constant intensity and frequency through a loudspeaker, recording the response of the microphone as the position of the loudspeaker was varied. In this way, he was able to construct a map of "iso-intensity" lines for each frequency, showing the position of "highs" (places from where the sound was loud) and "lows" in the space surrounding the ear. The owl's sensitivity was found to rise sharply to a maximum along the line of sight. A low in one ear always corresponded to a direction of good hearing for the other ear, and this was interpreted as a special adaptation to sound localization, because it would have the effect of sharpening the frontally directed regions of maximum sensitivity.

Barn owl (photo: Zoological Society of London).

Comparisons of positions at which regions of maximum and minimum sensitivity for a given frequency occurred in the vertical plane revealed that in the right ear such regions are roughly 10–15 degrees higher than in the left ear, presumably as a result of the marked asymmetry of the external ears. A sound which was loud to the right ear could therefore be soft to the left. If the owl then tilted its head in the vertical plane, the relative loudness of the sound in the two ears would change, and this too seems to be an adaptation to reduce ambiguity about the position of a sound.

One of the most characteristic features of an owl, the heart-shaped face, may also be connected with its hearing ability; it may act as a parabolic reflector to catch sound.

Repairing Mispairing of DNA

IN next Wednesday's *Nature New Biology* a simple observation is described which may have profound genetical implications. Using the multicellular alga, *Ulva mutabilis*, Fjeld has produced evidence which lends support to the master-slave hypothesis of undirectional correction of mispairing of DNA and also gives an indication of the reasons for only complete mutations being found after ultraviolet irradiation.

It seems surprising that *Ulva mutabilis* has not been used more frequently for genetic studies because individual cells of this haplo-diplontic alga can be induced to form gametes which will germinate parthenogenetically giving haploid plants. Development starts as a single filament of cells which later becomes tubular, and the characteristic plant form is assumed by elongation of the basal cells to form the stem and the continued division of cells at the apex to form the blade. Chimaeras can be induced by irradiation of young algal germlings and Fjeld has found that there are two kinds which occur in equal numbers; one is genetic so that the gametophytic progeny also show the chimaeric characteristic and the other is merely phenotypic and the gametophytes appear wild type. The chimaeras examined were those with a basal part very like wild type but with a narrow protuberance at the apex which developed into a long ribbon, and those with a bulge somewhere on the blade.

In twenty-six plants with this first type of alteration the apical protuberance did not develop very far, and when the cells of these plants were induced to form gametes the change proved to be merely phenotypic. The apical protuberance on a further twenty-three plants, however, continued development into a long ribbon and analysis of these plants showed that the change in this instance was genetic. Those plants which responded to the ultraviolet light by producing a bulge on the blade also showed the genetic and phenotypic chimaeras to be produced in equal numbers.

It is easy to understand how the genetic alterations can be induced by ultraviolet light, but the nature of the temporary phenotypic alteration is more puzzling, and Fjeld's suggestion that this lies at the level of the gene is interesting. His explanation centres around the fact that only one strand of the DNA is involved in transcription and a mutational event occurring in this strand would be transcribed into an altered mRNA. This cell and any immediate descendants would thus be channelled into an abnormal developmental path giving rise to the chimaeras. Fjeld then goes on to suggest that if the strand which is being transcribed is corrected, using the other strand as template, a phenocopy appears, for all the gametophytic progeny from these cells, in which the abnormal development has been corrected, will be wild type. Up to this point his explanation means that the ratio of genetic to phenotypic chimaeras would be 2:1, for the probability of mutation and correction would be the same in both strands. Half of the mutations in the transcribed strand will thus revert to wild type and will register as phenotypic chimaeras, although if the same type of event occurs in that DNA strand which is not transcribed the mutational event will be unnoticed. Only mutations in this strand which do not revert and which register as genetic chimaeras will be recorded so that there would be an imbalance in favour of genetic changes.

There are many implausible ways of explaining away the discrepancy, but Fjeld's ideas are quite attractive for he chooses to believe that the transcribed strand is always corrected against the other strand but not vice versa. Mutations in the transcribing strand will thus always give rise to the phenocopies, whereas mutations in the non-transcribing strand will produce the true genetic alterations. The expected ratio will then be 1:1.

BACTERIA

The Hot Life

from our Microbiology Correspondent

THE biochemistry of microorganisms able to grow at high temperatures has fascinated biologists for a long time, and the discovery that some bacteria can grow in boiling water (T. D. Brock *et al., J. Bact.*, **107**, 303; 1971) is certain to redouble interest in this area of thermobiology. Brock and his colleagues have been studying for several years the microbiology of thermal springs in Yellowstone National Park and their recent work has centred on Boulder Spring which is fed by two superheated sources. An understanding of the bacteriology of the effluent channel is now beginning to emerge, this particular habitat has a temperature of about 91° C which, at the altitude of Yellowstone, means boiling water.

The bacteria which colonize these inhospitable waters are unknown and have not been cultured in the laboratory, but the elegant techniques which Brock has developed for investigations *in situ* have enabled many of the physiological properties of these organisms to be defined. Brock *et al.* have found, for example, that the generation times of the bacteria last for 3 to 7.5 h at temperatures exceeding 90° C. But their latest study reveals the significant fact that these temperatures are only slightly higher than the optimal temperatures for these organisms.

The technique for determining the environmental optima is very simple. Glass cover slips were suspended in the effluent stream until the colonizing bacteria had formed a dense covering and then the incorporation of various labelled organic substrates was followed in tightly closed vials. The amount of isotope uptake paralleled the amount of bacterial biomass but only occurred if sulphide, or some alternative reduced sulphur compound, was present. The optimal effect was produced with a sulphide level of 13 μg per ml., and although this concentration increased the uptake of all compounds tested, the most dramatic stimulation occurred with leucine, acetate and thymidine (Boulder Spring water itself contains about 3 μg sulphide per ml.). Brock *et al.* conclude that these bacteria can utilize sulphides, sulphite and metabisulphite as energy sources and resemble bacteria like *Beggiatoa* which can incorporate organic matter when reduced sulphur is available. The temperature optimum for isotope uptake stimulated by sulphide ranged from 80° to 90° C, irrespective of the organic substrate, and significant incorporation even occurred at the higher temperature of the stream, 91.5° C.

At least two types of Gram negative bacteria were observed to colonize the cover slips and these could be distinguished by their filamentous or bacillary morphologies. But are the physiological data representative of both bacteria? Autoradiography was unfortunately unable to provide the answer because of the interaction of the sulphide with the photographic emulsion. Although attempts to cultivate these organisms have failed so far, success in this endeavour will be essential to elucidate their peculiar capacity of being able to adapt to boiling water.

ICHTHYOLOGY

Red Sea Migrants

from our Marine Vertebrate Correspondent

ALTHOUGH the Suez Canal is blocked to shipping it is still open as a passageway for fish. Two more Red Sea fishes have been discovered recently on the Israeli Mediterranean coast (A. Lourie and A. Ben-Tuvia, *Israel J. Zool.*, **19**, 203; 1970). One of them, *Pelates quadrilineatus*, is a small, longitudinally striped fish which is found in the coastal waters of the Indo-Pacific region from southern Africa to Australia. It has long been known from the Red Sea but no reports of its occurrence in the Suez Canal are known. This contrasts with the history of the second species, *Crenidens crenidens*, a sea bream which is common in the Red Sea and was reported as early as 1902, within 30 years of the cutting of the canal, as one of the most common fishes in the canal and in the Bitter Lakes, and had reached as far as Port Said. Lourie and Ben-Tuvia, who work at the Sea Fisheries Research Station, Haifa, collected in February 1970 many specimens of *C. crenidens*, up to 15 cm in body length, in a shallow hypersaline lagoon on the northern coast of Sinai.

Other Red Sea immigrants to the Mediterranean have not proved to have such limited habitats and several of the species are common enough to appear regularly in the commercial fish catches of Israel, the Lebanon and Egypt. According to a recent check list of the Mediterranean fishes of Israel (*Israel J. Zool.*, **20**, 1; 1971) by Ben-Tuvia, as many as thirty of the 284 species are immigrants from the Red Sea. The fish fauna of the eastern basin totals only a little more than half of the total of 540 Mediterranean species known, and although this is partly the result of the comparative sparsity of field work and study of critical areas, it is clear that it is a relatively impoverished fauna. Fishes of Red Sea origin have perhaps gained a place in the eastern basin more

RNA.DNA Complexes and Antibody Production

SYNTHETIC hybrid nucleic acids containing DNA and RNA chains are familiar tools of biochemists investigating the properties of various nucleic acid polymerases, and measuring the extent to which natural RNAs hybridize with DNA is a technique widely used by molecular biologists probing gene expression in one cell system or another. But complexes between RNA and DNA which occur naturally in cells have seldom been identified, and for that reason alone the experiments reported by Koros, Koster and Mowery in next Wednesday's *Nature New Biology* will attract considerable interest.

Koros and his colleagues have been investigating the spleen cells of mice, immunized with sheep erythrocytes, which are capable of producing IgM antibodies against sheep red cells and can be assayed *in vitro* by their ability to lyse these erythrocytes. Using an autoradiographic technique they have followed the incorporation by these cells of pulses of ^{3}H-thymidine of ^{3}H-5-uridine. As anticipated, the thymidine label is incorporated into material which can be completely digested by deoxyribonuclease and is therefore almost certainly DNA.

The uridine incorporated by these antibody forming cells, by contrast, responds to digestion by deoxy and ribonucleases in an unusual way. Little if any of the label is removed from the cells by deoxyribonuclease which indicates that little of the uridine can have been converted by the cells to the DNA precursor deoxycytidine. But only about half of the incorporated uridine is digested by ribonuclease, and half the label survives this procedure. If, however, the cells are first digested with deoxyribonuclease and then with ribonuclease all the labelled uridine is digested.

Koros *et al.* believe that these results indicate that in antibody forming spleen cells much of the RNA which is being made remains somehow complexed with DNA and is thereby protected from ribonuclease digestion until, of course, the DNA is digested. Furthermore, they report that spleen cells which are not making antibody incorporate uridine into RNA, 98 per cent of which can be digested by ribonuclease alone. This result, they claim, shows that RNA. DNA complexes are far more abundant in cells making antibody than in similar cells which have not been stimulated to antibody production. As they point out, these DNA.RNA complexes may be involved in antibody synthesis, acting perhaps as messengers, and they are now trying to test this fascinating idea.

Crenidens crenidens.

easily on account of this relative poverty of the native fauna, there being more unfilled ecological niches than there would be in the western basin. Their spread within the present century has, however, been rapid and at least sixteen species must now be regarded as common, some even as far afield as the coasts of Cyprus and Anatolia.

Very little seems to be known about the effects of this influx of Red Sea forms on the native fish fauna. As Ben-Tuvia observes, these fish, and especially the common ones, must be important in food chains and the general biological balance in the area. It may be significant that better than average catches of the immigrant lizard-fish, *Saurida undosquamis,* coincide with years when the native Atlanto-Mediterranean hake, *Merluccius merluccius,* is scarcer than usual. The connexion may not be direct, for *Saurida* and the Red Sea red mullet, *Upeneus moluccensis,* are noticeably more abundant in years of above average temperatures than in cool years in Israeli waters.

It is clear, however, that although this form of biological pollution of the eastern Mediterranean has reached considerable proportions, it is still continuing. With the drastic control exerted by the Aswan dam on the freshwater inflow of the Nile to the Mediterranean further changes in the marine fauna of this area could well take place.

TOXINS

The Perfect Poison

from our Molecular Biology Correspondent

VENOMS, it seems, are frequently more effective than their separated toxins. The ingenious rattlesnake for example, as Hendon and Fraenkel-Conrat (*Proc. US Nat. Acad. Sci.*, **68**, 1560; 1971) now show, secretes a pair of proteins, which act concertedly, one evidently softening up the victim for the other. Crotoxin, as isolated, behaves as a single component in the usual fractionation systems. When, however, amino-groups are chemically modified, an insoluble fraction appears, which is a basic protein, separated from its acidic partner. In ion-exchange chromatography crotoxin separates into two peaks, which, however, seem functionally identical. A tryptic digest of the material from these peaks shows only small differences, suggesting that at least one of the two proteins is heterogeneous, but the acidic and basic components have not separated. In 6 M urea, however, the results are very different, two protein peaks appear of very different amino-acid compositions—one acidic and one basic—eluting at widely different positions. The molecular weights, by detergent gel electrophoresis, are about 8,400 and 13,000.

The acidic protein is not neurotoxic, and does not cause haemolysis, though it is able to cleave lecithin. The basic protein, on the other hand, carries all the haemolytic activity of the venom. Between them the two proteins manifest ferocious neurotoxicity. As Hendon and Fraenkel-Conrat note, enzyme systems that function only when two components are both present are by no means unknown, one subunit species in an oligomer directing the specificity of the other. Alternatively it might be conjectured that lysis of a membrane is necessary before the neurotoxin can go to work.

A rather similar situation has been uncovered by Lowy, Sarmiento and Mitchell (*Arch. Biochem.*, **145**, 338; 1971) in a study of bee venom. This contains a variety of proteinaceous materials, including a hyaluronidase, a phospholipase, and several physiologically active peptides. This package turns out to be lethal to larvae. Lowy *et al.* have purified two peptides—meittin, which is well characterized and has been sequenced, and is the principal component of the venom and one other—and have examined their effects on *Drosophila* larvae. The new peptide is called minimine, for its physiological effect is nothing less than to minimize the insect. Its molecular weight is some 6,000 and it contains cysteine, or cystine. In appropriate doses it does not kill the larvae, but induces a condition of lethargy, in which the effort of feeding seems to be beyond the creature's will-power.

In a second article, Mitchell *et al.* (*ibid.*, 344) report the effects of melittin, which is an oligopeptide of twenty-six residues (molecular weight, 2,800). It is known to possess haemolytic activity, and it now seems that it is also a neurotoxin for the larvae. It is a non-competitive inhibitor of acetylcholinesterase, antagonized by cations—the first case, apparently, of such an effect by a polypeptide.

Cooperation Between Hapten and Carrier *in vitro*

IN recent months, cellular immunologists have been turning their attention to the hapten–carrier. Some of the questions being asked are do B (bone marrow derived) cells and T (thymus derived) cells respond to hapten and carrier respectively, are they specific in their responses, and what are the receptors on the responding cell populations?

An ingenious combination of techniques using *in vivo* and *in vitro* systems has been adopted by Christina Cheers and her colleagues to investigate these problems and they report their results in next Wednesday's *Nature New Biology*. 3.5-Dinitro, 4-hydroxyphenylacetic acid (NNP) was coupled to fowl gammaglobulins (or ovalbumin as a carrier specificity control), and the hapten protein conjugate thus formed was injected into mice, of which the spleens were later harvested. Primed spleen cells in culture in the presence of antigen were shown to be antibody producing by a plaque assay technique using sheep erythrocytes coupled to NNP as target cells for lytic antibody.

In the initial experiments the carrier used for priming *in vivo* and recall *in vitro* was varied, and it was shown that homologous carrier was required to obtain a satisfactory recall response. On the other hand, if cells primed to heterologous carrier were added to cultures in which recall was being attempted by the use of a heterologous carrier–hapten antigen, significant enhancement of the anti-hapten antibody was observed. If either the carrier primed cells or the hapten primed cells were incubated with an anti-immunoglobulin serum there was a marked inhibition of the immune response. The inference here is that both the carrier and hapten primed cells have immunoglobulin-like receptors for the antigens to which they are primed. When combinations of parental and F_1 cells were used in conjunction with specific anti-allosera it was possible to show that the antibody was derived chiefly from the hapten primed cells.

In a further series of experiments Cheers *et al.* injected thymocytes and antigen into irradiated mice. The primed thymocytes were harvested from these animals and were shown effectively to substitute for carrier primed whole spleen cells in enhancing the anti-hapten response of cells primed to a heterologous NNP–protein conjugate.

This whole series of experiments tends to confirm and extend the results of, among others, Raff (*Nature* **226,** 1257; 1970) and his colleagues. There is little doubt that in the fullness of time studies *in vitro* of immune responses in association with the use of cell populations of defined anatomic origins will greatly assist understanding of the nature of immunological responsiveness.

SEAFLOOR SPREADING

Direct Measurement

from our Geomagnetism Correspondent

ON the face of it the direct measurement of the rate of seafloor spreading is an unlikely activity bearing in mind that most of the spreading material is inaccessible, that spreading rates are low and thus that on the oceanic scale the absolute distance of spreading is small even within the human life span. But because of its position astride the mid-Atlantic Ridge Iceland offers a unique opportunity for the direct determination of spreading rate—or at least something closely akin to it—when combined with modern methods of distance measurement. Decker *et al.* (*Science*, **173**, 530; 1971) have now reported the results of just such an experiment to measure the extent of rifting between 1967 and 1970 within the rift zones of Iceland.

During 1967 fifty-eight survey lines, making up a total distance of about 157 km, were measured with a geodimeter. Most of these lines were joined to form two profiles, one across the eastern rift zone and one across the Thingvellir zone, although two, used as calibration lines, lay outside the rift zones in an aseismic area of Tertiary volcanics near the west coast. Seventeen of the survey lines were then remeasured in 1970. A different geodimeter was used here; but systematic errors between the two instruments were resolved by reference to the calibration lines. All of the remeasured lines had apparently increased in length between 1967 and 1970 by distances ranging from 6 to 45 mm. After taking account of an 18 millimetre cross-instrumental difference the lines were found to have lengthened on average by (20 ± 2) millimetres where the quoted error is the standard error of the mean.

The problem with this sort of thing is, of course, that, notwithstanding the sophistication of modern equipment, errors are likely to be large and not easily assessed; and random errors are likely to be particularly troublesome. So how much of the apparent lengthening is random error and how much represents a real extension of the rift zone? Random errors of measurement for eighty repeated observations made in 1967 and for forty-five repeated observations made in 1970 have a normal distribution with a standard deviation of 5.7 mm. Previous experience in California and Hawaii shows such a standard deviation to be reasonable for lines 2 km long ; and this is, of course, much less than the 20 mm average lengthening observed. Moreover, various statistical tests carried out by Decker *et al.* indicated extremely low probabilities for the pattern of extensions from line to line within the profiles to have arisen by chance. Accordingly, the net apparent extension of the eastern rift zone profile between 1967 and 1970 was calculated to be (65 ± 31) millimetres, or about 2 cm a year on average. By contrast, the Thingvellir lines showed an average contraction of 2 mm but this was well within the expected errors of measurement.

Is it possible to use this data to determine the nature of rifting in Iceland? Einarsson (*J. Geophys. Res.*, **73**, 7561 ; 1968) noted the en echelon pattern of rift fractures and related it to shear motions paralleling the rift zones; and in one sense the new data could be taken to support this view. In the eastern rift profile the first three and last lines indicated a net contraction whereas the middle seven gave a net extension (giving an overall net extension). This situation could arise from left-lateral shear movements; but Einarsson had already concluded (*Soc. Sci. Isl.*, **38**, 128; 1967) that the en echelon pattern of fractures near Hekla was caused by right-lateral shear. Einarsson's interpretation and the Decker data are thus inconsistent; and this has led Decker and his colleagues to support the alternative interpretation of Icelandic rifting put forward by Bödvarsson and Walker (*Geophys. J.*, **8**, 285; 1964) in which the rifts are related to crustal extension perpendicular to the strike of the fractures. In this case the measured extension of the eastern rift zone is explicable without complication.

BIOLOGICAL MATERIALS

Standardized Standards

PREPARATIONS of a variety of biological materials are now available free of charge from the National Institute of Medical Research in London, for use as standards to define units of activity or as reference materials. The substances, which are not for human consumption, include angiotensin, antilymphocyte serum and interform, and are available to scientists in any country.

Unambiguous standard units, which define the biological activity of a compound, have been adopted internationally to ensure that experimental results can be meaningfully communicated among research groups throughout the world. Many units can be defined on paper ; for example, enzymatic activity is expressed as the amount of reaction product formed in unit time in precisely described physical and chemical conditions. The activities of other substances, however, cannot be completely characterized by physical and chemical means.

This problem has been overcome by the Expert Committee on Biological Standardization of the World Health Organization, which has established reference preparations for substances used in the prophylaxis, diagnosis and treatment of human and animal diseases, so that their potency can be expressed in uniform terms throughout the world. These preparations are distributed free of charge by the World Health Organization to member countries, and scientists may receive them on request. Further information about this service can be obtained from the WHO International Laboratory for Biological Standards, NIMR, Mill Hill, London NW7 1AA. NIMR also has limited quantities of hormones and antisera, for use in immunoassays, which are available only to people working in the United Kingdom.

These biological standards are intended for calibration, by comparative assay, of the potency of existing national and laboratory standards and are not intended for administration to humans or for general experimental purposes. The list of substances available in reference ampoules ranges widely from pituitary hormones to insulin and immunoglobulins.

Microwave Molecular Line Astronomy

IN next Monday's *Nature Physical Science*, David Buhl and Lewis Snyder describe the techniques and antennae used by the NRAO's group at Green Bank, West Virginia in the study of molecular lines originating in interstellar space at millimetre and centimetre wavelengths. The interstellar environment provides a unique set of "laboratory" conditions with very low temperatures and densities in clouds travelling at high velocity and occupying regions several light years across so that these observations are of interest to chemists and physicists as well as astronomers.

Tiny dust particles in the gas clouds provide surfaces on which chemical reactions can take place, and the discovery so far of twenty-one molecules in space has been a great stimulus to the development of radio astronomy in recent years.

The most exciting aspect of this work has been the discovery of many organic molecules, including those of formaldehyde, hydrogen cyanide and cyanoacetylene, which are of great biological significance. Now the field has developed to such an extent that increased sensitivity from improved instrumentation seems likely to provide the means for the detection of weaker lines and for the resolution of known clouds at frequencies corresponding to the stronger known lines to an accuracy of better than one arc minute.

NRDC and the Environment for Innovation

G. E. HAIGH, A. W. PEARSON & D. S. WATKINS
R & D Research Unit, Manchester Business School

M. GIBBONS
Department of Liberal Studies in Science, University of Manchester

This article describes the historical development in Britain of the National Research Development Corporation, the evolution of its policy and the environment in which it now operates.

In recent years the execution of public policy towards science and technology has been increasingly scrutinized by many, including Parliament. The Parliamentary Select Committee on Science and Technology, for example, recently examined the exploitation of Britain's carbon fibre technology[1], and earlier this year the Secretary of State for Trade and Industry, Mr John Davies, announced that "Mr Docksey, formerly general manager of the Research and Technology Development Department of BP Limited, has agreed to advise me on the arrangements for the exploitation of inventions resulting from public research and the support by the National Research Development Corporation (NRDC) for the development and exploitation of inventions from other sources"[2].

One of the more important changes in the external environment of the NRDC was the outcome of the general election in 1970. The present British Government takes a far less interventionist view of its role than did its predecessor and this has engendered a determination to hive off some of the more commercially oriented activities which government has accumulated over the years. For example, when British Nuclear Fuels Limited was established recently as a wholly owned subsidiary of the UK Atomic Energy Authority (UKAEA), the government stated its intention that at some future date private enterprise would be invited to participate in the equity of the new company[3].

This disengagement has also been demonstrated by government policy on the services to industry which it provides, particularly those of the type previously the responsibility of the Ministry of Technology. Thus the white paper on the reorganization of central government stated of Mintech's successor, the Department of Trade and Industry (DTI): "It will be a major function of the new department, for which special machinery is provided, to review the size and methods of the various services now provided to help industry, in order to determine whether they are a proper charge on the State and, if so, whether the present system of organization is cost-effective and likely to produce the required results or whether other forms of organization would carry out these functions more effectively. These services include . . . government encouragement of industrial research"[4]. One outcome of this new philosophy might be the hiving off of NRDC, which has been functioning during its two decades or so of existence by means of a loan from what is now the DTI, its operating deficit being financed from the public purse. Such a procedure would of necessity entail alteration of the statutes under which the organization now operates. In particular, a way of accommodating (or dispensing with) NRDC's present obligation to act in the national interest would have to be devised; even within organizations which are wholly publicly owned, the problem of catering for the national interest in bodies which are expected to cover their costs on a relatively short term basis is well recognized. Thus the green paper published by the last Labour administration setting out proposals for a British Research and Development Corporation (BRDC) stated "in the civil R & D establishments, despite a rising demand for their services . . . a considerable and deliberate effort has been made to redirect programmes of work towards problems of immediate concern to industry, and likely therefore to bring early benefits to the economy of the country"[5]. Any definition of national interest of course depends on an accurate forecast of the future social and economic needs of the community, one of the problems which the Programmes Analysis Unit (PAU), established at Harwell in 1967, was designed to consider. The proposed BRDC would have brought together the civil research and development laboratories of the UKAEA and those of the then Ministry of Technology under a single management, with the intention that the organization should undertake work for both government and industry on a contract basis. NRDC would have been "assimilated into this new organization so that its expertise in the exploitation of invention would be brought to bear directly on the new corporation's work". (Fears were expressed at the time that this loss of independence might cause NRDC to become little more than a sales organ of BRDC. With the change of government, the BRDC proposals themselves may be deemed to have lapsed, although many of the issues raised in the debate on the green paper are germane to the discussion of any potential alternative organizational forms for government research which the present administration might propose.) Although NRDC's value seems to have been well recognized, however, the area of expertise was not that which had been foreseen when the organization was set up. It is therefore interesting to note the way in which this apparent dichotomy has arisen as a result of changes in both the environment for innovation and the responses of successive governments to meet them[6].

Origins of NRDC

The Second World War ended in an air of general optimism about the possibilities of scientific research and technological development. Nevertheless there was great concern in the United Kingdom that this force for potential good be effectively harnessed in the nation's interest. This concern was focused on four problem areas.

Since the adoption of the Haldane principle of independent research facilities, the government had been responsible for by far the greater part of research expenditure in the United

Kingdom, most of this research being undertaken in the government's own research establishments or in universities. Concentration of effort on specific objectives during the Second World War had accelerated the application of scientific and technological knowledge, techniques and inventions; the peacetime problem was to harness these same activities to industrial needs. Although the Department of Scientific and Industrial Research and the associated government-aided, autonomous industrial research associations (RAs) were active in the application of research to commercial ends, there was no obvious way in which economically valuable research results arising from government-funded research in other institutions could be exploited. Thus a Nuffield College report[7] recognized that "when universities become possessed of knowledge of high commercial value, there is a need for them to secure utilization of such discoveries in the interests of economic progress and the public", whereas there was a tacit acceptance of the fact that universities themselves were both unwilling and incapable of successfully commercializing the results of their research. It seemed obvious that some new form of institution was necessary.

A second area of discontent concerned the operation of the patent system. "From the 1930s there had been a feeling among left wing scientists that private inventions were suppressed by corporate business and finance, and that many inventors would come forward if funds were available to them"[8]. The same Nuffield College report had also expressed the view that the patent system could be used to check invention and exploitation rather than to stimulate it. The Swan Committee Report[9] of 1947 found "no satisfactory evidence of particular examples", but, perhaps influenced by the romantic image of the lone inventor then widely accepted, was "of the opinion that future legislation should be aimed not only at the prevention of 'abuse of monopoly' but also at ensuring the fullest practicable use of the rights conferred by patents towards the development of British production and trade".

The other two areas of discontent were more subjective and chauvinistic. One was voiced by Herbert Morrison in 1944 when he said that "(in the field of scientific research) there is an impression, not without some evidence to support it, that in America and Russia things are happening which we shall do well to emulate. It is generally believed that, in the field of what is called 'fundamental research', British science has as fine a record as that of any country in the world . . . but there is some uneasiness about the application of science to current problems of many kinds, including notably problems of technological development in industry"[10]. This feeling that Britain was good at invention but poor at exploitation was compounded by the case of penicillin, which was seen at this time by some as an example of the exploitation of a fundamental British discovery by foreign competition. Any discussion of the reasons for this foreign application of British inventions leads to an examination of the problems of development and the factors influencing innovation in general; this in fact constituted the fourth principal area of discussion.

Central to the problem was the view that innovation or the commercial application of research results could be modelled as a straightforward linear chain, commencing with the "flowering of the tree of human knowledge"[11]. Although the existence of feedback and the problems of attitude, market needs and so on were noted in passing when delineating this innovation chain through research, development, production and application, the chief preoccupation and concern were with the first stages of research and development. The general acceptance of a simplistic model may have engendered the belief that the efficacy of these various stages could be considered in isolation.

These then were the seminal influences leading the government to announce in 1944 that it was to examine "the need for the establishment of a fund to meet the cost of developing new inventions and of providing facilities for testing new ideas for industry" and how best to integrate this with the existing work of cooperative research associations[12].

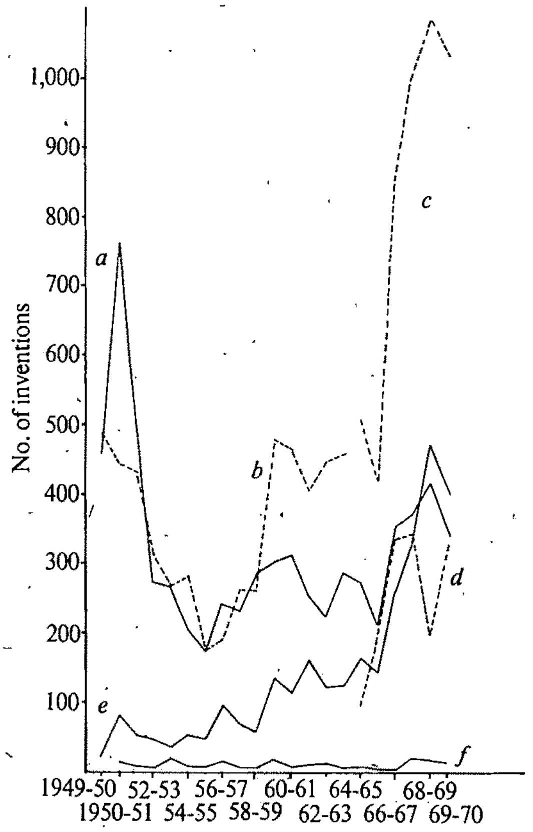


Fig. 1 Number of inventions communicated to NRDC each year (from annual reports). *a*, Research councils and government departments; *b*, total British companies and individuals; *c*, private individuals; *d*, private companies; *e*, universities; *f*, industrial research associations.

Creation of the Corporation

Introducing the Development of Inventions Bill in 1948, the then President of the Board of Trade, Mr Harold Wilson, claimed that the creation of the NRDC "filled an important gap in our scientific and economic life"[13]. The resulting act charged NRDC with (*a*) securing, where the public interest so requires, the development or exploitation of inventions resulting from public research and of any other invention as to which it appears to the corporation that it is not being developed or exploited or sufficiently developed or exploited; (*b*) acquiring, holding, disposing of and granting rights (whether gratuitously or for consideration) in connexion with inventions resulting from public research and, where the public interest so requires, in connexion with inventions resulting from other sources[14].

The NRDC was to operate with an initial loan of £5 million for five years; by securing levies on sales in its exploitation activities, it was hoped that the corporation would eventually be able to balance its accounts by taking one year with another. Its aim was to be commercially minded while endeavouring at all times to consider the national interest. During these early years, the emphasis was primarily on the organization's rôle as an agency concerned with the exploitation of publicly funded research. Consequently NRDC was anxious to establish good liaison with government departments, bodies such as the Medical and Agricultural Research Councils, the universities, and the RAs[15]. The universities and RAs were expected to be fruitful sources of the type of developments which NRDC had been set up to exploit; thus much effort was spent establishing good working relationships with these groups[16].

During its early years of existence, the corporation received large numbers of submissions from private individuals, but its "first five years of experience has failed to confirm that a multiplicity of meritorious private inventors stand in need of public assistance. Overoptimistic hopes of the possibilities of effecting a spectacular increase in the national wealth by supporting them have accordingly been disappointed"[17] (see Table 1).

At this time, NRDC already covered a very wide range of activities from its nurturing of the British computer industry to the development of a potato harvester. Although its continuing concern was with the commercial problems of development and the exploitation and licensing of inventions, it was increasingly recognized that much of the corporation's investment was of a long term nature and, furthermore, was not clearly identifiable with either development or research. Thus in 1954, when the Development of Inventions Act was extended for a further five years, NRDC's research function was clarified, thereby enabling it to support research likely to lead to invention[18].

Table 1 Inventions Considered by NRDC up to 1954

Source of inventions	Total No. of inventions submitted (up to 1954)	Total No. of patent rights assigned for development/exploitation (up to 1954)
Research councils and government departments	2,254	1,322 *
Nationalized industries	5	0
Industrial research associations	45	39
Universities	240	89
Private firms and individuals	1,942	20

* This high take-up rate is chiefly due to the statutory transfer of rights accumulated in previous years.

Industrial Involvement

When the Development of Inventions Act was reviewed in 1958, NRDC's loan ceiling was raised from £5 million to £10 million, and the duration of this advance was extended from ten years to twenty years[19]. By 1960, the corporation's licence income more than covered its administrative expenses, but this was still not sufficient to finance all the development projects which it wished to fund without recourse to this Board of Trade loan. The licence income derived from only 3% of the inventions communicated to the corporation and 9% of the patent rights assigned by the corporation for exploitation[20].

As traditional sources of capital seemed to be reluctant to invest in innovation at the development stage, NRDC felt that it could benefit the nation by encouraging joint development projects with industry.

By 1964, the managing director of NRDC, then Mr John Duckworth, could state that "our experience is that there are good ideas latent in industry which can be brought forward to the stage of cooperative development after a great deal of joint investigation and planning. . . . The projects only materialize when joint investigations are made in the new climate induced by the realization that the risk of financing development work can be shared. Therefore we need a lot of hard work in close cooperation with industry to bring forward projects worthy of support. The 'conventional' development contract approach for projects chosen by government bodies is most unlikely to lead to profitable enterprises, but contracts based on risk sharing with industry on projects selected jointly with industry can have a significant future"[21].

More than one third of NRDC's new projects in 1964 were joint ventures and these covered a very wide field, including automatic foundry equipment, diesel engines and fish finders.

At that time it was felt that NRDC had been most useful "in cases where the fruits of invention had been rather long term, or rather indirect, and therefore not of particular commercial concern"[22,23], so an increase in the corporation's joint development activities with industry was deemed a desirable objective. These factors were borne in mind when the review of NRDC's activities leading to the 1965 Development of Inventions Act was carried out[23].

Stimulating Innovation

The 1965 act was the culmination of a debate about ways in which the corporation's contribution as a stimulus to technological innovation in industry could be increased. In essence, the proposals on which the act was based were those made by the President of the Board of Trade in the former administration, Mr Edward Heath, in 1964. Among other things, the act provided that the limit on the loan to NRDC be increased to £25 million, removed the time limit within which advances to it may be made and decreed that government departments should repay expenditure and/or losses incurred on projects carried out on their behalf by the corporation. It also made changes in NRDC's accountability procedures by empowering the responsible minister to give relief in respect of eight years interest on any advance made to the corporation and permitting him, with Treasury approval, to remit its liability to repay loans corresponding to projects written off by the corporation[24].

The incoming Labour government's proposed drive to modernize British industry was to be spearheaded by greater use of institutions such as NRDC and the RAs and the use of devices such as civil development contracts[25]. This approach was perhaps epitomized by the creation of the Ministry of Technology.

The NRDC, its resources expanded by the 1965 act, reorganized in the belief that "the main part of the corporation's business will continue to be concerned with the support of technological development, but that this will increase markedly and will be more broadly based"[26]. Responsibility for NRDC was transferred from the Board of Trade to the Ministry of Technology, but its operational independence was maintained.

As NRDC's outgoings increased, in large measure through more joint development activities and projects involving increased levels of expenditure, and as its forward commitments became larger, its loan ceiling was soon approached. Increasing exploitation receipts again failed to keep pace with this higher level of activity and NRDC's loan ceiling has since been increased to £50 million.

It would seem, however, that NRDC's own experience after more than twenty-one years and more than 23,000 submissions tends to support the view that "the main reason for the modest scale of NRDC's activities seems simply to be that there are very few inventions worth carrying through the development stage which are not carried through for the want of money"[27].

Changing Environment

—It has already been pointed out that NRDC was formed as a result of the complex interaction of several stimuli, among which the following may be singled out.

—There was no agency to exploit developments resulting from publicly funded research.

—There was a general lack of finance for development activities.

—British research results were being exploited abroad.

—It was felt that the patent system might not be encouraging exploitations of inventions originating with lone individuals in particular.

At the time of NRDC's creation, it was felt that, for some reason, foreigners were often more adept at exploiting the results of British inventiveness than the British themselves. Subsequent studies have shown that the availability of development funds is not of itself sufficient to ensure successful innovation; above all, a clearly identifiable market is essential. Thus in the case of penicillin, which was widely discussed when NRDC was set up, the resources to develop and produce the

Pure Che [illegible]

drug on a large scale were made available only as a result of the intensely felt need for the drug during wartime. It has also been appreciated in recent years that licensing British ideas abroad may itself have an appreciable impact on the balance of trade[28]. To cite one well known example, Pilkingtons now receive as great an income from royalties on the float glass process as from the production of glass itself using this process[29]. In fact NRDC has often been criticized for failing to pursue a more vigorous licensing policy abroad[30].

An important source of the ideas exploited by foreign competitors was believed to be the lone inventor who, it was thought, had no access to sources of development capital and was not well placed to take full advantage of the benefits of the patent system. The NRDC itself recognized at a comparatively early stage that the number of commercially viable ideas being put forward by lone inventors was negligible. Although NRDC is largely associated in the public mind with the exploitation of an invention from a private individual—namely, the hovercraft—both this and the Bacon fuel cell (similarly acclaimed as a great technological achievement) have so far been important net drains on the corporation's resources, chiefly because there does not seem to be a significant market for either invention in this country at the present time.

It was not the lone inventor, but publicly funded research, which was regarded as its principal source of developments when NRDC was created. Although these have been numerically the most significant source, the corporation could recently report, with the benefit of hindsight, that "the inventions acquired from government establishments and other parts of the public sector did not provide significant revenue, and only the emergence of cephalosporin from Oxford University as an important revenue earner in recent years changed this situation"[31]. Cephalosporin has proved to be NRDC's most significant revenue earner by far.

Computer Software

It should not necessarily be assumed from this that little exploitable research is carried out in government and university laboratories[32]; nor does this mean that all such research is exploitable through patent and licensing arrangements. The most obvious example of the last of these types of activity is the development of computer software (which is not patentable) and research in fields such as operations analysis which may not give rise to a definite product. Even in areas where the results are patentable, it does not follow that a separate agency is required to exploit them.

Thus the best way to utilize an area of research expertise (with or without associated patents) may be to create a new organization within the university or government establishment for the specific purpose of exploiting that expertise commercially. Examples of exploitation in this broader sense are the rapid growth in the contract research activities undertaken by government establishments[33], the creation of university-owned companies such as Loughborough Consultants[34] and SWIRL (at Bath University) and the formation of spin-off companies such as Oxford Instruments Ltd and Optel Instruments Ltd by people attempting to exploit their university research expertise directly. Even when it is possible to embody research results in the form of a patent, it could be argued that exploitation activities should be carried out at the level of the innovating institution as is done, for example, by certain of the larger universities or groups of universities in the United States. The cutback in the quantity of real resources available for research may mean that universities and government laboratories will increasingly have to seek this kind of extra income to set against their overheads or subsidize their traditional research activities. And the position of a "middleman" in such situations has recently been the subject of extensive debate following the comments of the Select Committee about NRDC's role in the exploitation of carbon fibres[1], although it could be argued that such a middleman is more insulated from political pressures, as well as being a source of advice.

Two areas in which NRDC has interests at present were not envisaged as its specific concern when it was set up; these are the support of research likely to lead to invention and the support of development within industry—joint development ventures—which have become increasingly important since the 1965 Development of Inventions Act.

In both these areas NRDC was a pioneer and has undoubtedly fulfilled a useful role. But other institutions now exist specifically to fulfil these two functions. The Science Research Council (SRC) recently noted that its objective was to create scientific and technological assets through the support of "research whose timeliness and promise are likely to lead to the discovery of new knowledge and techniques of significant scientific, economic or social benefit"[35]. The SRC recently set up a separate engineering board so that "added point and impetus could be given . . . to encourage . . . the application of high standards of intellectual discipline not only to the furtherance of knowledge, but to its creative development in the best interests of society at large"[36].

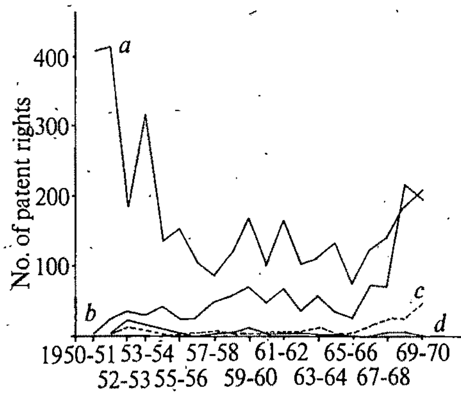


Fig. 2 Patent rights assigned to the corporation each year for further development and/or exploitation. *a*, Research councils and government departments; *b*, universities; *c*, British companies and individuals; *d*, industrial research associations.

As well as providing funds for research likely to lead to commercial exploitation, NRDC has also found it advantageous to participate in the finance of developments in private industry; when the corporation was set up, the possibility that industry itself would be an important source of undeveloped or unexploited new inventions or processes was not then contemplated[37]. This involvement has taken two forms, participation in the "equity in a development" and participation in the equity of the innovating company itself.

In the former case involving a company which is presumably trading profitably, NRDC has been prepared to contribute as much as 50% of the development cost of a particular project with the hope that it will recoup its investment by a levy on sales. (In this respect, NRDC's activities are unique. No other organization in the UK is known to undertake the support of a particular project within a firm in conditions which allow it to recoup its investment only by a levy on sales resulting from the successful outcome of that project. Grossfield[38] has discussed some of the merits of this and alternative systems of project finance.) The advantages of this scheme to the company are two-fold; first, projects can be undertaken on a risk-sharing basis in a situation in which, for one reason or another, the company does not feel it can underwrite the whole cost of development; second, it provides a method whereby a company can undertake development projects over and above those which it could finance from retained earnings or normal borrowing, possibly allowing the firm to broaden its product range more quickly. From the point of view of NRDC the investment is hopefully less risky because the development is carried out within the user company, and possibly more

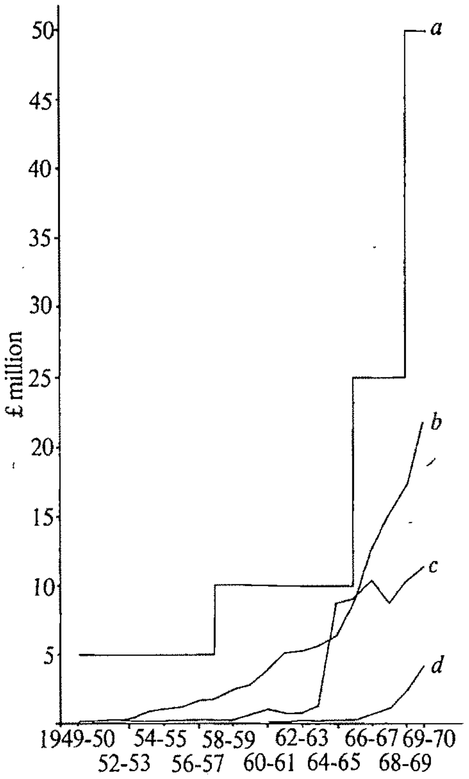


Fig. 3 Finances of NRDC (from annual reports). *a*, Government loan ceiling; *b*, cumulative government loan; *c*, additional forward commitments that year; *d*, total income. The cumulative loan is the difference between total incomings and outgoings. Total income includes sale of patent rights, revenues from exploitation of inventions and levies from joint venture projects.

profitable than some of the corporation's other activities, such as investment in start-up situations.

In the latter case, NRDC takes equity in the associated or subsidiary company—examples include Revenue Systems Ltd (46% holding), which is developing automated credit cards, and Tracked Hovercraft Ltd (100% holding) (see ref. 39 for a complete list). Although NRDC invests in these situations at an earlier stage of development than traditional institutions would usually consider, it presumably adopts very similar investment appraisal, selection and monitoring criteria. Indeed in some cases NRDC has invested in projects jointly with non-government funded institutions; for example, Technical Development Capital Ltd also holds equity in Revenue Systems Ltd.

The development of the NRDC in the context of British scientific and industrial institutions since the end of the Second World War can be seen in terms of a growing awareness of the complexities of technological innovation. It now seems clear that the continual stream of technological development, which forms an important basis for economic growth, cannot be obtained through the simple expedient of nurturing scientific and technical ideas in the hope that their commercial relevance will be apparent to the industrial sector. The temptation is very great to judge an idea or sophisticated gadget on its own merits, without adequate attention to its usefulness and its ultimate commercial viability. With the benefit of hindsight it is now evident that scientific and technical ideas must be seen as one element in the matrix of innovation—more consideration and effort in terms of both money and manpower must be expended in the production and marketing of ideas and hardware.

An apparent paradox, experienced by the NRDC over the last twenty years, thus emerges. On the one hand, there is no doubt that many eventually successful products are dependent on the use of sophisticated technologies and that these should therefore be nurtured; on the other hand, the relationship between technology and the form of organization seeking to exploit it is so intimate that it could draw the government into almost every phase of its operations. In the present political climate, at least, this is regarded as undesirable. The simple fact seems to be that, considerations of national interest apart, it is difficult to see why projects which are judged to be commercially viable should need to be financed from public funds. The difficulty is to ensure that the community does not lose the potential benefits which might arise through innovations resulting from NRDC-type activities which produce a consumer surplus without any organization apparently obtaining any greater or smaller profits[38]. Certainly the government's stated policy of disengagement from industry—witnessed by, for example, the disbandment of the Industrial Reorganization Corporation—is dependent (if industrial development is to continue) on private sources of capital acquiring the same kinds of expertise and taking the same kinds of calculated techno-economic risks that are now being taken by NRDC.

G. E. H. acknowledges a Science Research Council advanced course studentship in the Department of Liberal Studies in Science; D. S. W. holds a Midland Bank Finance Corporation research fellowship at the Manchester Business School Research and Development Research Unit.

[1] *Select Committee on Science and Technology, Carbon Fibres Report*, HC 157 (HMSO, 1969).
[2] House of Commons written answers, *Hansard* (February 17, 1971).
[3] *Financial Times*, 6 (April 1, 1971).
[4] *The Reorganisation of Central Government*, Cmnd. 4506 (HMSO, 1970).
[5] *Industrial Research and Development in Government Laboratories* (HMSO, 1970).
[6] Haigh, G. E., thesis, Univ. Manchester (1970).
[7] *Problems of Scientific and Industrial Research*, 6 (Nuffield College, 1944).
[8] Vig, N. J., *Science and Technology in British Politics* (Pergamon, Oxford, 1968).
[9] *Swan Committee Report on the Patents and Designs Acts*, Cmnd. 7206 (1947) and Cmnd. 6789 (1946).
[10] Hobhouse, L. T., *Science and Administration in Modern Government*, Memorial Trust Lectures, No. 14 (London, 1944).
[11] Larke, Sir W., *Industry and Research* (Federation Brit. Industries Conf., 1946).
[12] Attlee, C., *Hansard* (April 19, 1944).
[13] Wilson, H., *Hansard* (June 11, 1944).
[14] *Development of Inventions Act* (1948).
[15] *Treasury Circular*, T.C. No. 5/50 (1950).
[16] *NRDC Report and Statement of Accounts 1949/1950* (HMSO, 1951).
[17] *NRDC Annual Report, 1953/4*, 2 (HMSO, 1955).
[18] *Development of Inventions Act* (1954).
[19] *Development of Inventions Act* (1958).
[20] Grossfield, K., *Econ. J.*, **72**, 12 (1962).
[21] Duckworth, J., *Economist*, 5 (January 4, 1964).
[22] *Estimates Committee, Third Report*, 52 (1964/5).
[23] *NRDC Annual Report 1964/65* (HMSO, 1966).
[24] *Development of Inventions Act* (1965).
[25] *National Plan*, Cmnd. 2764, 49 (1964/5).
[26] *NRDC Annual Report 1964/65*, 6 (HMSO, 1967).
[27] Edwards, R. S., and Townsend, H., *Business Enterprise: Its Growth and Organisation*, 364 (Macmillan, 1965).
[28] Caplan, B., *New Scientist*, 326 (May 6, 1971).
[29] *Economist*, 85 (May 1, 1971).
[30] *Times Business News* (March 16, 1971) and reply by Dr B. Bard, NRDC managing director (March 22, 1971) and associated correspondence.
[31] *NRDC Annual Report 1969/70* (HMSO, 1971).
[32] Gibbons, M., and Watkins, D. S., *J. R. and D. Management*, **1**, 11 (1970).
[33] Marshall, W., *The Role of Harwell in Industrial Research and Innovation*, Conf. industrial innovation through contract research, University of Newcastle upon Tyne, April 1970.
[34] *New Technology*, No. 33 (October 1969).
[35] *Selectivity and Concentration in Support of Research*, SRC pamphlet, 1–70 (Science Research Council, London, 1970).
[36] *Science Research Council Annual Report 1969/70* (HMSO, 1970).
[37] *NRDC Annual Report 1966/67*, 6 (HMSO, 1968).
[38] Grossfield, K., *National Provincial Bank Review*, **77**, 6 (1967).
[39] *NRDC Annual Report 1969/70*, 79, 80 (HMSO, 1971).

Lithium in Psychiatry

I. B. PEARSON
University Department of Psychiatry, Sheffield S10 3TL

F. A. JENNER
MRC Unit for Metabolic Studies in Psychiatry, University Department of Psychiatry, Sheffield S10 3TL

Lithium salts have been reported to have valuable therapeutic effects in psychiatric disorders. But although some impressive results have been obtained, there is still much to be learnt about the effects and metabolism of lithium.

LITHIUM salts have been used medicinally in various ways for more than a century, and their history has been one of mistaken enthusiasm. Garrod recommended them for the treatment of kidney stones, gout and other rheumatic conditions in 1859[1], but without appreciable success. Subsequently lithium bromide was given as an anticonvulsant in the treatment of epilepsy, but it was no more effective than other bromides. At the end of the nineteenth century lithium salts were first tried in the treatment of mental illness. Lange[2] considered that lithium was helpful in mental depression, but fifty years were to elapse before further and more extensive psychiatric uses developed. More recently lithium salts have been offered as a substitute for sodium chloride in patients requiring a restricted sodium intake, but there were fatalities, and it is now recognized that cardiac and renal decompensation are both indications that the use of lithium should be avoided. Psychiatric interest was revived after work reported by Cade in 1949[3] when, following an accidental observation of a sedative effect on guinea-pigs, lithium was found to be helpful in controlling mania. The modern psychotropic use of lithium dates from that time, but dissemination has been slow and probably reflects a natural caution after the earlier fatalities, combined with the introduction of many new and effective psychoactive compounds.

During the past two decades lithium has been used increasingly in the treatment of manic-depressive illnesses in which the mood of the patient is elevated in mania and lowered in depressions. Results so far suggest that lithium salts have considerable potential in the treatment of manic depressive illnesses, although it is far from clear how they exert their effects.

Very occasionally the patient's mood changes in a regular clock-like manner, but it is more usual to find only occasional brief episodes of mania interspersed in a series of depressive episodes. The remarkably regular patients, though very rare, offer unusual opportunities to prove that lithium salts have a psychopharmacological effect. This has been done, but the relevance of such findings to the more usual clinical studies requires a more complicated statistical approach, taking proper account of the variable natural history of the condition.

It is difficult to classify these illnesses since unipolar affective disorders are much commoner than the bipolar type, and patients who have recurrent episodes of depression for several years may then have a manic episode. Depressions also merge into anxiety and other psychiatric states. The natural history and heterogeneity of the clinical group studied present all sorts of obvious difficulties to the investigator, and it is these types of problem which have influenced much of the debate about lithium in psychiatry. Schou, who produced a useful guide to the literature in 1968[4], has been the most active and assiduous student in this field.

The effectiveness of lithium in the treatment of mania is now well recognized, and has been confirmed by controlled studies. But this treatment requires daily doses of the order of 1.5–2 g of lithium carbonate, when therapeutic and toxic levels closely approximate. In recent years the prophylactic use of lithium has been advocated, using rather smaller doses in the range 0.75 to 1 g daily of lithium carbonate, when toxic effects are reduced. The efficacy of lithium salts in the prophylaxis of manic depressive illness has been the subject of some debate. It has been claimed that when effective, lithium salts diminish the change in mood and in addition reduce the frequency of relapse in affective illness. Angst *et al.*[5] have demonstrated a natural tendency in manic depressive illness for the frequency of episodes to increase, but the pattern of relapse in affective illness is variable, although it does tend to show a progression of increasing frequency which is greater in bipolar illness than in recurrent depressive illness.

Blackwell and Shepherd[6] properly challenged some of the earlier favourable reports, pointing out that with such patients seen at a time of frequently recurring illness, a reduced rate of relapse might be accounted for solely by chance, and certainly this could be so with patients having frequent episodes, even if there is an overall tendency for the cycles to shorten. They suggested that the prophylactic value of lithium could only be determined by a comparative trial. Subsequently several clinical studies have been reported, including double blind trials, and most of them support the original conclusion that prophylactic lithium is of value. In general it seems that the frequency of relapse is appreciably reduced and that the natural trend of increasing frequency is reversed.

Lithium belongs to the group of alkali metals which includes sodium and potassium and it shares several properties with the other members of this group, but Birch has pointed out that in certain respects it resembles magnesium and calcium to which it bears a diagonal relationship in the periodic table[7]. It is absorbed promptly and virtually completely from the gastro-intestinal tract, and this is independent of whether lithium is given as carbonate, citrate or acetate. Lithium is not bound to plasma proteins, but is distributed freely in the tissues, and equilibration occurs, although this is somewhat slower in bone and brain. Distribution in the tissue differs sharply from that of sodium and potassium since lithium is more evenly divided between intracellular and extracellular compartments. Concentrations in the serum reflect those in tissue and can be used satisfactorily to monitor treatment. Lithium salts are largely excreted by the kidneys, and only small amounts are lost in faeces and sweat. Renal lithium excretion is proportional to the plasma concentration over a wide range; renal clearance is of the order of 15–30 ml./min, but decreases with age, and values of 10–15 ml./min are not uncommon in elderly people. Lithium excretion decreases when sodium intake is restricted.

The principal disadvantage of lithium therapy lies in the narrow safety margin between therapeutic and toxic doses. Therapeutic plasma concentrations are usually considered to be between 0.8 and 1.6 mequiv./l.; at these concentrations only minor side effects occur. The commonest of these effects are gastro-intestinal disturbances, muscular weakness, tremor, drowsiness and a dazed feeling. When plasma concentrations are higher, serious toxic effects are liable to ensue, and poisoning results in coma, hyperreflexia, muscle tremor, attacks of hyperextension of the limbs and convulsions; fatalities have occurred. Haemodialysis is the most effective treatment if available but where this is not possible patients should be treated by forced diuresis. Long term treatment may give rise to small goitres and hypothyroidism but these usually respond to modest doses of thyroxine. Teratogenic effects have now been reported in two out of forty patients who received lithium during pregnancy[8]. Clearly lithium should be used with care, and concentrations in the blood should be monitored regularly.

Mode of Action

The mode of action of lithium in affective illness remains obscure, and most theories have related to interference in electrolyte metabolism or to effects on the metabolism of brain amines. More recently evidence has been adduced to suggest a specific effect of lithium on the limbic system of the brain, an area known to be associated with emotion[9]. These uncertainties about the action of lithium reflect the unsatisfactory state of understanding of the biological disturbance associated with manic-depressive illness.

When the fundamental importance of sodium and potassium for neuronal activity is considered together with the similarity of their ions to those of lithium, the suggestion that lithium interferes with the metabolism of these ions seems to be reasonable. Evidence is accumulating that disturbance of electrolyte and water balance accompanies manic-depressive illness although it is not clear whether this is causal or consequential. It has been shown that the administration of lithium leads to changes in the body water spaces, and this observation is supported by the natriuresis following the intake of lithium, which is more striking in normal people than in manic-depressive patients. More lithium is retained by patients than by normal people, and the retention is greater in mania and in patients who respond to lithium therapy. In addition a proportion of patients taking lithium develop persisting polyuria and thirst. This may be explained by the fact that lithium ions specifically and reversibly inhibit the action of the antidiuretic hormone on the kidney. Conceivably the consequences of this inhibition are relevant to the therapeutic effects.

Further work on this particular action has demonstrated that lithium probably blocks the action of the cyclic AMP released by vasopressin[10]. This raises the possibility that inhibition of the effect of vasopressin is just one example of an action on the effects of all hormones releasing cyclic AMP in target cells. In addition to its action on cyclic AMP, lithium also inhibits adenyl cyclase necessary for the production of cyclic AMP from ATP, but in the kidney this only occurs with high doses[11].

Perhaps a more exciting observation is an increase of α-ketoglutaric acid (shown by Bond *et al.*) in the urine of patients receiving lithium. This observation, although unequivocally established, still requires explanation. Does lithium, for example, inhibit enzymes in the tricarboxylic acid cycle and so cause a build-up of α-ketoglutaric acid? Is it a response to alkalosis and does it occur in brain? This, like many other questions, is being actively explored. It could also be relevant to Delgado and DeFeudis's observation[9] that lithium leads to an increased level of brain glutamate.

Inhibition of several enzyme systems could be the result of effects of lithium on tissue magnesium and calcium which have now been observed. As these changes may also occur in brain, possible hypotheses of the consequences are legion.

The work of Smith, Balagura and Lubran[12] demonstrates an apparently direct action of lithium on the lateral nuclei of the hypothalamus. There is stimulates drinking behaviour and presumably thirst. Application of lithium ions directly to the hippocampus and amygdala of monkeys[9] evoked rather specific EEG effects which could be associated with the reduction of aggression reported in man, rodents and fish[13–15]. These are among the few reported direct effects of lithium on nervous tissue, and it is not surprising that neural function can be altered in this way, but the significance of these observations in relation to the therapeutic actions of lithium is not entirely clear.

Lithium does, however, have very clear effects on the electroencephalogram in man. In particular, it increases the amplitude of the alpha rhythm and causes slow waves to appear. After quite usual doses of lithium carbonate have been discontinued, the effects on the electroencephalogram persist for up to 6 weeks.

Lithium, like so many other agents which have therapeutic uses, is like a heavy spanner in a delicate piece of machinery. Like electroconvulsive therapy, it changes so many things that clues to as what is relevant are difficult to assess. Among other effects which require further study, one must include inhibition of carbohydrate transport[16–18] across various membranes including the inhibition of the accumulation of myoinositol by the lens[19] and kidney[20]. The latter substance may well have a special role in the central nervous system.

The most popular current theories suggest that brain monoamine metabolism is altered in manic-depressive illness, but again, as with electrolytes, it is uncertain whether this is cause or effect; moreover, it is not clear which of the amines is related to changes in mood. Lithium has effects on brain monoamine metabolism, but these are confined to noradrenaline and the function of noradrenergic neurones. Noradrenaline metabolism is shifted from O-methylation to intraneuronal deamination with an increase in noradrenaline destruction; noradrenaline uptake by synaptosomes is increased and the release of synaptic neurotransmitters may be inhibited. Unfortunately these findings do not easily fit into models developed to account for the actions of psychotropic drugs, since such drugs change mood in one direction only, whereas lithium restricts mood swing in both directions. One school of thought, however, considers mania as a more profound form of depression[21].

The pharmacological actions of lithium salts are curiously complex, and knowledge about them is far from complete. Indeed, there is ample room for further research designed to increase our understanding of the effects of lithium, which may in turn shed more light on the biological mechanisms possibly involved in manic-depressive illnesses.

[1] Garrod, A. B., *Gout and Rheumatic Gout* (Walton and Maberly, London, 1859), cited by Ottosson, J. O., *Acta Psychiat. Scand.*, Suppl. 207 (1969).

[2] Lange, C., *Bidrag til urinsyrediates ens klinik. Hospitalstidende*, 5, 1 (1897), cited by Ban, T., *Psychopharmacology* (Williams and Wilkins, 1969).

[3] Cade, J. F. J., *Med. J. Austral.*, **36**, 349 (1949).

[4] Schou, M., *J. Psychiat. Res.*, **6**, 67 (1968).

[5] Angst, J., Weis, P., Grof, P., Baastrup, P. C., and Schou, M., *Brit. J. Psychiat.*, **116**, 604 (1970).

[6] Blackwell, B., and Shepherd, M., *Lancet*, i, 968 (1968).

[7] Birch, N. J., *Brit. J. Psychiat.*, **116**, 461 (1970).

[8] Schou, M., and Amdisen, A., *Lancet*, i, 1391 (1970).

[9] Delgado, J. M. R., and DeFeudis, F. V., *Exp. Neurol.*, **25**, 255 (1969).

[10] Harris, C. A., and Jenner, F. A., *J. Pharmacol.* (in the press, 1971).

[11] Dousa, T., and Hechter, O., *Life-Sci.*, **9**, 1, 765 (1970).

[12] Smith, D. F., Balagura, S., and Lubran, M., *Physiol. Behav.*, **6**, 209 (1971).

[13] Sheard, M. H., *Nature*, **230**, 113 (1971).

[14] Sheard, M. H., *Nature*, **228**, 284 (1970).

[15] Weischer, M. Z., *Psychopharmacologia*, **15**, 245 (1969).

[16] Bhattacharya, G., *Biochim. Biophys. Acta*, **93**, 644 (1964).

[17] Bihler, I., and Adamic, S., *Biochim. Biophys. Acta*, **135**, 466 (1967).

[18] Bosackova, J., and Crane, B. K., *Biochim. Biophys. Acta*, **102**, 423 (1965).

[19] Varma, S. D., Chakrapani, B., and Reddy, V. N., *Invest. Ophthal.*, 9, 794 (1970).

[20] Margolis, R. U., and Heller, A., *Biochim. Biophys. Acta*, **98**, 438 (1965).

[21] Coppen, A., and Shaw, D. M., *Lancet*, ii, 805 (1967).

Perspectives in Organometallic Chemistry

F. G. A. STONE

School of Chemistry, University of Bristol

Professor Stone describes the state of the art of organometallic chemistry and suggests that it will continue to be a growth point in the chemical sciences.

ORGANOMETALLIC compounds are those substances containing organic groups attached to metal atoms by carbon–metal bonds. The metal may be from the main groups of the periodic table (for example, magnesium or lead), or it may be a transition element such as titanium or iron. The organic groups are of many different types and include both the familiar radicals of organic chemistry such as CH_3 or C_6H_5 which bond both to main group metals and to transition metals, and also stable molecules such as CO, C_2H_4 or C_6H_6 which can coordinate to transition metals. One of the many interesting facets of this field has been the discovery that transition metals can form stable bonds with entities such as cyclobutadiene or trimethylenemethane which are incapable of an independent existence. Countless organometallic compounds are known, and many have played an important role in the history, theory, technology and laboratory practice of chemistry. For example, the discovery[1,2] of ferrocene *bis*(π-cyclopentadienyl)iron, $(\pi\text{-}C_5H_5)_2Fe$ (Fig. 1), brought about a renaissance in the study of organo-derivatives of the transition metals. The elucidation[3] of the polymeric structure of $[(CH_3)_2Be]_n$ did much to stimulate work on electron deficient compounds. In these the number of bonding orbitals exceeds the number of available electron pairs, so that some bonding orbitals embrace more than two atoms—that is, they are multicentred. The Ziegler–Natta catalysts[4] have had a profound effect on the technology of macromolecules, and organoboron compounds are now widely used in organic syntheses[5].

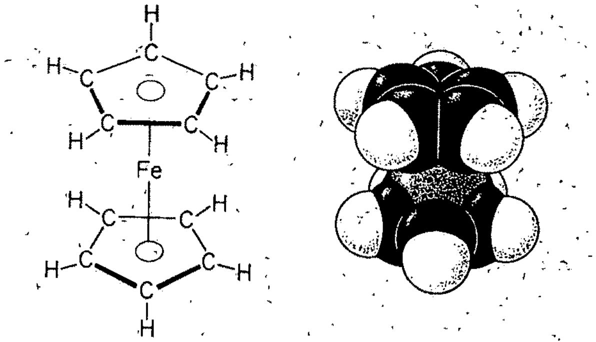


Fig. 1 The structure of ferrocene.

In the chemical literature, references to organometallics first appeared in the early nineteenth century. In 1827 the Danish chemist Zeise[6,7] found that boiling platinum chloride with ethanol and adding potassium chloride produced a substance of composition $KCl{\cdot}PtCl_2{\cdot}C_2H_4{\cdot}H_2O$. More than 100 years were to pass before it was realized that Zeise's salt should be formulated as a coordination compound of platinum, that is $K[C_2H_4PtCl_3].H_2O$, with carbon–metal bonding. Early historical landmarks were Bunsen's synthesis[8] of cacodyl $(CH_3)_2AsAs(CH_3)_2$ in 1842, Frankland's synthesis[9] of alkylzinc compounds in 1849, Mond's synthesis[10] of nickel carbonyl $Ni(CO)_4$ in 1890, and Grignard's discovery[11] of organomagnesium compounds (for example, C_2H_5MgBr) in 1900.

No account of recent developments in organometallic chemistry would be complete without a brief summary of current views on such matters as stability, bonding and structure. Indeed, it is in these areas that some of the most significant recent advances have been made. Studies in this field, as in organic chemistry, have been greatly aided by the application to structural problems of such techniques as nuclear-magnetic resonance spectroscopy and single crystal X-ray crystallography. The discovery of many novel compounds with unusual bonding of organic groups to metals has done much to stimulate preparative work in this field. The application of valence theory, particularly consideration of molecular rather than atomic orbitals, has led to a better understanding of the reactivity of compounds with important consequences for further chemical syntheses.

Stability, Bonding and Structure

All organometallic compounds are thermodynamically unstable to oxidation, the driving force for which is the large favourable free energy change in the conversion into metal oxide, carbon dioxide and water. In this respect organometallic compounds are no different from purely organic compounds except that an additional product (metal oxide) is involved. As in organic chemistry, however, kinetic factors are often decisive in inhibiting oxidation in ambient conditions. Instability to air can often be associated with the presence of empty low lying metal orbitals which can interact with molecular oxygen thereby providing a pathway to decomposition involving little activation energy. Similar considerations apply to hydrolytic instability. Hydrolysis often proceeds by initial bonding of a water molecule, a process facilitated by an empty metal orbital which can accept an electron pair from the substrate. Thus the simpler organo-derivatives of lithium, beryllium or aluminium are readily, sometimes even explosively, decomposed by water or oxygen. The high reactivity can be traced to the ability of these metals first to coordinate a reactant molecule; this is followed by interaction of the bonded substrate with a polar carbon–metal bond. Indeed, whether or not decomposition follows attachment of a substrate molecule depends on the nature of the carbon–metal bond as determined by electronic and steric factors. With electropositive metals such as aluminium the bond may be sufficiently polar in the sense $\overset{\delta+}{M}\text{—}\overset{\delta-}{R}$ that the organic group becomes susceptible to electrophilic attack (for example, from a proton of coordinated water to release RH). Most organometallic compounds are, however, highly covalent in nature because the electronegativity differences between the organic groups and the metal atoms are small. Indeed, the least electropositive metals, such as lead or mercury, form organo-derivatives (for example $(C_2H_5)_4Pb$ or $(CH_3)_2Hg$) which provide excellent examples of covalent compounds with classical two electron, two centre σ bonds. In accord with this they are volatile, hydrocarbon soluble and immiscible with water. By contrast with the behaviour of the light metals mentioned above, chromium forms a cationic benzyl complex $[C_6H_5CH_2Cr(H_2O)_5]^{2+}$ sufficiently stable to have a half-life of about 1.5 day in solution[12]. Even though water molecules are present in the coordination sphere of the metal in the cation, the C–Cr σ bond is insufficiently polar to release the benzyl group as toluene. Addition of acid does, however, decompose the complex.

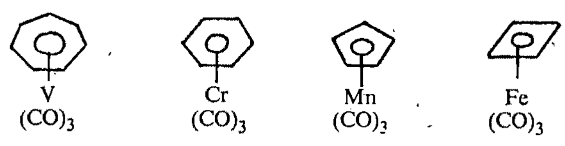
Carbon–mercury bonds form readily from mercury salts in a variety of reactions. These bonds once formed are relatively stable in ambient conditions because the R–Hg group has little affinity for oxygen or water. Organo-mercurials are very poisonous, and the chemical robustness of CH_3Hg and related groups can have serious consequences if they become accumulated in natural food chains through careless discharge of industrial waste. One process for converting acetylene into acetaldehyde depends on a mercury catalyst and effluent from a factory using this process was responsible for several deaths from the so-called Minamata Bay disease. Shellfish removed from the sea for human consumption were subsequently found to contain the compound CH_3HgSMe (ref. 13). The death of birds in Sweden was traced to the use of phenylmercury compounds in the pulp and paper industry; degradation produced elemental mercury which was converted to methylmercury by bacterial action—after ingestion into fish the mercury became concentrated in sea birds. Organomercury compounds have been used in fungicides used in the treatment of wheat and in this manner mercury has been transmitted to humans by way of hogs in New Mexico[14].

The thermal stability of organometallic compounds varies considerably. Some such as the tetraalkyl derivatives of tin or ferrocene are very stable. The last of these survives heating to over 400° C in the absence of air. The robustness of a particular organometallic can be related to the energy required to bring about loss of the organic group as a molecule or a radical and this in turn depends on the activation energy needed to promote a bonding electron from the highest filled molecular orbital of the compound into an anti-bonding orbital. In this way a molecule C_nH_n could dissociate from π-C_nH_nM, and a radical R, which could irreversibly dimerise to R_2, might form from σ-RM (where M is a metal atom with attendant ligands). In the last case an alternative mode of decomposition involves release of olefin through the reaction $\sigma\text{-}CH_3CH_2M \rightarrow C_2H_4 + HM$. The reverse of this reaction is important in the synthesis of organometallic compounds.

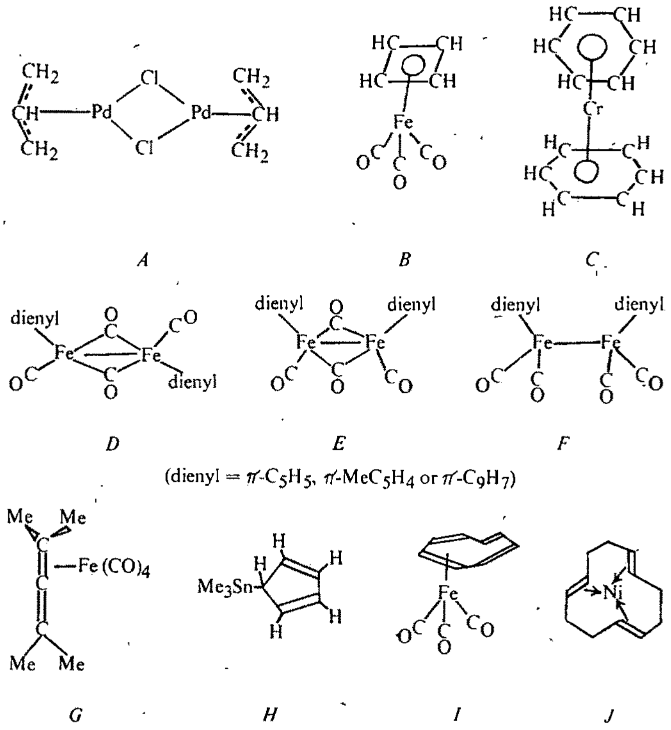
Evidently the energy required for decomposition is very small for a molecule such as $(CH_3)_4Ti$ which decomposes above −78° C, whereas it is high for a molecule such as ferrocene or $(Ph_3P)_2Pt(CH_3)_2$. Phosphines are especially effective ligands for stabilizing carbon–metal σ bonds and it has been suggested that they modify the relative energies of the orbitals so that the energy difference between the highest occupied and lowest unoccupied orbital is increased[15,16]. The stability of ferrocene may be correlated with the complete electron occupation of all bonding orbitals (the iron atom acquires a pseudo-krypton electron configuration according to the "effective atomic number rule") coupled with the relatively high energy of the anti-bonding orbitals, the transfer of electrons to which would destabilize the compound.

π-Complexes

Ferrocene[17,18] is but one of many so-called "π-complexes", most of which have been prepared in the past ten or fifteen years. π-Complexes are coordination compounds in which unsaturated organic systems are associated with metals, which may or may not have other ligands present. Examples include Zeise's salt (mentioned earlier), allylpalladium chloride[19–21], cyclobutadieneiron tricarbonyl[22] and *bis*(benzene)-chromium[23] (Fig. 2*A*, *B* and *C*). Generally the bonding of these unsaturated groups to the metals is interpreted in terms of donation of π electrons from the hydrocarbon into vacant d orbitals of the metal, coupled with transfer of electrons from the metal to the ligand by way of overlap of filled orbitals of the metal with empty anti-bonding (π^*) orbitals of the hydrocarbon. The components of the bond thus involve opposing electron flows and as such tend to strengthen one another (a synergic effect). This concept stems from consideration of the nature of the bonding of the ethylene to platinum in the anion of Zeise's salt $[(\pi\text{-}C_2H_4)PtCl_3]^-$ (refs. 24, 25). The overall effect on the stereochemistry of the complexes is that all the carbon atoms of the unsaturated systems are equivalently bonded to the metal atoms, for example



It follows that if retrodative bonding is important for the existence of the compounds, metals will generally only form π-complexes when they are in low oxidation states because only then will filled d orbitals be available on the metal atoms to partake in bonding with π^* orbitals of the hydrocarbon groups. In support of this concept, it has been found experimentally that the new organic chemistry of the transition elements is most extensive when the metal is in a relatively low oxidation or valence state. Moreover, the discovery of countless π complexes has brought about a convergence of organometallic and coordination chemistry. Thus nickel is formally zerovalent not only in the well recognizable coordination complexes $K_4[Ni(CN)_4]$ and $Ni(PF_3)_4$ but also in the π-complex $Ni(\pi\text{-}C_8H_{12})_2$ (C_8H_{12} is cycloocta-1,5-diene).



(dienyl = π-C_5H_5, π-MeC_5H_4 or π-C_9H_7)

Structural Studies

Although infrared and nuclear magnetic resonance (NMR) spectroscopy are used extensively in the study of organometallic compounds, the increasing complexity of the complexes discovered makes the X-ray crystallographic method the final arbiter of structures. This analytical technique has been greatly facilitated by computer processing of data, and about 150 new organometallic structures are now determined by this technique each year. Important though the X-ray study of single crystals is, however, it has become increasingly clear in the past decade that the structure of an organometallic in the solid state is not necessarily the same as that operating in solution. This is because many of the molecules are stereochemically non-rigid. The intramolecular rearrangements can take two forms; the first involves tautomerism wherein non-equivalent structures are detectable, often by infrared spectroscopy. Thus the cyclopentadienyliron carbonyl complex $[Fe(CO)_2(\pi\text{-}C_5H_5)]_2$ has the centro-symmetric structure (Fig. 2*D*) in the crystal with two bridging and two terminal CO ligands[26]. The infrared spectra of this and related complexes (dienyl = π-MeC_5H_4 or π-C_9H_7(indenyl)) in solution are, however, solvent dependent, and vary in a manner that indicates an equilibrium between the forms indicated in Fig. 2*D*, *E* and *F*; the last form is present only in small amounts[27]. A second phenomenon, even more widely observed, involves interconversions between equivalent structures which are therefore of equal free energy content. Such molecules are termed fluxional[28]; atoms of a given element exchange sites within the molecule at a speed which can be detected by n.m.r. spectroscopy. This spectroscopic technique operates on a time scale 10^{-1} to 10^{-9} s, similar to that of the dynamic processes whereby molecules pass from one configuration to another involving a different permutation of the nuclei.

Several types of fluxional organometallic molecules are known. An interesting example in the acyclic olefin category is the tetramethylallene complex[29] (Fig. 2*G*). The instantaneous structure shown should give rise to a proton NMR spectrum with three sets of resonances (relative intensity 1 : 1 : 2), whereas a single sharp signal is observed at 25° C. It is reasonable to assume that the $Fe(CO)_4$ group moves from end to end of the allene group at a speed greater than the time scale of the NMR experiment. Such intramolecular processes should slow down when the temperature is lowered and in accord with this at −60° C the spectrum of the structure shown in Fig. 2*G* appears as the expected three signals.

Many non-transition metal and transition metal compounds containing σ-bonded cyclopolyene groups show fluxional behaviour. For example, the chemical behaviour of the cyclopentadienyl compound $Me_3Sn(\sigma\text{-}C_5H_5)$ (Fig. 2*H*) suggests the 1,3-diene system shown, which would imply three different environments for the protons. The spectrum nevertheless shows only a single peak indicating a rapid movement of the carbon–metal bond among the five possible positions.

Among the most intensively studied fluxional organometallics are those involving coordination of a cyclic polyolefin to a metal carbonyl group, the cyclic olefin having more π-electrons than the metal needs to achieve the effective atomic number rule (that is, 4 for the $Fe(CO)_3$ group and 6 for the $Mo(CO)_3$ group). A classic example of this behaviour is the cyclooctatetraene complex (Fig. 2*I*). The structure shown has been established in the crystal state by X-ray methods[30]. The diffraction of X-rays takes place on a time scale of 10^{-18} s and so this technique "sees" the instantaneous structure. At room temperature the proton NMR spectrum of $(\pi\text{-}C_8H_8)Fe(CO)_3$ shows a single peaking indicating that all protons are equivalent which is not in accord with a rigid structure as shown in Fig. 2*I*. Apparently the locus of the metal attachment to the four carbon atoms of the ring shifts at such a speed that the protons appear to be equivalent. On lowering the temperature to −150° C the spectrum becomes more complex; for example, that of the ruthenium analogue $(\pi\text{-}C_8H_8)Ru(CO)_3$ displays four bands as the intramolecular rearrangement slows down[31,32]. The mechanism by which this occurs—for example, a 1,2-shift of the $M(CO)_3$ group around the ring, is not known with certainty partly because of the experimental difficulties of knowing whether the limiting spectrum corresponding to rigidity has been reached even at ~ −150° C (ref. 33).

Chemical Reactivity

The rapid expansion of organometallic chemistry in recent years has had many important consequences which have stimulated research. It has become clear that there are numerous ways in which organic groups can be combined with metals and because metals vary so much in their chemistry, quite different properties are found for organometallic compounds involving the same organic group but different metallic elements. The extraordinary development of the organic chemistry of the transition metals has followed in part from the recognition that the properties of organometal groups are influenced by the presence of other ligands in the coordination sphere of the metal. In other words, because of their electronic and steric effects, particular ligands can act as fine tuners in determining the reactivity of others.

In dealing with such wide variations of chemical behaviour in the context of developments in recent years it is convenient to separate the organo-derivatives of the main group metals from those of the transition metals. Such a separation is, however, somewhat arbitrary because many compounds are known containing both main and sub-group metals associated with organic moieties.

Main Group Metals

During the first half of this century it became widely recognized that organolithium and organomagnesium compounds were valuable in synthesis. In 1956 it was discovered that diborane (B_2H_6), generated *in situ* from sodium borohydride and a boron halide, added rapidly to olefins in the presence of ether (for example, diglyme $(CH_3OCH_2CH_2)_2O$) (refs. 5, 34, 35).

$$12RCH:CH_2 + 3NaBH_4 + 4Et_2O.BF_3 \xrightarrow{\text{diglyme}} 4(RCH_2CH_2)_3B + 3NaBF_4 + 4Et_2O$$

It has been found possible to cleave the C–B bonds thus formed in a variety of ways, and this reaction, termed hydroboration, has become the basis of many organic syntheses comparable in importance with the earlier discovered Grignard and lithium reagents. Thus treatment of a triorganoborane with alkaline hydrogen peroxide yields an alcohol RCH_2CH_2OH, whereas protonation by acid gives RCH_2CH_3, that is, an overall hydrogenation of a double bond. Oxidation of $(RCH_2CH_2)_3B$ with chromic acid gives ketones or acids, whereas treatment with bromine and sodium methoxide gives RCH_2CH_2Br (an overall anti-Markownikov addition of HBr to $RCH:CH_2$). Many related organic syntheses depend on the hydroboration of acetylenes or dienes as the first step, and with olefins, acetylenes or dienes the substituents on the double or triple bonds can be complex organic groups so that the reaction has very wide scope throughout organic chemistry.

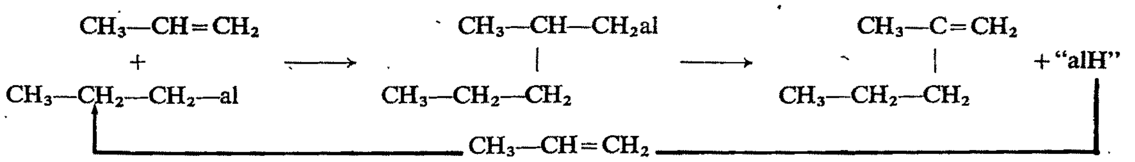
Hydroboration is formally related to the so-called "direct syntheses" of aluminium alkyls developed by Ziegler and his coworkers[4]:

$$Al + 3H_2 + 3(1\text{-olefin}) \rightarrow alkyl_3Al.$$

Moreover, aluminium alkyls themselves react with olefins in a stepwise growth reaction:

$$R_3Al + 3nC_2H_4 \rightarrow [R(CH_2CH_2)_n]_3Al.$$

As aluminium alkyls are readily oxidized to alcoholates $(RO)_3Al$ which in turn can be hydrolysed, this process forms the basis for a conversion of ethylene into long chain primary alcohols. The importance of the aluminium alkyls does not, however, end with the synthesis of alcohols needed for the detergent industry. Propylene when heated with tripropylaluminium under pressure is converted into 2-methyl-1-pentene



$((C_3H_7)_2AlH = alH)$

Rearrangement of 2-methyl-1-pentene into 2-methyl-2-pentene followed by elimination of methane by cracking affords the important monomer isoprene (synthetic rubber).

Transition Metals

Spurred by the recognition of the sandwich structure of ferrocene and the discovery within the following decade of such compounds as dibenzene chromium (Fig. 2*c*, ref. 23) and cyclododeca-1,5,9-trienenickel (Fig. 2*J*, refs. 36, 37), new organo-complexes of the transition metals were characterized very rapidly[38]. This period of research activity was accompanied by a growing awareness of the many ramifications of this field. Among the more important of these may be mentioned the following.

(i) It is relatively easy to prepare metal complexes containing hydrocarbon groups if certain other ligands are also present, particularly carbon monoxide and phosphine groups. Moreover, it is also possible to prepare complexes containing only hydrocarbon ligands if either the metal is preconditioned by a reductive process in the presence of a hydrocarbon which then uses its π electrons to fill the metal's bonding orbitals, or a salt is treated with a suitable carbanion so that in the organometallic produced the metal remains in the same stable valence state. Such syntheses are illustrated by the preparation of the nickel complex $Ni(\pi\text{-}C_8H_{12})_2$ by reducing nickel acetylacetonate with aluminium alkyls in the presence of cycloocta-1,5-diene (ref. 39), and the preparation of *bis*-(π-allyl)nickel by the reaction[40]

$$2C_3H_5MgBr + NiCl_2 \xrightarrow[-10^\circ C]{\text{ether}} Ni(\pi\text{-}C_3H_5)_2 + 2MgBrCl \quad [Ni(II)d^8]$$

(ii) The mode of bonding of an organic group to a metal can change from the σ to the π form or vice versa, for example

$$(\sigma\text{-}CH_2 : CHCH_2)Mn(CO)_5 \underset{\text{pressure}}{\overset{\text{u.v.}}{\rightleftarrows}} (\pi\text{-}C_3H_5)Mn(CO)_4 + CO$$

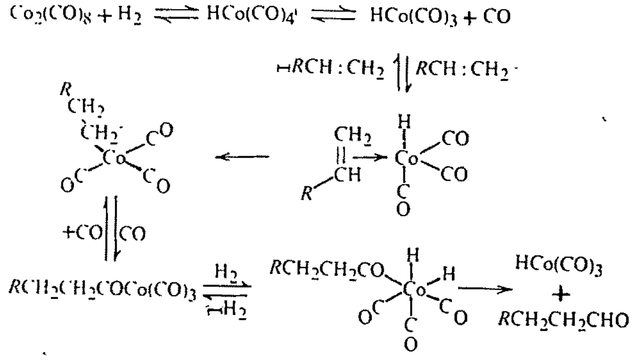
$$(\sigma\text{-}CH_2 : CHCH_2)Fe(CO)_2(\pi\text{-}C_5H_5) \xrightarrow{H^+} [(\pi\text{-}CH_3CH : CH_2)\ Fe(CO)_2(\pi\text{-}C_5H_5)]^+ \underset{Ph_3C^+BF_4^-}{\overset{NaBH_4}{\rightleftarrows}} (\sigma\text{-}(CH_3)_2CH)Fe(CO)_2(\pi\text{-}C_5H_5)$$

Such transformations are the key to certain catalytic reactions of technological importance to be described later. Moreover, these rearrangements fall into the category of reactions of coordinated ligands, an area of coordination chemistry which has been developed significantly in recent times[41].

(iii) It has been amply demonstrated that reactions can be carried out on organic groups coordinated to transition metals, and indeed the metal can either donate or withdraw electron density from an organic moiety so that its substituent effect is profound. Metals are also multisite substituents with the sites disposed in accord with the metal's coordination characteristics. There has been a growing appreciation of the chemical consequences of the control of organic reactions by metals in the past decade, so that the mechanisms of a number of processes catalysed by transition metals are better understood; and some principles have been developed which have made the search for new catalysts less empirical.

Even before the discovery of ferrocene and related π-complexes, it was known that some organic syntheses were catalysed by transition metal complexes. Roelen[42] studied the mechanism of the Fischer–Tropsch synthesis and discovered the oxo or hydroformylation reaction which generates aldehydes and alcohols from olefins, carbon monoxide, and hydrogen in the presence of cobalt catalysts. Reppe *et al.*[43] discovered that acetylene in the presence of nickel compounds can be cyclized to cyclooctatetraene and almost concurrently with the isolation of ferrocene, Ziegler *et al.*[4,44] found that the addition of transition metal halides to triethylaluminium produced a catalyst capable of polymerizing ethylene or propylene under mild conditions; this polymerization occurs in a sterically regular way[45]. It has come to be recognized recently that all these processes have in common one of the most basic of all ligand reactions, namely the combination of two ligands within the coordination sphere of the metal in a so-called "insertion" step. This is well illustrated by the presently favoured mechanism for hydroformylation[46] shown in the scheme below. The catalyst is believed to be the hydridocarbonyl $HCo(CO)_3$ which can bond an olefin molecule to form a π-complex because the cobalt atom is only four-coordinate ("coordinatively unsaturated"). Addition of the Co–H bond to the olefinic double bond affords an alkylcobalt tricarbonyl in an insertion step which involves rearrangement of a π into a σ-complex. The σ-complex is again coordinatively unsaturated, and reacts with CO to give an acylcobalt tricarbonyl in another insertion reaction. From mechanistic studies this is more properly regarded as a migration step in which an attacking CO ligand bonds to a vacant site on the cobalt atom thereby promoting transfer of the RCH_2CH_2 group onto one of the terminal CO ligands. The acylcobalt tricarbonyl complex involves $Co^I(d^8)$ and as such is able to cleave hydrogen molecules to form, by so-called oxidative addition, a cobalt (III) (d^6) complex in which the components necessary to produce the aldehyde and regenerate the catalyst are brought together in close proximity in the metal's coordination sphere.

The generality of the insertion process is evident from the fact that it may be used to explain the function of the Ziegler–Natta catalysts. Stereospecific coordination of an olefin molecule to a vacant site on a titanium atom adjacent to a σ-bonded growing polymer chain can continue indefinitely for as long as the coordination step is followed by an insertion.



Catalysis

Studies on hydroformylation, hydrogenation and isomerization of olefins, and the oligomerization of dienes and acetylenes by transition metal complexes have, in the past, been predominently empirical in nature in that one of a wide range of complexes tested has been found capable of promoting a certain type of reaction. As yet we are far from being able to design catalysts for a given purpose because knowledge of the kinetic and thermodynamic factors which lead to formation of a complex and its subsequent breakdown in a desired way is too imprecise. Recent work has, however, amply demonstrated the importance of vacant coordination sites, σ–π transformations of organic groups, the widespread occurrence of insertion reactions—in particular those involving metal–hydrogen and metal–alkyl bonds—and the fact that there are a large group of syntheses which depend on the ability of transition metals to pass readily from one oxidation state to another. A good example of this ability is provided by the compound $(Ph_3P)_3RhCl$, an effective hydrogenation catalyst which operates because rhodium(I) bonds olefins and can readily be "oxidized" by molecular hydrogen to a rhodium(III) hydrido-species which transfers hydrogen to an olefin molecule so that the catalyst is regenerated[47,48].

Many of the principles now associated with the reactions of organic groups bonded to transition metals are also applicable to biological processes where "metal-containing" enzymes catalyse isomerization and transmethylation reactions. As yet there is little evidence that metal–carbon bonds play any extensive role in biological chemistry though vitamin B_{12} forms C–Co bonds in its conversion to coenzymes (ref. 49 and references therein). In biological processes involving metals, the important ligand atoms are nitrogen and oxygen, but as both areas are part of the broader field of coordination chemistry as such, the study of one is likely to have some relevance to the other. And in the context of reactions of coordinated ligands, irrespective of whether metal–carbon, metal–nitrogen, or metal–oxygen bonds are involved, recent studies have shown that metal ions can assist either nucleophilic or electrophilic attack on a ligand depending whether the metal is in an electron-withdrawing or an electron-donating environment. These characteristics depend on the metal involved, its oxidation state, and also on the nature of all the ligands, and almost infinite variations in reactivity seem possible.

Olefins are not normally subject to nucleophilic attack. By contrast, coordination of, for example, ethylene to Pd^{+2} makes possible a reaction with water to yield acetaldehyde. This is the basis of the technologically important Wacker process[50,51]. The overall reaction ($C_2H_4+\frac{1}{2}O_2\rightarrow CH_3CHO$) results from a combination of several steps. Thus in one variation of this basic reaction, ethylene and oxygen are passed through an aqueous solution of palladium and cupric salt.

$$C_2H_4+PdCl_2+H_2O\rightarrow CH_3CHO+Pd^\circ+2HCl \quad \text{(product forming reaction)}$$

$$Pd^\circ+2CuCl_2\rightarrow PdCl_2+2CuCl \quad \text{(Pd oxidation)}$$

$$\underline{2CuCl+2HCl+\tfrac{1}{2}O_2\rightarrow 2CuCl_2+H_2O} \quad \text{(CuCl oxidation)}$$

$$C_2H_4+\tfrac{1}{2}O_2\rightarrow CH_3CHO$$

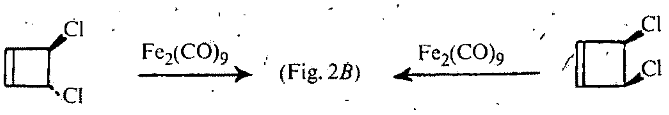
In the first reaction it is assumed that $[(\pi\text{-}C_2H_4)PdCl_3]^-$, closely related to the anion of Zeise's salt, is an intermediate. Ligand exchange of H_2O for Cl^- then gives $[(\pi\text{-}C_2H_4)Pd(H_2O)Cl_2]$ which releases a proton (H_3O^+) and affords $[(\pi\text{-}C_2H_4)PdCl_2(OH)]^-$. In the last of these species ethylene donates π-electrons to an electron accepting palladium (oxidation state +2) thereby promoting nucleophilic attack of OH^- (already within the metal's coordination sphere) on one of the carbon atoms to give a σ-bonded organo-palladium intermediate $[\sigma\text{-}CH_2\text{-}OHCH_2PdCl_2]^-$. The nominally vacant coordination site (it may be solvent occupied) on palladium would subsequently promote transfer of a β hydrogen atom, by way of transient Pd-H, to give an unstable carbonium ion $CH_3\overset{+}{C}(H)(OH)$ which affords acetaldehyde and a proton (hence HCl). Palladium metal is also formed and is reoxidized by cupric chloride. Addition of acetic acid as a reactant leads to the formation of vinyl acetate by attack of OAc^- on coordinated ethylene. Vinyl acetate is important as a monomer for lattices in paints, fibres and resins. Unfortunately there are important corrosion problems in its production by the liquid-phase palladium chloride process.

In other reactions π-complexes are susceptible to electrophilic rather than nucleophilic attack. In these cases the metal is in a low oxidation state so that electron density is available for transfer to the π^* orbitals of the ligand which thus becomes susceptible to Friedel–Crafts type substitutions, protonation or loss of hydride. The latter process, first reported in 1958 (ref. 52) has been used extensively in organometallic syntheses. For example, cycloheptatrienemolybdenum tricarbonyl reacts with trityl borofluoride $Ph_3C^+BF_4^-$ to give Ph_3CH and the cationic complex $[(\pi\text{-}C_7H_7)Mo(CO)_3]^+$. Interestingly, in the cation the effect of the metal, now in a more positive oxidation state, is to make the C_7H_7 group susceptible to attack by nucleophiles (for example, $R^-=CN^-$, OMe^-, Ph^- and so on) thereby producing cycloheptatriene derivatives $(\pi\text{-}RC_7H_7)Mo(CO)_3$ (ref. 53).

Many olefin–metal complexes can be protonated[38] so that a hydrogen atom becomes attached to the hydrocarbon group. As well as rendering an olefin susceptible to protonation it is probable that the metal plays a rather direct role, through metal–hydrogen bond formation, in transferring the proton to the ring[54,55]. Much research needs to be done to establish mechanistic details of metal-assisted electrophilic reactions in general, but it is clear that reactions of this kind have considerable potential in organic syntheses. Thus cyclooctatetraene does not undergo electrophilic reactions to give substituted derivatives. This is not true for cyclooctatetraene-iron tricarbonyl which undergoes both addition and substitution reactions with electrophiles (for example, $POCl_3$ in dimethylformamide, or CH_3COCl and $AlCl_3$) to give cyclooctatetraene derivatives coordinated to an $Fe(CO)_3$ group[56]. Treatment of the products with ceric salts liberates the substituted cyclooctatetraene. Pettit and his coworkers[57] first used this method for removing coordinated olefins from metals, and subsequently applied the technique in elegant studies on cyclobutadiene and its derivatives[58,59].

Cyclobutadiene Chemistry

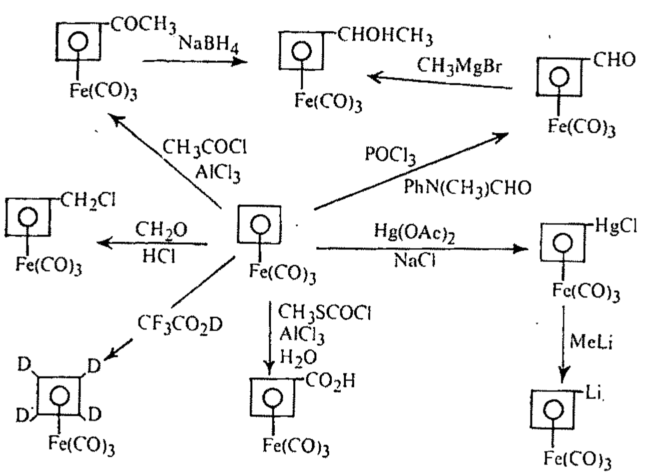
Mention was made earlier of the advances in organometallic chemistry which have led to the isolation of normally unstable organic moieties by coordination to a transition metal. The practical and theoretical problems associated with the cyclobutadiene molecule have concerned organic chemists for a hundred years. In perhaps the only example in organometallic chemistry where theory has been used to predict an experimental result, instead of accounting for it after it has been observed, Longuet-Higgins and Orgel[60] suggested that in spite of the instability of cyclobutadiene itself it would act as a good ligand. This suggestion was confirmed by the isolation of a stable tricarbonyliron complex of tetraphenylbutadiene by a serendipitous method[61] (many of the best discoveries in the field have been made by chance), and by the characterization of a dimeric form (bridging chloride ligands) of tetramethylcyclobutadiene-nickel (ref. 62). The parent ligand was prepared by the synthesis[58,59]



Cyclobutadieneiron tricarbonyl is an aromatic system in that it readily undergoes electrophilic substitution reactions. Moreover, the substituents introduced in this way undergo functional group chemistry without destabilization of the iron–cyclobutadiene bond (see structure 4).

The oxidative decomposition of cyclobutadieneiron tricarbonyl with Ce^{4+} has revealed several novel compounds through Diels–Alder type reactions, as indicated in Fig. 3.

The product from phenylacetylene is a molecule in which one ring has the Dewar benzene structure whereas the other has the Kekulé structure. Although this is somewhat of a laboratory curiosity, cyclobutadiene–iron tricarbonyl chemistry



Structure 4.

does have considerable potential in organic syntheses. Thus in recent years there has been much interest in cubane chemistry, and this synthesis of a dicarboxylic acid derivative is relatively simple. The ring system has previously been accessible only by elaborate reaction sequences.

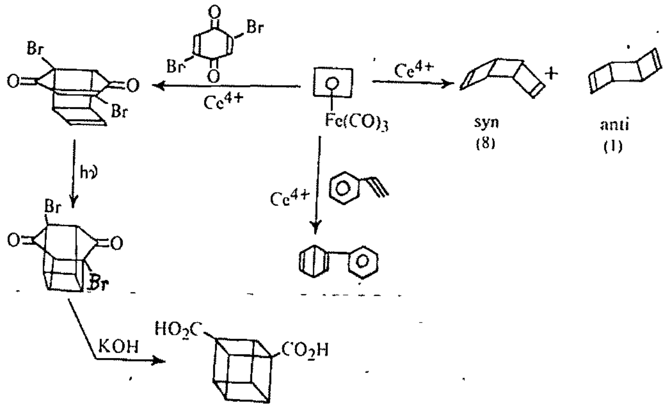


Fig. 3 Compounds revealed by the oxidative decomposition of cyclobutadieneiron tricarbonyl.

Predicting the Future

Although only a few organometallic compounds are widely used for specific purposes (for example, silicone polymers, tetraalkyl leads), their value in synthesis either as intermediates or as catalysts is great, and likely to increase in the future; and as organometallic chemistry impinges on both inorganic and organic chemistry research in this area helps to unify chemistry. But many problems remain to be solved. Previously insufficient attention has been given to a mechanistic approach in the study of organometallic reactions. Our knowledge of the bonding in organometallic compounds, particularly those involving transition elements, remains at best imprecise. Although some significant advances have been made in the design of homogeneous catalysts often little is known about the exact composition of the catalysts under the working conditions. Studies of a synthetic and structural nature of polynuclear metal complexes containing hydrogen and organic groups as ligands may eventually allow a better understanding of heterogeneous reactions, particularly the reactions of hydrocarbons on metal and metal oxide surfaces. It is difficult to predict the future of this field but it certainly has more momentum than many areas of chemistry.

[1] Miller, S. A., Tebboth, J. A., and Tremaine, J. F., *J. Chem. Soc.*, 632 (1952).

[2] Kealy, T. J., and Pauson, P. L., *Nature*, **168**, 1039 (1951).

[3] Rundle, R. E., *J. Phys. Chem.*, **61**, 45 (1957).

[4] Ziegler, K., *Adv. Organometal. Chem.*, **6**, 1 (1968).

[5] Brown, H. C., *Hydroboration* (Benjamin, New York, 1962).

[6] Zeise, W. C., *Pogg. Ann.*, **9**, 632 (1827).

[7] Zeise, W. C., *Pogg. Ann.*, **21**, 497 (1831).

[8] Bunsen, R., *Annalen*, **42**, 14 (1842).

[9] Frankland, E., *Annalen*, **71**, 171, 213 (1849).

[10] Mond, L., Langer, C., and Quincke, F., *J. Chem. Soc.*, 749 (1890).

[11] Grignard, V., *CR Acad. Sci.*, **130**, 1322 (1900).

[12] Anet, F. A. L., and Leblanc, E., *J. Amer. Chem. Soc.*, **79**, 2649 (1957).

[13] Ui, J., and Kitamura, S., *Marine Pollution Bull.*, **2**, 56 (1971).

[14] Curley, A., Sedlak, V. A., Girling, E. F., Hawk, R. E., Barthel, W., Pierce, P. E., and Likosky, W. H., *Science*, **172**, 65 (1971).

[15] Chatt, J., *Proc. Chem. Soc.*, 318 (1962).

[16] Chatt, J., *Record Chem. Prog.*, **21**, 147 (1960).

[17] Wilkinson, G., Rosenblum, M., Whiting, M. C., and Woodward, R. B., *J. Amer. Chem. Soc.*, **74**, 2125 (1952).

[18] Fischer, E. O., and Pfab, W., *Z. Naturforsch.*, **7b**, 377 (1952).

[19] Moiseev, I. I., Federovskaya, E. A., and Syrkin, Y. K., *Russ. J. Inorg. Chem.*, **4**, 1218 (1959).

[20] Smidt, J., and Hafner, W., *Angew. Chem.*, **71**, 284 (1959.)

[21] Hüttel, R., Kratzer, J., and Beckter, M., *Angew. Chem.*, **75**, 456 (1959).

[22] Emerson, G. F., Watts, L., and Pettit, R., *J. Amer. Chem. Soc.*, **87**, 132 (1965).

[23] Fischer, E. O., and Hafner, W., *Z. Naturforsch.*, **10b**, 665 (1955).

[24] Chatt, J., and Duncanson, L. A., *J. Chem. Soc.*, 2939 (1953).

[25] Dewar, M. J. S., *Bull. Soc. Chim. France*, **18**, C79 (1951).

[26] Mills, O. S., *Acta Cryst.*, **11**, 620 (1958).

[27] McArdle, P. A., and Manning, A. R., *J. Chem. Soc.*, A, 1498 (1969).

[28] Cotton, F. A., *Accnts. Chem. Res.*, **1**, 257 (1968).

[29] Ben-Shoshan, R., and Pettit, R., *J. Amer. Chem. Soc.*, **89**, 2231 (1967).

[30] Dickens, B., and Lipscomb, W. N., *J. Chem. Phys.*, **37**, 2084 (1962).

[31] Bruce, M. I., Cooke, M., Green, M., and Stone, F. G. A., *Chem. Commun.*, 523 (1967).

[32] Bratton, W. K., Cotton, F. A., Davison, A., Musco, A., and Faller, J. W., *Proc. Nat. Acad. Sci.*, **58**, 1324 (1967).

[33] Cooke, M., Goodfellow, R. J., Green, M., Maher, J. P., and Yandle, J. R., *Chem. Comm.*, 565 (1970).

[34] Brown, H. C., and Subba Rao, B. C., *J. Amer. Chem. Soc.*, **78**, 5694 (1956).

[35] Brown, H. C., and Subba Rao, B. C., *J. Org. Chem.*, **22**, 1136 (1957).

[36] Wilke, G., *Angew. Chem.*, **72**, 581 (1960).

[37] Wilke, G., *et al.*, *Angew. Chem. Internat. Ed.*, **2**, 105 (1963).

[38] Green, M. L. H., *Organometallic Compounds*, **2**, *The Transition Elements* (Methuen, 1968).

[39] Bogdanovic, B., Kroner, M., and Wilke, G., *Annalen*, **699**, 1 (1966).

[40] Wilke, G., and Bogdanovic, B., *Angew. Chem.*, **73**, 756 (1961).

[41] Busch, D. H., *Science*, **171**, 241 (1971).

[42] Roelen, O., *Angew. Chem.*, **60**, 62 (1948).

[43] Reppe, W., Schlichting, O., Klager, K., and Toepel, T., *Annalen*, **560**, 1 (1948).

[44] Ziegler, K., *Angew. Chem.*, **76**, 545 (1964).

[45] Natta, G., *Angew. Chem.*, **76**, 553 (1964).

[46] Heck, R. F., *Accnts. Chem. Res.*, **2**, 10 (1969).

[47] Montelatici, S., van der Ent, A., Osborn, J. A., and Wilkinson, G., *J. Chem. Soc.*, A, 1054 (1968).

[48] Osborn, J. A., Jardine, F. H., Young, J. F., and Wilkinson, G., *J. Chem. Soc.*, A, 1711 (1966).

[49] Parshall, G. W., and Mrowca, J. J., *Adv. Organometal. Chem.*, **7**, 203 (1968).

[50] Smidt, J., Hafner, W., Jira, R., Sieber, R., Sedlmeier, J., and Sabel, A., *Angew. Chem. Intern. Edn.*, 80 (1962).

[51] Aguilo, A., *Adv. Organometal. Chem.*, **5**, 321 (1967).

[52] Dauben, H. J., and Honnen, L. R., *J. Amer. Chem. Soc.*, **80**, 5570 (1958).

[53] Munro, J. D., and Pauson, P. L., *J. Chem. Soc.*, 3475, 3479, 3484 (1961).

[54] Winstein, S., Kaesz, H. D., Kreiter, C. G., and Friedrich, E. C., *J. Amer. Chem. Soc.*, **87**, 3267 (1965).

[55] Young, D. A. T., Holmes, J. R., and Kaesz, H. D., *J. Amer. Chem. Soc.*, **91**, 6968 (1969).

[56] Johnson, B. F. G., Lewis, J., and Randall, G. L. P., *J. Chem. Soc.*, A, 422 (1971).

[57] Pettit, R., and Emerson, G. F., *Adv. Organometal. Chem.*, **1**, 1 (1964).

[58] Pettit, R., *Pure Appl. Chem.*, **17**, 253 (1968).

[59] Ward, J. S., and Pettit, R., *J. Amer. Chem. Soc.*, **93**, 263 (1971).

[60] Longuet-Higgins, H. C., and Orgel, L. E., *J. Chem. Soc.*, 1969 (1956).

[61] Hübel, W., and Braye, E. H., *J. Inorg. Nucl. Chem.*, **10**, 250 (1959).

[62] Criegee, R., and Schröder, G., *Annalen*, **623**, 1 (1959).

Lightning Observations by Satellite

J. G. SPARROW & E. P. NEY

School of Physics and Astronomy, University of Minnesota, Minneapolis, Minnesota 55455

The data from OSO-5 confirm the OSO-2 result that storm complexes at night are chiefly found over land. The North Atlantic Ocean seems to have more lightning storms associated with it than other regions of ocean whereas the principal desert regions display a marked scarcity.

OBSERVATIONS of the positions of night-time lightning storms during the new Moon periods from February to October 1965 have already been reported[1] from the satellite OSO-2. A similar instrument was also flown on OSO-5, launched in January 1969, and this article reports the new data.

The Minnesota experiment[4] on OSO-5 consisted of six photometers, four of which can be used to detect lightning strokes. This analysis is based on data from two of these, each having a 10° angular diameter field of view looking along the spin axis of the satellite in the direction opposite to that of the solar sail. Each photometer is sensitive to a broad spectral region, astronomical blue (approximately 3500 to 5000 Å) and red (approximately 6000 to 8000 Å) respectively. The photometers are sampled three and two times per 0.32 s when the satellite is in darkness. The time constants of the two telescopes are each approximately 0.1 s. The photometers are protected from excessive light levels by protective photodiodes which disconnect the high voltage supply at times when the light level is above a minimum value. This effectively limits the observational periods to times near new Moon.

Storm Complexes

The data were searched by the computer (CDC 6600) to separate abrupt increases that might be interpreted as lightning. Manual examination further refined the selection, taking into account the expected time constant of the signal decay, and the positions of lightning storm complexes were plotted. These were taken to be areas (several square degrees) containing one or more active lightning cells which gave rise to two or more lightning flashes visible at the satellite. This criterion (to reject a single flash seen by two telescopes) became necessary to remove some abrupt increases in the apparent light level that cannot be interpreted as lightning. The distribution of the lightning storm complexes (Fig. 1) is similar to that found previously, confirming the result from OSO-2 that the distribution of night-time storms is heavily biased towards land areas rather than similar areas of sea. As the satellite coverage is relatively uniform in bands of constant latitude, Fig. 1 may be interpreted directly as an indication of non-uniformity. About 1,000 storm complexes have been plotted in Fig. 1 from the data on some 7,000 lightning strokes. As the results from the new Moon period February to September 1969 (20 to 24 h local time) were similar to those from January to July 1970 (00 to 04 hLT), the data have been combined in one plot.

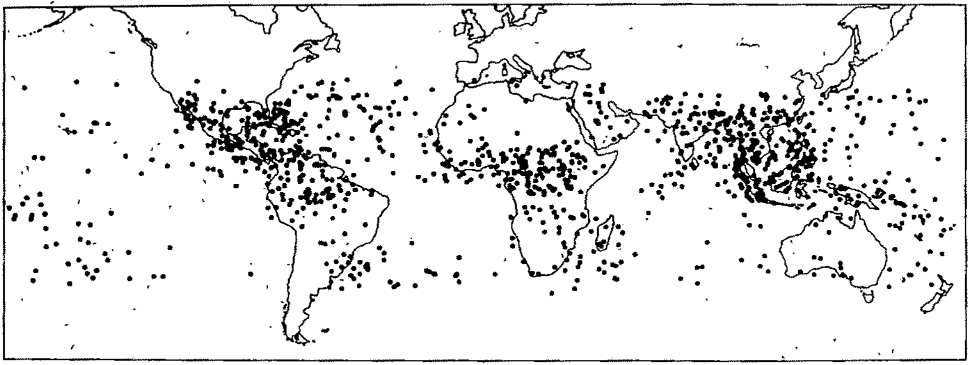


Fig. 1 Distribution of night-time lightning storm complexes observed by photometers on board satellite OSO-5.

It is evident that these data are in agreement with Schonland's statement[2] that "thunderstorms are not very frequent over the oceans; exceptions are the areas off the south-east coasts of Brazil and South Africa, and the seas surrounding the islands of the East Indies and Madagascar". It is also apparent that the North Atlantic Ocean has more lightning storms than similar areas of other oceans. There is a marked scarcity of lightning storms in the principal desert regions (Sahara and Kalihari Deserts, Great Australian Desert and the area in central South America) even compared with those over the North Atlantic Ocean.

The field of view of the photometers on the Earth ranges from approximately one square degree when the photometers view vertically to a maximum value of approximately 12 square degrees just before the field of view leaves the Earth. From the total time scan (~200 h) and the area-time scan (~400 degree^2 h) the mean area of the field of view is found to be approximately 2 square degrees. The number of lightning strokes in each storm complex varied from a minimum value of two to about thirty strokes seen in 2 min; most of the last group were observed simultaneously by both telescopes.

Spurious Events

On some occasions apparent "lightning strokes" were detected that could not be assigned as such because at the time the fields of view of the telescopes were well above the Earth. Some of these were detected by as many as three photometers simultaneously when the photometers were looking above the satellite. The decay time indicates that the signal probably originated forward of or in the photomultiplier preamplifier systems. Because the electronics associated with each photomultiplier are isolated from each other (although sharing the primary spacecraft power) it is unlikely that these apparent increases are electronic in nature. Furthermore, if the increases were initiated by feedback through the spacecraft batteries then all six photometers would probably show similar effects. This has occurred at other times when noise is present in the data but in these cases the characteristic time constant is not seen in each photometer signal. On the occasions under discussion, only those photometers viewing in the anti-sail direction and not those viewing in the opposite direction have been affected. The possibility of spurious reflexions from the front light baffles of each photometer (for example, from lightning, meteors and so on) can probably be eliminated because of the large attenuation in the scattered signal compared with the direct signal when the photometers view close to the Moon. The distribution of these signals over the Earth's surface is approximately random, suggesting that the source is not lightning. Single occurrences are seen about once every ten orbits. No acceptable mechanism has yet been found to explain these anomalous data spikes.

These increases, which seem to be due to short duration (<0.1 s) "light" flashes, arise from processes other than lightning and must be eliminated from the data before any discussion of lightning distribution can be entered into. This has effectively been done by accepting as storms only those occasions when two or more strokes were observed. In this way some seventy occasions when one stroke only was seen simultaneously by the two telescopes—when their fields of view were on the Earth—have been eliminated.

No Change in Frequency

The earlier experiment on OSO-2 was conducted during solar minimum conditions (1965) whereas these later measurements were made at a more active solar period. If a substantial change in the worldwide frequency of night-time lightning storms occurred between these two periods it should be detectable from a comparison of the frequencies observed by the two satellites. That such a relation might exist between solar activity and increased storminess has been suggested by Ney[3]. Taking into account the increased sensitivity in the later experiment it does not seem that any significant change was detected (the accuracy of the comparison is not better than perhaps a factor of two). A search for solar activity effects can also be made by looking for a correlation between lightning storm occurrence and K_p or another magnetic index within the data from one of these experiments. No significant correlation was found for lag/lead times of as much as 24 h between the time of storm occurrence and the three hourly value of K_p in the data of this experiment or in the less statistical data of OSO-2. These findings are not unexpected, for Markson[4] has shown, from data supplied by Lethbridge, that there is no apparent correlation between annual thunderstorm activity in the US and the solar cycle. Brooks[5] and Likhter *et al.* (quoted by Markson[4]) have also shown that there is low correlation between these parameters for low latitude stations.

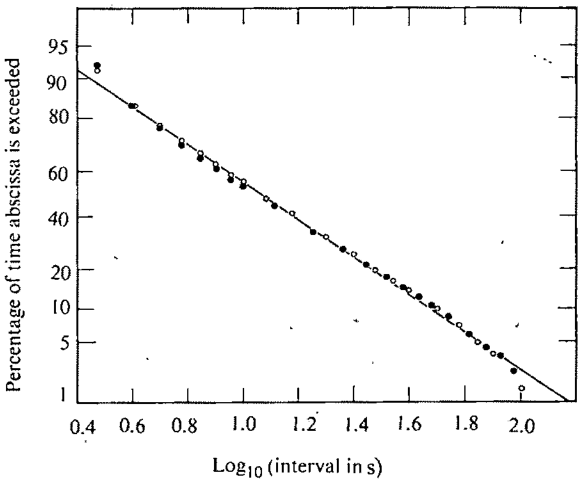


Fig. 2 Time intervals between successive lightning flashes plotted separately for the periods February to September 1969 and January to July 1970.

To reduce the computer time required for the original selection of likely lightning strokes, the computer was instructed to skip forward ten data points after each stroke was found (for one of the photometers). This restriction (to intervals longer than 2 s) avoided the detection of the rapid variations of intensity within a "single stroke". Individual lightning strokes have typical mean durations of 200 to 500 ms (ref. 6). It has been found that the logarithm of the intervals between lightning flashes (as well as the logarithm of the duration of the flashes) within a given storm, or within any of a number of simultaneously occurring storms, are normally distributed[6,7]. The data reported here correspond to those from one thousand storms distributed over 60° of latitude (at all longitudes) throughout most months of the year (1969 to 1970). It is apparent from Fig. 2—in which the time intervals between individual lightning flashes for the periods February to September 1969 and January to July 1970 are plotted—that these data also follow a lognormal distribution. The intervals for both periods belong to the same lognormal distribution with a median flash interval of 11.5 s and a standard deviation of 0.48 log$_{10}$ units.

This work was supported by NASA and the US Office of Naval Research. A detailed description of the experiment will be published later.

Received July 12, 1971.

[1] Vorpahl, J. A., Sparrow, J. G., and Ney, E. P., *Science*, **169**, 860 (1970).
[2] Schonland, B., *The Flight of Thunderbolts* (Clarendon Press, Oxford, 1964).
[3] Ney, E. P., *Nature*, **183**, 451 (1959).
[4] Markson, R., *Pure Appl. Geophys.*, **84**, 161 (1971).
[5] Brooks, C. E. P., *Quart. J. Roy. Meteor. Soc.*, **60**, 153 (1934).
[6] Lakshminarayan, K. N., *J. Sci. Indust. Res.*, **21D**, 228 (1962).
[7] Dennis, A. S., *J. Atmos. Sci.*, **27**, 170 (1970).

Formation of New Connexions in Adult Rat Brains after Partial Deafferentation

P. D. WALL & M. D. EGGER

MRC Cerebral Functions Research Group, Department of Anatomy, University College, Gower Street, London WC1E 6BT

Mapping of the receptive fields of cells in the thalamus and cortex after section of the dorsal columns in the rat reveals evidence of functional reorganization in the central nervous system.

BRAIN damage in adult mammals may be followed by remarkable recovery or by a depressingly permanent defect. Any understanding of this sometime plasticity would be important not only for therapy but also for understanding how the central nervous system adjusts to its environment during development and learning. During experiments designed for a different purpose, we came across evidence that a group of cells which had been deprived of their major input began to respond to quite novel stimuli after a few days. In these experiments, the cell bodies of the normal major input had been destroyed. The one class of explanation which cannot be invoked is true regeneration in which cells had divided and replaced the destroyed cells. In the mammalian adult central nervous system (CNS) there is no evidence for nerve cells which are still capable of division, growth and differentiation: following destruction of nerve cells, non-neural elements divide and migrate into the region of damage, phagocytosing the wreckage and replacing it with an impenetrable scar. Even if the cell bodies are intact but their axons are severed, there is no evidence of successful regeneration of axons within the CNS.

Our experiments involved the dorsal columns, through which cutaneous sensory afferents travel from the hind limbs to terminate on cells of nucleus gracilis. From this nucleus, axons decussate and project to the ventral posterior nucleus of the thalamus (VPL). Similar projection systems pass from the forelimb by way of the cuneate nucleus and from the face through the trigeminal nucleus. Within this region of the thalamus, the entire contralateral body surface is represented in an exact somatotopic map (Fig. 1). Recent experiments have suggested that discriminative cutaneous sensation survives the destruction of the dorsal columns[1]. One explanation for this lack of effect is that other systems converge onto the thalamic nucleus and transmit the necessary afferent information by alternative pathways. To test this hypothesis, experiments were in progress to examine the response of cells in VPL before and after part of the dorsal column–medial lemniscus system had been destroyed. Responses had been recorded in VPL immediately before and after section of thoracic dorsal columns in eleven rats, and after destruction of one nucleus gracilis in eight rats. We suspected that the full potentiality of the cells might not be revealed immediately after deafferentation and therefore decided to leave the animals for various times after the lesion before examining the thalamus. We encountered the unexpected result that cells in VPL which normally respond to leg stimulation began to respond to arm stimuli some time after destruction of nucleus gracilis. We shall report elsewhere the effect of acute and chronic deafferentation on the response of VPL cells to leg and body stimulation. Here we concentrate on those cells whose receptive fields switched from leg to arm.

Recordings were made in seventy-seven adult rats, ASH–CSE strain, weighing 200–400 g, under urethane (25%) anaesthesia, 0.65 ml./100 g. The head was held in a stereotactic machine with the lambda vertically above the ear bars in which position the centre of VPL is A 4.5, L 3.0 and 5.0 mm below cortical surface. The nucleus is crescent shaped in cross section and extends 1.4 mm rostrocaudally and mediolaterally. Thalamic recordings were made with glass-covered platinum microelectrodes whose impedance was 600–800 kohms at 1,000 Hz. When marking of electrode sites was required, recording was first carried out with silver plated steel microelectrodes followed by electrolytic deposition of iron which was later located by the prussian blue reaction. Cortical unit recordings were made with glass microelectrodes filled with 3 M KCl with impedance of 2–4 M ohms. When latencies were required, electrical stimuli were applied to the cutaneous receptive field of the unit through intradermal 30 gauge needles. Chronic lesions of nucleus gracilis or dorsal columns were made in sterile conditions with the animal anaesthetized with 'Equithesin'. With the head fully flexed and the cisterna magna open, nucleus gracilis can be observed directly without further dissection. The nucleus was destroyed by repeated maceration with honed jeweller's forceps. At various times after this lesion, thalamus or cortex was mapped and then the extent of the lesion was determined from serial frozen sections stained with darrow red–luxol fast blue. Only those animals with the lesion restricted to gracile nucleus are reported. In some, the extreme caudal end of the nucleus was spared.

Distribution of Receptive Fields

The distribution of receptive fields of cells in VPL was mapped in sixteen intact animals. In a further thirty-five animals maps were made of the intact VPL on one side so that these maps could be compared with those from the opposite VPL which had been partially deafferented by nucleus gracilis destruction. Successive recording points were distributed in a regular grid separated by 100 μm vertically and 200 or 250 μm rostrocaudally and mediolaterally. For each station on this three dimensional grid, the entire body surface was brushed with medium pressure and the position was noted of any area of skin which evoked unit responses with an amplitude greater than 50 μV.

In the normal VPL individual cells had small discrete receptive fields. Neighbouring cells recorded at a single electrode site had overlapping receptive fields restricted to some small area of the contralateral body surface. In 35% of the recording sites, cells responded to stimulation of trunk or proximal portions of the limbs or tail and these cells lie in the dorsolateral part of the nucleus. Twenty-four per cent responded to lower hind leg or feet or toes and lie ventrolaterally. Forty-one per cent responded to lower forearm or hands or fingers and are located ventromedially. The ventral surface of the nucleus is made up of a lamina about 200–400 μm thick which contains cells that respond to passive movement of the limbs. Ventral to the arm area these cells respond to arm movements and the cutaneous leg zone has leg movement cells ventral to it.

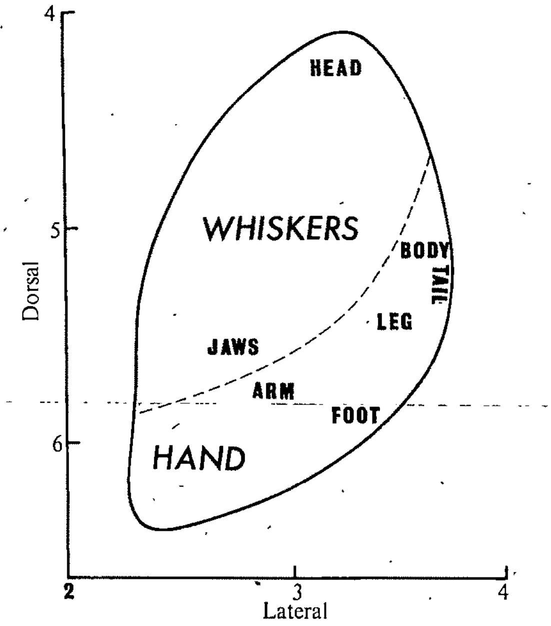


Fig. 1 Diagram of the representation in rat thalamus of the contralateral body surface. The region in which arm, trunk, leg and tail is represented in nucleus ventralis-posterior-lateralis. Maps are produced by placing microelectrodes successively at many locations in a single transverse plane in the region. For each recording point, the face and body surface is mechanically stimulated. Cells close to the recording electrode respond to a small area of skin. A combination of the receptive fields for all the points provides a map of the entire skin surface. The transverse plane is 4.5 mm rostral to external auditory meatus and the areas show distances in mm lateral to the midline and vertically below the cortical surface.

Cross-sectional maps were made first of one side and then continued across the midline to investigate the opposite nucleus. Fifty-seven pairs of maps were made in twenty animals at various stages after destruction of n. gracilis. Five variables were compared in the normal nucleus and its partner which had lost afferents: (1) the stereotactic coordinates of areas responding to particular parts of the body; (2) the ratio of the number of points on each side which responded to certain stimulus sites; (3) the monopoly by hind limb cells of the rostral pole of the normal nucleus; (4) the map in the ventral lamina of movement detection cells, which was unaffected by gracilis lesions and therefore provided another marker; (5) the receptive fields and latencies of selected single cells.

In rats mapped 1 or 2 days after destruction of the n. gracilis the picture is the same as immediately after the lesion (Fig. 2). The lower arm–hand–finger area is unchanged. In the leg–foot–toe region, there is an almost complete disappearance of sites where stimuli evoked activity. We shall describe the nature of these scattered responses to leg stimuli and those in the body region in another paper. In eight of nine animals mapped 3–17 weeks after the lesion, responses to stimuli on trunk, tail and hind leg remained essentially the same as in the acute and short survival animals but there was an expansion of the arm area into regions normally responding to leg stimuli. Sites responding to body and proximal limb stimuli averaged 25% fewer than normal, those for leg averaged 87.5% fewer but the number of arm points increased by 24%. Examination of the maps in detail shows that this expansion is produced by a lateral movement of the lateral edge while the medial part remains unchanged (Fig. 3). This lateral zone subserves the lower arm and wrist and these sites increased by an average of 132%. The change was most marked at the rostral pole where the zone normally monopolized by leg cells was now occupied by arm cells. Arm cells now lay dorsal to cells which responded to leg movement. The 16 and 17 week survival animals showed no greater expansion than the 3 week survivors. In no animal

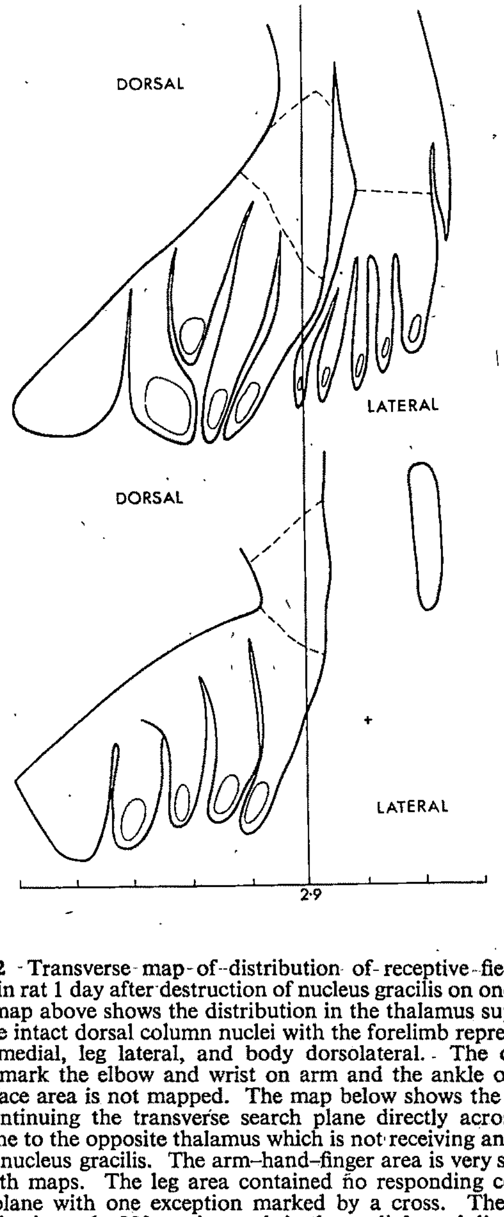


Fig. 2 Transverse map of distribution of receptive fields in VPL in rat 1 day after destruction of nucleus gracilis on one side. The map above shows the distribution in the thalamus supplied by the intact dorsal column nuclei with the forelimb representation medial, leg lateral, and body dorsolateral. The dotted lines mark the elbow and wrist on arm and the ankle on leg. The face area is not mapped. The map below shows the result of continuing the transverse search plane directly across the midline to the opposite thalamus which is not receiving an input from nucleus gracilis. The arm–hand–finger area is very similar in both maps. The leg area contained no responding cells in this plane with one exception marked by a cross. The horizontal axis marks 200 μm intervals in the mediolateral direction. The vertical line marks electrode tracks 2.9 mm from the midline penetrating both left and right thalamic maps. These tracks passed through the lateral arm region in each thalamus and show how similar the two arm regions are.

was the entire area which normally responded to hind limb invaded by the forelimb area. In a ninth animal with 3 week survival there were signs of expansion of the body area.

Time Course and Nature of the Change

The simplest explanation for the expansion would be the shrinking of deafferented cells with a simple moving over of the neighbours. In this case there would be no new connexions and the cortical somatotopic map would not be distorted. Successful maps were completed of the arm and leg area of main sensory-motor cortex on both sides in animals 2, 5 and 6 weeks after destruction of one n. gracilis. The animals were anaesthetized with urethane and the body surface stimulated as in the thalamic recording experiments. In order to obtain a sufficiently detailed map it was necessary to record the receptive fields of cortical units by penetrating 500 μm into cortex with glass, 2–4 Mohms, KCl filled microelectrodes. Maps were constructed from a grid of recording points separated by 500 μm. In all three animals, the arm area had expanded into the leg area and it must therefore be concluded that thalamic cells projecting to cortex had changed their peripheral receptive fields.

The simplest explanation for the appearance of new receptive fields would be that the deafferentation had unmasked existing afferents which are normally inhibited. This is unlikely because in this type of preparation even massive electrical stimuli to arm in the intact animal fail to excite cells with discrete receptive fields on leg. Furthermore, there is a time delay in the switching of receptive fields. During this period when the former leg cells fail to respond to peripheral stimuli, ongoing activity continues in them at at least the frequency of normally connected cells showing that the deafferented cells themselves do not go through a period of lowered excitability.

To postulate the formation of new connexions, it is necessary first to discover the time course of their development. Eleven animals were examined 1–7 days after the lesion. At 1 and 2 days there was no significant change. At 3 days, two of three animals showed some expansion but without invasion of the rostral pole. At 4 days one animal showed both expansion and rostral invasion while a second showed no change. Of two animals at 5 days, one showed the full picture of expansion and the other some expansion. A 6-day survivor was inconclusive but the 7-day animal showed the same full picture as those examined after 3 weeks. We conclude that the changeover begins at 3 days and is fully established between 1 and 3 weeks. The cell bodies of the changed cells lay 200–400 μm from the original boundary of the normal area but one must remember that the dendrites of the innervated and deafferented cells might be intermingled so that any shift of connexion might be over very small distances.

It is conceivable that the stimulus for the change of connectivity is not the degeneration of the terminals but the absence of the normal afferent impulses which bombard the thalamic cells. This possibility was ruled out by sectioning the dorsal columns just caudal to n. gracilis leaving the nucleus itself intact. The section of the fibres was made to spare the more lateral component of dorsal columns which supplies the cuneate nucleus. Thalamic maps from this animal taken 7 days after the lesion showed no signs of expansion of the arm area into the silent region which no longer responded to leg stimuli. It is true that the thalamus would still be bombarded by the ongoing activity of the deafferented nucleus gracilis but this is presumably slight by comparison with that transmitted in the normal freely moving animal.

A possible explanation for the newly effective connexion is that a background of ineffective fibres from the cuneate nucleus lie scattered through the area normally innervated by n. gracilis and that these fibres mature and successfully stimulate the cells vacated by degenerating axons. The change could be either presynaptic or postsynaptic. If such a diffuse system of fibres existed, one might expect the receptive fields of the newly connected cells to differ from those in the established part of the nucleus. In fact we were unable to differentiate the receptive fields of cells in the presumably newly invaded region from those medial to them either in terms of field size or threshold. It is a particularly suitable region for examining receptive fields because many cells respond to movement of one or more of the long whisker-like hairs protruding from the rat's volar wrist. Cells with precisely this type of receptive field seemed to be duplicated in the new territory. Another possible mechanism is that the cells respond to an indirect input perhaps by way of cortex rather than by way of axons originating in cuneate nucleus. This seems unlikely because the latency of response of the switched cells was identical to that in the unchanged zone.

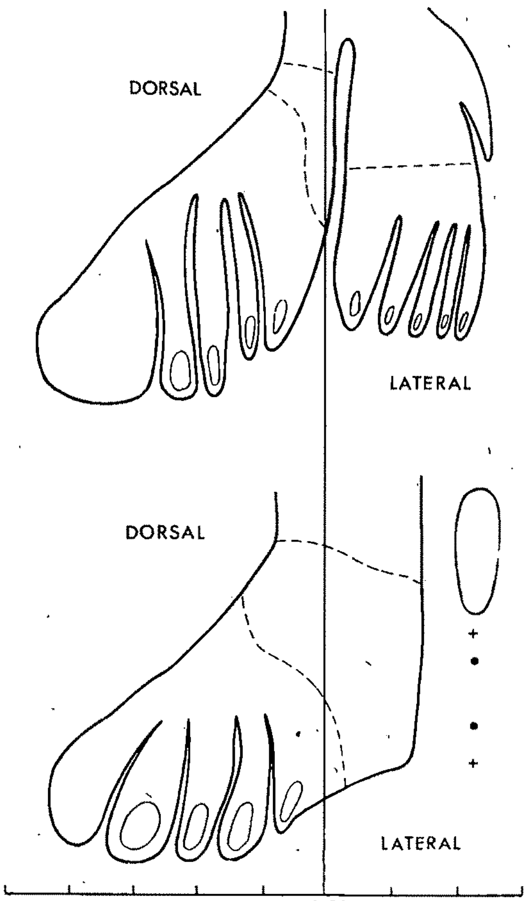


Fig. 3 Transverse map of distribution of receptive fields in intact VPL (above) and the map produced by continuing the mapping plane across the midline into the opposite VPL studied 7 weeks after destruction of the n. gracilis which projected to this nucleus (below). The vertical line marks an electrode track 2.8 mm from the midline which samples a similar region of the thalamus on the intact side to the vertical line shown in Fig. 2. The thalamus on the medial side of the line contains a similar map on both the intact and deafferented side. But the region representing the arm, especially the lower arm, has expanded on the operated side to invade a region which responds to leg on the intact side. At the lateral edge of the nucleus four cells were encountered which responded to body or leg stimulation but most cells in this region failed to respond to any peripheral stimuli.

Collateral Sprouting

Probably the most likely explanation is that sprouts have grown from the terminal arborization of intact axons from cuneate nucleus and have established successful contact with deafferented neighbours. Collateral sprouting and new contact formation have been shown to occur in the periphery both in denervated muscle[2] and in sympathetic ganglia[3]. In the central nervous system, light microscope evidence of sprouting into denervated regions has been provided for dorsal root fibres[4] and for optic nerve fibres[5]. Raisman[6] has shown clear electron microscope evidence for sprouting and end knob formation in the partially denervated septal nuclei of adult rat. But in the case of collateral sprouting, one must still explain the small receptive fields of the newly connected cells. The distance over which the sprouts would have to extend is not known, because although the cell bodies of the switched cells can be as much as 400 μm from the intact area it may be that the dendritic fields of the denervated cells overlap with those of the intact cells. If a sprout had only to grow a few-μm to occupy a new cell, a pioneer sprout might lay down a pathway along which its neighbours could grow. Another way in which a restricted receptive field might be formed would be if the fibres which fire together grow together. This would require a mechanism by which the presence of more or less coincident impulse traffic in terminals would exaggerate the growth of terminals which sensed impulses in themselves and their neighbours.

Clearly an explanation of the phenomenon reported here will have to await an electron microscope examination of morphological changes in the region of changed response. In assessing the significance of the phenomenon of new connexions, one must give certain warnings. These observations were made in the rat, an animal which continues to grow throughout adult life, perhaps therefore retaining certain embryological characteristics. The distance over which new connexions could be established is evidently limited since there were no signs of occupation of vacant cells in the lateral edge of the nucleus. The new connexions do not represent recovery from the original lesions because information from arms is now channelled into a system presumably specialized to handle leg information. However, one might speculate that if scattered single cells were destroyed, as in an encephalitis, sprouting from associated collaborating neighbours might provide some functional advantage.

Received April 8; revised July 30, 1971.

[1] Wall, P. D., *Brain*, **93**, 505 (1970).
[2] Edds, jun., Mac V., *Quart. Rev. Biol.*, **28**, 260 (1953).
[3] Guth, L., and Bernstein, J., *J. Exp. Neurol.*, **4**, 59 (1961).
[4] Liu, C. N., and Chambers, W. W., *Arch. Neurol.*, **79**, 46 (1958).
[5] Goodman, D. C., and Horel, J. A., *J. Comp. Neurol.*, **127**, 71 (1966).
[6] Raisman, G., *Brain. Res.*, **14**, 25 (1969).

Temperature Changes associated with Adiabatic Decompression in Geological Processes

D. R. WALDBAUM

Department of Geological and Geophysical Sciences, Princeton University

Joule-Thomson coefficients of crystals indicate that temperatures may increase during irreversible adiabatic release of pressure. Isenthalpic decompression provides a mechanism for "self-heating" in igneous and metamorphic processes.

When a substance is forced through a constriction in adiabatic conditions, the decompression is thermodynamically irreversible, occurring at constant enthalpy, *H*. For a normal fluid, the result may be either an increase or decrease in its temperature according to the initial and final pressures of the process[1-3]. Joule and Thomson[1] showed that the effect may be measured directly or calculated from the relation

$$\left(\frac{\partial T}{\partial P}\right)_H = \frac{V(T\alpha_P-1)}{C_P}$$

where T is thermodynamic temperature, P is pressure, α_P is isobaric thermal expansion $(1/V)\,(\partial V/\partial T)_P$, V is volume at P and T, and C_P is isobaric heat capacity.

This paper is concerned with the isenthalpic decompression coefficients (Joule–Thomson coefficients) of minerals, and their application to geological processes.

Decompression Coefficients of Crystals

For a crystalline phase, isenthalpic decompression produces an increase in its temperature inasmuch as $T\alpha_P \ll 1$. Table 1 lists values of $(\partial T/\partial P)_H$ at 1,000° C and 1 atm for minerals of basaltic and ultramafic rocks calculated from physical property measurements[4]. The sign and magnitude of the isenthalpic coefficients indicate that the temperature of a rock consisting of these minerals will change by approximately +220° C if the rock is decompressed across a pressure difference of 10 kbar by a strictly isenthalpic process, whether it be by extrusion or by some other mechanism.

For example, peridotite (or pyrolite) extruded from 20 kbar on an oceanic temperature–depth curve[5] to 10 kbar would be heated sufficiently to intersect an experimentally determined "dry" solidus for basalt or peridotite[6,7] (curve *A*, Fig. 1). Extrusion experiments by Bridgman[8] on antimony metal, discussed in the present context by Adams[9], suggest that a magma obtained by extrusion of crystalline rock might be ejected with nearly explosive force to still lower pressures, possibly to the surface.

It is relatively unimportant whether decompressed material melts or undergoes any phase changes. If decompression ends without a phase change, the heat exchanged between the decompressed material and its new surroundings may still cause anomalous heat flow at the surface[10] and phase changes (metamorphism) in the surrounding rocks.

The process represented by the isenthalpic decompression curve *A* in Fig. 1 does not differ in principle from the assumption that magmas can form as a result of the rapid release of pressure from material, so that its temperature subsequently intersects a melting curve from the high pressure side[11,12]. Such decompression requires at least a partially adiabatic process, otherwise the temperatures of the material would imitate those of the normal geothermal gradient. It is therefore usually implied that the pressure is released rapidly enough for the material moving upward to be always at a higher temperature than distant surrounding rocks at the same depth. But it is also assumed that the limiting maximum temperature in such a rapid release of pressure is that of an isentropic process[7,12] (for example, curve *B*, Fig. 1). Fig. 1 illustrates quantitatively that all forms of adiabatic decompression of minerals do not necessarily lead to cooling.

Table 1 Adiabatic Decompression Coefficients of Some Minerals

Mineral	Formula	$(\partial T/\partial P)_H$ (°K/kbar)
Spinel	Mg_2AlO_4	−21.0
Forsterite	Mg_2SiO_4	−23.7
Fayalite	Fe_2SiO_4	−23.0
Clinoenstatite	$Mg_2Si_2O_6$	−21.0
Diopside	$CaMgSi_2O_6$	−25.4
Jadeite	$NaAlSi_2O_6$	−23.2
Albite	$NaAlSi_3O_8$	−30.2
Anorthite	$CaAl_2Si_2O_8$	−29.6
Magnetite	$FeFe_2O_4$	−21.9
Chromite	$MgCr_2O_4$	−23.0
Corundum	Al_2O_3	−19.9
Periclase	MgO	−21.6
Rutile	TiO_2	−24.8
Graphite	C	−23.4
Diamond	C	−13.4
α-Iron	Fe	−13.2
Nickel	Ni	−18.9
Platinum	Pt	−29.1

The isentropic coefficient $(\partial T/\partial P)_S$, or "adiabatic gradient", is $+1.0 \pm 0.5$ K/kbar for these substances. Pressure does not significantly affect the above isenthalpic coefficients in the range of Fig. 1. The effects of temperature are important, and will be discussed in a later paper; for silicates, the isenthalpic coefficients decrease by about 20% from 500° to 1,500° C. The effects of *P* and *T* are small enough that the above values serve as good estimates of the slopes of the true isenthalpic *P*, *T* curves from 800° to 1,200° C, in which case the line connecting the initial and final pressures of an irreversible decompression process is nearly coincident with the isenthalp itself.

It should not be inferred that a single stage isenthalpic process is required to produce heating. In any complex geological environment, when adiabatic decompression occurs, it is likely to consist of a series of isenthalpic and isentropic steps as shown by curve *C* in Fig. 1. A process involving only a small fraction of isenthalpic decompression steps could still result in an increase in temperature because

$$|(\partial T/\partial P)_H| \gg |(\partial T/\partial P)_S|$$

Recognizing that both isenthalpic and isentropic decompression represent limiting aspects of an adiabatic process, and that many other factors may affect the net result of pressure changes in geological processes, there are other geological situations where the isenthalpic contribution may be significant.

It is emphasized that the thermodynamic coefficients in Table 1, as with any thermodynamic property, may be applied to real or hypothetical processes whose physical mechanisms differ considerably in detail. It is not necessary to limit the application of isenthalpic decompression to conditions of high-velocity flow[1,2,9]. The only condition to be met is conservation of enthalpy in the material undergoing decompression.

Flow Processes

Extrusion and throttling are examples of a large class of phenomena where a stationary flow is maintained in an isolated part of a mass bounded by streamlines, or through a tube of varying cross-section (as, for example, in the mantle convection model of Turcotte and Oxburgh[13], although they assume an isentropic model). Enthalpy remains constant along such an adiabatically enclosed "tube" of flowing material[3,14]. The isenthalpic coefficients of minerals indicate that the temperature of an isolated mass will rise during decompression and flow, even in the idealized case where friction, viscosity, and turbulence are ignored.

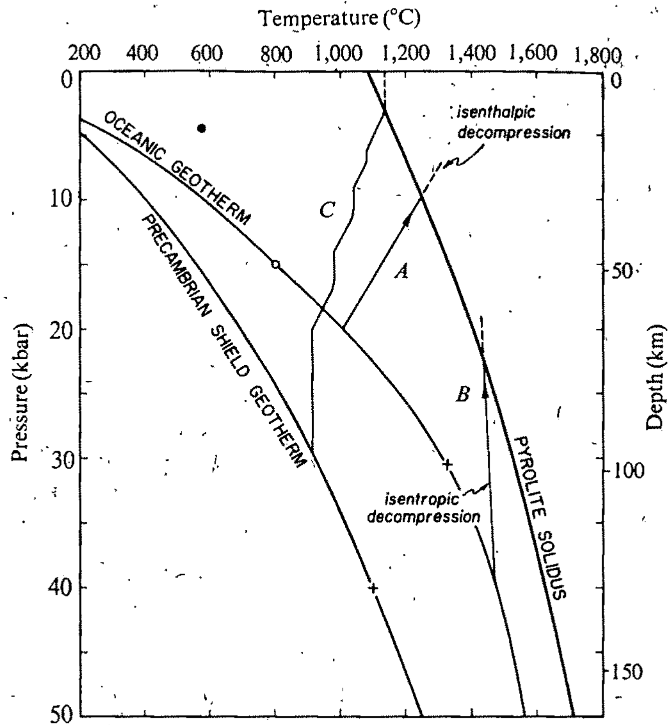


Fig. 1 Diagram comparing isenthalpic decompression (*A*), isentropic decompression (*B*), and a composite decompression curve (*C*) for a rock consisting of silicate minerals in Table 1. Temperature–depth curves are taken from ref. 8; the curve representing beginning of melting of pyrolite III composition is taken from ref. 10. The decompression curves are extended as a dashed line beyond their intersection with the melting curve, due to possible complications and uncertainties in the enthalpies of fusion, physical properties of the fluid, and the rate of migration of the fluid from the point of extrusion. Open circle denotes minimum depth from which decompression could cause a rock of pyrolite composition to begin melting in the absence of water. Small dot represents andalusite–kyanite–sillimanite triple point. Crosses indicate approximate depths from which forsterite would have to be decompressed at constant *H* to form a liquid at or near the surface.

The sign and magnitude of the isenthalpic coefficients thus provide a possible quantitative thermodynamic basis for the close association often observed between deformation, regional metamorphism, and partial melting of sedimentary rocks[15,16]. The values are such that the observed effects of regional metamorphism can be obtained without necessarily requiring the addition of energy from external sources such as magmatic intrusions, by fluid transfer[17], or by frictional heating[18]. The effects of adiabatic decompression, however, are only likely to be of importance in those areas where evidence for forced flow can be demonstrated[19]. Dewey and Pankhurst[16] summarized studies on the distribution of metamorphic temperatures in time and space in the final episode of the Caledonian orogeny. The form of the proposed geothermal curves and their variation in time are consistent with a possible origin by isenthalpic flow. Similar observations summarized by Niggli[20] and Wenk[21] for the last stage of orogenic activity in the Central Alps may also be interpreted in this way.

As with any extrusion process, the resultant increase in metamorphic grade from simple isenthalpic decompression will depend on the initial temperature and pressure, the pressure difference causing flow, and the energy required for phase changes within the rock mass. Models of regional metamorphism based on thermochemical fluid dynamics will probably yield more satisfactory results than those based on a simple heat conduction model for an igneous intrusion[22].

Considering the emplacement of alpine-type ultramafic rocks from this viewpoint, the observations of Raleigh[23], Dickey[24], and others may be extended to include the possibility that some peridotites and dunites originated as "peridotized" serpentinites[25] (which later reserpentinized, in part). Serpentine has an isenthalpic decompression coefficient of 26° C per kbar of differential (hydrostatic) pressure at 500° C, and calculations based on thermodynamic data[4] support the hypothesis of Carpenter and Phyfer[25]. An isenthalpic decompression of 9 kbar of partially serpentinized peridotite (initially one part serpentine to one part olivine by volume at 550° C and 10 kbar) yields an anhydrous peridotite at 1 kbar and 670° C. This result includes the conservation of enthalpy in dehydrating serpentine to less hydrous or anhydrous phases during decompression.

Isenthalpic decompression may be a mechanism in the lunar interior where heat sources of sufficient magnitude have not yet been conclusively identified for producing magma[26]. This mechanism may contribute significantly to the formation of subsurface magma associated with the impacts of large meteorites[27] that produce fractures through which solid material might extrude. This mechanism allows for the possibility of early or recent lunar volcanism in a way that does not conflict with the hypothesis that the interior of the Moon has been relatively cold since the early stages of its formation. A similar mechanism could account for the origin of subsurface-derived magmas associated with the Sudbury (Canada) complex[28] and other terrestrial structures[29] thought to have resulted from meteorite impacts.

Shock Decompression

Another possible isenthalpic decompression process is that which occurs during the passage of a shock wave in a solid, although it is sometimes assumed, by analogy with the behaviour of gases, that compression alone causes the heating. Shock compression and decompression are largely adiabatic and irreversible, so these processes may occur at constant enthalpy. For example, any of the silicates in Table 1 would cool from room temperature to near absolute zero with only 12 kbar of compression at constant H (if such a process occurs). Enthalpy is gained, however, in the transfer of kinetic energy to the solid, so that very low temperatures may never be reached, but any net temperature rise on compression will be small. Subsequent decompression of only 100 kbar would form the high temperature crystalline phases and vesicular glass observed in shock-wave experiments on anhydrous minerals[30]. Igneous rocks associated with impact structures[28,29] may thus originate from two possible isenthalpic processes: shock decompression acting on the surface rocks and extrusion leading to melting at depth.

Isenthalpic shock-decompression may also cause magma to form from crystalline rock *in situ* at depth in seismically-active zones. The shock pressures required for high residual temperatures may be much less than previously estimated[29,31] if attributed to isenthalpic rather than isentropic decompression.

To summarize, the sign and magnitude of the coefficients in Table 1 indicate that any process leading to a completely or partially isenthalpic decompression of minerals can, at least in principle, lead to an increase in their temperature. The prevailing opinion that the adiabatic parts of geological processes are entirely isentropic (reversible) requires some reconsideration. They are also likely to be irreversible in part, in which case data such as those in Table 1 apply. It should be possible to explore these hypotheses in decompression experiments[32] by attempting to duplicate the textures and mineralogy found in some of the geological environments described here. Some use could be made of high-pressure mineral synthesis or rock-deformation experiments that terminated unexpectedly by rapid decompression. Detailed petrographic study of these "unsuccessful" run products should yield information on textural and kinetic aspects of decompression that cannot be predicted by thermodynamics alone.

I thank R. B. Hargraves, R. E. Openshaw, L. M. Cathles, and L. S. Hollister for comments on the manuscript.

Received December 23, 1970; revised April 28, 1971.

[1] Joule, J. P., and Thomson, W., *Phil. Trans. Roy. Soc.*, **144**, 321 (1854).

[2] Adams, L. H., *J. Geol.*, **32**, 191 (1924); Barton, jun., P. B., and Toulmin, P., *US Geol. Survey Prof. Paper* 424D, D-350 (1961).

[3] Pippard, A. B., *The Elements of Classical Thermodynamics*, (Cambridge University Press, 1957).

[4] Kelley, K. K., *US Bureau Mines Bull.*, 584 (1960); Skinner, B. J *Geol. Soc. Amer. Mem.*, **97**, 75 (1966); Robie, R. A., and Waldbaum, D. R., *US Geol. Survey Bull. 1259* (1968).

[5] Ringwood, A. E., Boyd, F. R., and Macgregor, I. D., *Carnegie Inst. Wash. Yearbook*, **63**, 147 (1964).

[6] Yoder, H. S., and Tilley, C. E., *J. Petrol.*, **3**, 342 (1962).

[7] Green, D. H., and Ringwood, A. E., *Contr. Mineral. Petrol.*, **15**, 162 (1967).

[8] Bridgman, P. W., *Phys. Rev.*, **9**, 138 (1917).

[9] Adams, L. H., *J. Wash. Acad. Sci.*, **12**, 407 (1922).

[10] Horai, K., and Uyeda, S., in *The Earth's Crust and Upper Mantle* (edit. by Hart, P. J.), Geophys. Monog. 13, 104 (Amer. Geophys. Union, Washington, DC, 1969); Morgan, W. J., *Nature*, **230**, 42 (1971).

[11] Bowen, N. L., *The Evolution of the Igneous Rocks*, 315 (Princeton University Press, 1928).

[12] Turner, F. J., and Verhoogen, J., *Igneous and Metamorphic Petrology*, second ed., 443 (McGraw-Hill, New York, 1960).

[13] Turcotte, D. L., and Oxburgh, E. R., *J. Geophys. Res.*, **74**, 1458 (1969).

[14] Liepmann, H. W., and Roshko, A., *Elements of Gasdynamics*, 47 (Wiley, New York, 1957).

[15] Turner, F. J., *Evolution of the Metamorphic Rocks*, Geol. Soc. Amer. Mem. 30, 286 (1948); Ramsay, J. G., in *The British Caledonides* (edit .by Johnson, M. R. W., and Stewart, F. H.), 168 (Oliver and Boyd, London, 1963); Thompson, jun., J. B., Robinson, P., Clifford, T. N., and Trask, jun., N. J., in *Studies of Appalachian Geology: Northern and Maritime* (edit. by Zen, E., *et. al.*), 215 (Wiley, New York, 1968); Thompson, jun., J. B., and Norton, S., *ibid.*, 325; Spry, A., *Metamorphic Textures*, 253 (Pergamon, 1969).

[16] Dewey, J. F., and Pankhurst, R. J., *Roy. Soc. Edinburgh Trans.*, **68**, 361 (1970).

[17] Shimazu, Y., *J. Earth Sci. Nagoya Univ.*, **6**, 31 (1958); Shimazu, Y., *Jap. J. Geophys.*, **2**, 135 (1961).

[18] Reitan, P. H., *Lithos*, **1**, 161 (1968); Reitan, P. H., *Lithos*, **1**, 268 (1968).

[19] Haller, J., *Geol. Rundschau*, **45**, 159 (1956).

[20] Niggli, E., *Fortschr. Mineral.*, **47**, 16 (1970).

[21] Wenk, E., *Fortschr. Mineral.*, **47**, 34 (1970).

[22] Turner, F. J., *Metamorphic Petrology: Mineralogical and Field Aspects*, 377 (McGraw-Hill, New York, 1968).

[23] Raleigh, C. B., in P. J. Wyllie, *Ultramafic and Related Rocks*, **191** (Wiley, New York, 1967).

[24] Dickey, J., *Min. Soc. Amer. Spec. Paper No.* 3, 33 (1970).

[52] Carpenter, J. R., and Phyfer, D. W., *Geol. Soc. Amer. Abstracts with Programs*, **1**, Pt. 7, 261 (1969).

[26] Ringwood, A. E., *J. Geophys. Res.*, **75**, 6470 (1970); Wood, J. A., *J. Geophys. Res.*, **75**, 6507 (1970); Smith, J. V., Anderson, A. T., Newton, R. C., Olsen, E. J., and Wyllie, P. J., *J. Geol.*, **78**, 395 (1970); Turcotte, D. L., and Oxburgh, E. R., *J. Geophys. Res.*, **75**, 6549 (1970).

[27] Baldwin, R. B., *The Face of the Moon* (University of Chicago Press, 1949); Ronca, L. B., *Icarus*, **5**, 515 (1966); Mackin, J. H., *Bull. Geol. Soc. Amer.*, **80**, 742 (1969).

[28] Dietz, R. S., *J. Geol.*, **72**, 412 (1964); French, B. M., *Bull. Vulcanol.*, **34**, 466 (1971).

[29] French, B. M., and Short, N. M., *Shock Metamorphism of Natura Materials*, 43, 59, 219 (Mono Book Corp., Baltimore, 1968).

[30] Milton, D. J., and De Carli, P. S., *Science*, **140**, 670 (1963); Dobretsov, N. L., Deribas, A. A., and Maly, V. I., *Phys. Earth Planet. Interiors*, **3**, 352 (1970).

[31] Ahrens, T. J., Petersen, C. F., and Rosenberg, J. T., *J. Geophys. Res.*, **74**, 2727 (1969).

[32] Bridgman, P. W., Studies in Large Plastic Flow and Fracture, 174 (Harvard University Press, Cambridge, 1964); Pugh, H. Ll. D., and Low, A. H., *J. Inst. Metals*, **93**, 203 (1965).

LETTERS TO NATURE

PHYSICAL SCIENCES

Ehrenfest's Paradox

ATWATER's remarks[1,2] on the relativistic rotating disk problem (Ehrenfest's paradox[3]) have elicited considerable response[4–6] of a somewhat peripheral nature. Atwater concludes that recourse to experiment is highly desirable. Arzelies[7], while questioning the existence of relativistic constraints, also agrees with this view. The purpose of this communication is to point out what one might expect to observe as a disk rotates and to develop the magnitude in a practical case.

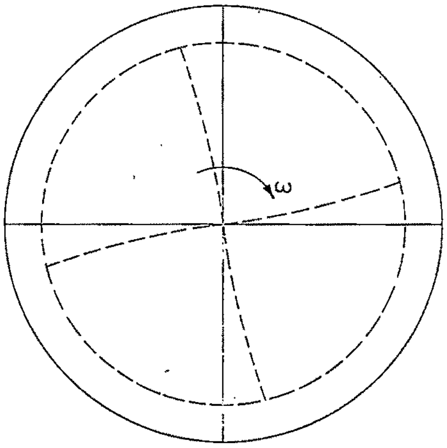


Fig. 1 A schematic representation of the rotating disk.

Suppose we have a disk of radius a rotating clockwise with angular velocity ω about an axis through its centre perpendicular to its plane. Take a radial element of the disk at distance r from the centre and consider a small section Δr along r. Then after one revolution, this section must have rotated counterclockwise with respect to its direction along r in consequence of the Thomas precession[8].

It is easy to see that this angle (in radians) is given by

$$\delta\theta = \pi \frac{\omega^2 r^2}{c^2} \qquad (1)$$

per revolution. Integrating along the whole element, we find it deflected counterclockwise by an angle

$$\theta = \frac{\pi}{3} \frac{\omega^2 a^2}{c^2} \qquad (2)$$

per revolution.

Thus the appearance of the disk to a fixed observer will be as shown by the dotted lines in Fig. 1. It will be noted that the disk is in torsion with consequent reduction of both radius and circumference. Thus the premises leading to the Ehrenfest paradox need not exist. This description should not be any more surprising than the well observed time dilation. A rotating disk is clock-like in character with the Thomas precession giving an accumulating measure of the number of revolutions. Further, the effect should be independent of the disk material, just as an equivalent clock is independent of its construction.

The relativistic constraints just introduced do, moreover, imply physical consequences for rotating bodies such as the Earth, pulsars, and so on, but this is considered elsewhere (D. H. W. and J. W. Kern, to be published). I wish to make clear that a practical laboratory experiment is entirely possible.

Take, for example, a disk 10 cm in diameter rotating 1,000 r.p.s. Then equation (2) yields $\theta \sim 10^{-12}$ per revolution. For a period of 30 days $\theta \sim 0.16°$. This should be well within currently available means of measurement when the position of a point on the circumference is compared with a point near the axis.

This measurement might be conveniently carried out, for example, by placing a fiducial mark on the edge of the disk with a similar mark in the stationary frame adjacent to the edge. These marks can then be compared during rotation when illuminated by a discharge lamp triggered by a sharp magnetic pulse, or other means, positioned at a radial distance b on the disk. This will modify equation (2) and it then becomes

$$\theta = \frac{\pi}{3}\frac{\omega^2}{c^2} a^2 \left(1 - \frac{b^2}{a^2}\right) \qquad (3)$$

per revolution.

With the passage of time, the mark on the disk will appear to move away from the fixed mark in accordance with equation (3) and counter to the direction of rotation.

D. H. WEINSTEIN

Superior Oil Company,
Houston, Texas, and
University of Houston,
Houston, Texas

Received June 14; revised July 23, 1971.

[1] Atwater, H. A., *Nature*, **228**, 272 (1970).
[2] Atwater, H. A., *Nature*, **230**, 197 (1971).
[3] Ehrenfest, P., *Phys. Z.*, **10**, 918 (1909).
[4] Suzuki, M., *Nature*, **230**, 13 (1971).
[5] Marsh, G. E., *Nature*, **230**, 197 (1971).
[6] Noonan, T. E., *Nature*, **230**, 197 (1971).
[7] Arzelies, H., *Relativistic Kinematics*, 236 (Pergamon, London, 1966).
[8] Thomas, L. H., *Nature*, **514**, 117 (1926).

Geomagnetic Dynamos in a Stable Core

Higgins and Kennedy[1] have suggested that the Earth's core is in stable equilibrium. If this is so there cannot be the large scale steady motions with a radial component which have usually been thought necessary for the dynamo theory of the Earth's magnetic field. A stable fluid can have internal wave motions and it seems worth considering whether a body of fluid can act as a dynamo and produce steady electric currents and magnetic fields even when the motion is purely oscillatory. The work of Moffat[2,3], Roberts[4] and Steenbeck and Krause[5] shows that this is so in an infinite fluid and it seems likely that it will be true in a sphere. The discussions of fluid dynamos are necessarily complex and we have therefore considered the very simple case of an oscillatory disk dynamo. This dynamo consists of a disk oscillating about its axis of symmetry with a coil connected to brushes on the axle and at the edge of the disk. Any current in the coil will produce a field, the motion of the disk in the field produces a current in the coil and maintains the field. The equation for the current, I, in such a system is easily shown to be[6]

$$L\dot{I} + RI = M\Omega I \qquad (1)$$

where L and R are the inductance and resistance of the circuit, $2\pi M$ is the mutual inductance of the coil and the disk and Ω is the angular velocity of the disk. The equation bears an analogy to Maxwell's equations for the field, $\mathbf{B}$, in a fluid of conductivity σ and permeability μ moving with velocity $\mathbf{v}$, which give

$$\dot{\mathbf{B}} - \nabla^2\mathbf{B}/\mu\sigma = \text{curl}\ (\mathbf{v}\times\mathbf{B}) \qquad (2)$$

In both cases the question is, can the interaction of the motion and the field, the term on the right, balance the second term on the left to give an average $\dot{I}$ or $\dot{\mathbf{B}}$ which is zero or positive ? If the motion is oscillatory the answer will depend critically on the relative phase of the motion and the current or field, that is of Ω relative to I in equation (1) or $\mathbf{v}$ relative to $\mathbf{B}$ in equation (2) (the mean of $\cos t \sin t$ is zero, but the mean of $\cos^2 t$ is $\frac{1}{2}$). The study of equation (2) needs elaborate mathematical techniques, since $\mathbf{B}$ is a function of space as well as time. For equation (1), however, there exists an elementary solution; it shows that the phase relations are wrong and that the current is not maintained. It is known, for steady motion, that two coupled disk dynamos with the current from each feeding the coil of the other possess properties not shown by the single dynamo[7,8]. We have studied such a system with oscillating disks and have shown that a current with a steady part may be maintained or caused to grow exponentially so long as some Fourier component of the oscillations of one disk is out of phase with the corresponding component of the motion of the other.

The simplest case is when each disk performs simple harmonic oscillations, the phase difference between the two being $\frac{1}{2}\pi$. The decay time of the current in the absence of motion is L/R, if this is large compared with the period of oscillation of the disks, then one coil has a steady current with a small ripple superposed and the other a much smaller alternating current. The calculations, which are very simple, suggest that dynamos with steady fields and oscillatory motions may exist in a wide variety of circumstances. Higgins and Kennedy, therefore, may not have seriously embarrassed the dynamo theory, even if their main contention is correct, as seems likely.

The detailed calculations will appear in a forthcoming book[9].

E. Bullard
D. Gubbins

Department of Geodesy and Geophysics,
Cambridge CB3 OE2

Received July 15, 1971.

[1] Higgins, G., and Kennedy, G. C., *J. Geophys. Res.*, **76**, 1870 (1971).
[2] Moffatt, H. K., *J. Fluid Mech.*, **41**, 435 (1970).
[3] Moffatt, H. K., *J. Fluid Mech.*, **44**, 705 (1970).
[4] Roberts, G. O., *Phil. Trans. R. Soc.*, **266**, 535 (1970).
[5] Steenbeck, M., and Krause, F., *Z. Naturf.*, **21a**, 1285 (1966).
[6] Bullard, E. C., *Proc. Camb. Phil. Soc.*, **51**, 744 (1955).
[7] Rikitaki, T., *Proc. Camb. Phil. Soc.*, **54**, 89 (1958).
[8] Allan, D. W., *Proc. Camb. Phil. Soc.*, **58**, 671 (1962).
[9] Bullard, E., and Gubbins, D. (in the press).

Variations in Magnetic Intensity and Climatic Changes

CORRELATION between the variations of the Earth's magnetic field, fluctuations in atmospheric radiocarbon activity, and climatic changes during the past 7,000 yr has been discussed extensively in the past[1-7]. In addition, Harrison[8] has suggested that reversals of the Earth's magnetic field could cause climatic changes. With an associate we have recently[9] been able tentatively to conclude on the basis of studies of deep-sea sediment cores that in the past 470,000 yr the magnetism of the Earth has modulated climate. We have now extended the correlation between variations of the magnetic intensity and evidence of climatic change from deep-sea sediment cores back to 1.2 m.y.

Many investigators have studied remanent magnetism in deep-sea sediment cores[10-15]. The study was conducted according to the method of Opdyke *et al.*[11]. The cores on which the remanent magnetism was measured were sampled at intervals of 2 to 10 cm and dried samples were cut into rough cubes measuring approximately 2 cm. Reversals were detected by finding the inclination of the remanent field of the series of samples with respect to the axis of the core. The samples were partially demagnetized in an alternating field of 150 oersted to remove unstable components.

Table 1 Details of the Cores that were Investigated

Core	Latitude (N)	Longitude (W)	Depth (m)	Length (cm)	Sedimentation rate (cm per 1,000 yr)
V12–122	17° 00′	74° 24′	2,800	1,090	2.32
V20–108	45° 27′	179° 14′	5,625	1,320	1.10
V26–41	19° 19′	26° 06′	4,340	1,400	1.16

The cores (Table 1) consist of a mixture of fine mineral particles and particles of calcium carbonate, except that core V20–108 contains no calcium carbonate.

Our climatic study is based on measurements of the abundance of the *Globorotalia menardii* group of planktonic foraminifera, including the four subspecies *G. menardii menardii*, *G. m. tumida*, *G. m. flexuosa* and *G. m. fimbriata*. Although Emiliani[16], on the basis of a study by Lidz[17], has maintained that the *G. menardii* group is unreliable as a climate indicator, we believe[18] that Lidz's conclusion is misleading and that as a consequence, Emiliani's conclusion is ill-formulated. Therefore, like others[19-21], we have taken the *G. menardii* group to be reliable for the study of climatic changes in most deep-sea sediment cores, with abundance indicating warm climate.

Fig. 1 shows correlation of the magnetic intensity curve of deep-sea sediment core V20–108 from the North Pacific, the *Globorotalia menardii* climate curve in core V26–41 from the North Atlantic, and the oxygen isotope climate curve of *Globigerinoides rubra* measured by van Donk[22] in core V12–122 from the Caribbean. The magnetic intensity measurements in core V20–108 are according to Ninkovich *et al.*[12] and Foster[23] with adjustment for the stretched out top section. Because this core contains no calcium carbonate we cannot make foraminiferal measurements of the climatic changes. The time scales for cores V20–108 and V26–41 are based on magnetic reversals at the Matuyama–Brunhes boundary and at the Jaramillo event. The age for the Matuyama–Brunhes boundary is given by Cox and Dalrymple[24] who consider that the most probable age for this boundary is 0.69 m.y. The age and duration of the Jaramillo event is given by Opdyke[25] who places the upper boundary at 0.86 m.y. and the lower boundary at 0.92 m.y. with a duration of approximately 60,000 yr. The palaeomagnetic stratigraphy of core V20–108 was determined by Ninkovich *et al.*[12] and by Foster[23] and of core V26–41 by Opdyke (personal communication). The time scale for core V12–122 was determined by Broecker and van Donk[26] based on thorium-230 dating by Ku and Broecker[27]. It is in general agreement with the protactinium dating of the same core by Sackett[28].

Because the intensity measurements in core V20–108 are per gram of sediment and not per gram of magnetizable material in the samples, some of the variability in the intensity measurements in the core may be due to differences in composition and abundance of magnetic minerals in the sediment samples. Although the magnitude of the remanent magnetic moment in the core may be related to a change in the sedimentary sequences brought about by dilution of detrital components by biogenic components, it is difficult to imagine a scheme in which the direction[9] of the recorded magnetic vector is changed systematically through depositional processes.

The changes in the sedimentary sequence in the North Pacific core, V20–108, which contains no calcium carbonate, could have been brought about by an influx of ice-rafted detritus which could have introduced a greater abundance of magnetic minerals in the cold periods. Therefore, we have plotted variations in magnetic intensity in this core against weight percentages of sediment coarser than 250 µm in particle diameter measured by Kent *et al.*[29]. According to these authors this size fraction has been transported by icebergs.

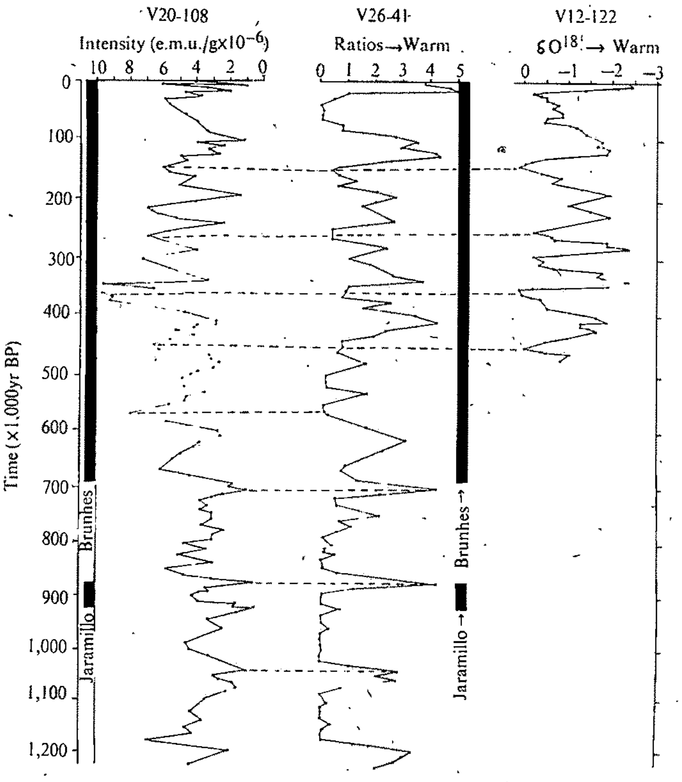


Fig. 1 Correlation of the magnetic intensity curve of deep-sea sediment core V20–108 from the North Pacific, the *Globorotalia menardii* climate curve in core V26–41 from the North Atlantic, and the oxygen isotope climate curve of *Globigerinoides rubra* measured by van Donk[22] in core V12–122 from the Caribbean. The magnetic intensity measurements and the magnetic stratigraphy of core V20–108 are according to Ninkovich *et al.*[12] and Foster[23] with adjustment for the stretched out top section. Because this core contains no calcium carbonate we cannot make foraminiferal measurements of the climatic changes. The magnetic stratigraphy of core V26–41 was determined by Opdyke (private communication). The time scales for cores V20–108 and V26–41 are based on magnetic reversals at the Matuyama–Brunhes boundary and at the Jaramillo event according to dating of the boundary by Cox and Dalrymple[24] and dating of the event by Opdyke. The time scale for core V12–122 was determined by Broecker and van Donk[26] based on thorium-230 dating by Ku and Broecker[27].

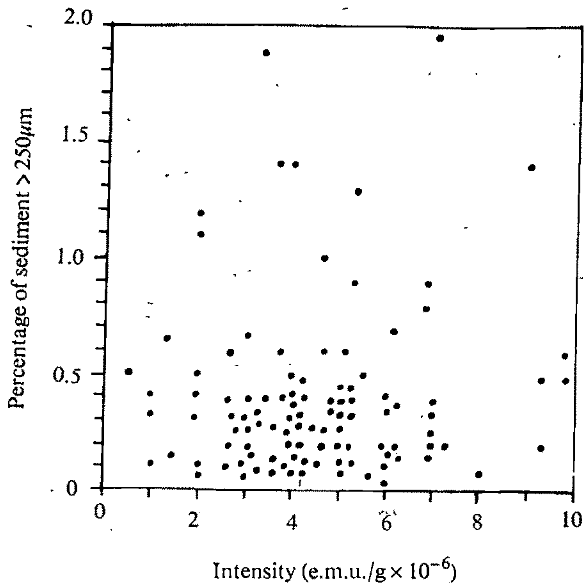


Fig. 2 Scatter plot showing that there is no correlation between magnetic intensity variations and changes in the sedimentary sequence in deep-sea sediment core V20–108 from the North Pacific. The changes in the sedimentary sequence could have been brought about by an influx of ice-rafted detritus which could have introduced a greater abundance of magnetic minerals in the cold periods. Therefore, we have plotted variations in magnetic intensity against weight percentages of sediment coarser than 250 µm in particle diameter measured by Kent *et al.*[29]. According to these authors this size fraction has been transported by icebergs.

As Fig. 2 shows, there is no correlation between the magnetic intensity variations and changes in the sedimentary sequence.

On the basis of the results shown in Fig. 2 we suggest that the intensity measurements in core V20–108 may represent meaningful approximations of changes in the intensity of the Earth's magnetic field although we have not attempted to estimate the percentages of magnetizable material in the core.

We have shown correlations between variations in magnetic intensity and climatic changes. On the basis of evidence reported here and additional evidence[9] we suggest that cause and effect relationship links changes of the Earth's magnetic field and climate. For instance, higher magnetic intensity seems to indicate colder climate. Thus we conclude that magnetism may modulate climate to some degree by the ability of the Earth's magnetic field somehow to provide a shield against solar corpuscular radiation.

We thank M. Ewing, J. Kukla, N. D. Opdyke, W. Pitman III, Bruce C. Heezen, J. van Donk, A. W. H. Be', D. Horn, J. H. Foster, and B. Horn for help, and Janet Wollin and Julie McGowan for assistance in laboratory investigations. The research was supported by the US National Science Foundation.

GOESTA WOLLIN
DAVID B. ERICSON
WILLIAM B. F. RYAN

Lamont-Doherty Geological Observatory,
Columbia University,
Palisades,
New York 10964

Received May 28; revised July 2, 1971.

[1] Elsasser, W., Ney, E. P., and Winckler, J. R., *Nature*, **178**, 1226 (1956).
[2] Libby, W. F., in *Proc. Symp. Radioactive Dating and Methods of Low-Level Counting*, Monaco, March, 1967, 2 (International Atomic Energy Agency, Vienna, 1967).
[3] Damon, P. E., in *Radiocarbon Variations and Absolute Chronology* (edit. by Olsson, I. U.) (Wiley Interscience, New York, 1968); *Met. Monog.*, **8**, 151 (1968).
[4] DeVries, H., *Konikl. Ned. Acad. Weteusch. Proc.*, **B61**, 94 (1958).

[5] Bucha, V., Taylor, R. E., Berger, R., and Haury, E. W., *Science*, **168**, 111 (1970).
[6] Suess, H. E., *J. Geophys. Res.*, **70**, 5937 (1965).
[7] Suess, H. E., *Bild Wiss.*, **6**, 121 (1969).
[8] Harrison, C. G. A., and Funnel, B. M., *Nature*, **204**, 566 (1964).
[9] Wollin, G., Ericson, D. B., Ryan, W. B. F., and Foster, J. H., *Science* (in the press).
[10] Harrison, C. G. A., and Funnel, B. M., *Nature*, **204**, 566 (1964).
[11] Opdyke, N. D., Glass, G., Hays, J. D., and Foster, J. H., *Science*, **154**, 349 (1966).
[12] Ninkovich, D., Opdyke, N. D., Heezen, B. C., and Foster, J. H., *Earth Planet. Sci. Lett.*, **1**, 146 (1966).
[13] Glass, B., Ericson, D. B., Heezen, B. C., Opdyke, N. D., and Glass, J. A., *Nature*, **216**, 437 (1967).
[14] Hays, J. D., Saito, T., Opdyke, N. D., and Burkle, L. H., *Bull. Geol. Soc. Amer.*, **80**, 1481 (1969).
[15] Kennett, J. P., and Watkins, N. D., *Nature*, **227**, 930 (1970).
[16] Emiliani, C., *Science*, **168**, 822 (1970).
[17] Lidz, L., *Science*, **154**, 1448 (1966).
[18] Wollin, G., Ericson, D. B., Ewing, M., in *Late Cenozoic Glacial Ages* (edit. by Turekian, K. K.), 199 (Yale University Press, 1971).
[19] Imbrie, J., and Kipp, N. G., in *Late Cenozoic Glacial Ages* (edit. by Turekian, K. K.), 71 (Yale University Press, 1971).
[20] Beard, J. G., in *The Marine Quaternary of the Caribbean and Gulf of Mexico Regions, SEPM Symposium*, 535 (1969).
[21] Schott, W., *Wiss. Ergebn. Deut. Atlant. Exped. Meteor.*, 1925–27, 3, 43 (1955).
[22] van Donk, J. thesis, Columbia Univ. (1970).
[23] Foster, J. H., thesis, Columbia Univ. (1970).
[24] Cox, A., and Dalrymple, G. B., *Earth Planet. Sci. Lett.*, **3**, 173 (1967).
[25] Opdyke, N. D., in *The Application of Modern Physics to the Earth and Planetary Interiors* (edit. by Runcorn, S. K.), 549 (Wiley Interscience, London, 1969).
[26] Broecker, W. S., and van Donk, J., *Rev. Geophys. Space Phys.*, **8**, 169 (1970).
[27] Ku, T. L., and Broecker, W. S., *Science*, **151**, 48 (1966).
[28] Sackett, W. M., in *Symp. Marine Geochem.*, Graduate School of Oceanog. Univ. Rhode Island Occasional Pub. No. 3, 29 (1965).
[29] Kent, D., Opdyke, N. D., and Ewing, M. (in the press).

Spontaneous Fission of Heavy Transuranium Elements in the Surface Layers of the Peculiar A Star HR 465 explains Promethium Abundance

THE possibility that transuranium elements are present in the surface layers of peculiar A stars was raised last year[1,2] after Jaschek and Malaroda[3] announced evidence for the presence of uranium lines in the Ap star 73 Dra. Further information about the abundances of transbismuth elements in peculiar A stars is now available[4–6] and there is strong evidence[7,8] for the presence of promethium lines in the peculiar A star HR 465. All these facts imply, in the framework of the Guthrie–Van den Heuvel theory of the origin of Ap stars[9,10], that a search for transuranium elements in the spectra of those stars, at least in those in which uranium or promethium has been detected, should give positive results. It may even be possible to find the spectral lines of superheavy elements from the island of stability[11] near $Z=114$.

The implications resulting from the presence of uranium are self-explanatory[1,2] whereas this is not so if promethium is present. We therefore present some qualitative arguments in favour of our hypothesis that the promethium which is observed in HR 465 is continually produced in the spontaneous fission of very heavy transuranium elements, possibly superheavy elements which have been sought for in numerous experiments.

The longest lived promethium isotopes, ^{145}Pm ($T_{1/2}=17.7$ yr) and ^{146}Pm ($T_{1/2}=5.55$ yr), are neutron-deficient and cannot be produced in any neutron capture chains. The other Pm isotopes which could have been produced in the r process have even shorter half-lives. It is thus unrealistic to attribute the promethium abundance in the Ap star HR 465 to fast neutron capture which occurred either in the primary star of the binary system or in the surface of the recent peculiar star. The amount of promethium that should have been produced could not survive until now. We could think about some kind of continual production of promethium in surface reactions either with helions or with protons that might be accelerated in areas of high magnetic field density. This would be some *ad hoc* extension of previous concepts[12,13] and it seems very improbable. From a detailed analysis of the various possible production modes of Pm (Kuchowicz, work in preparation) it seems that only spontaneous fission of very heavy nuclides provides a natural mechanism for this. This is because the two promethium isotopes ^{147}Pm and ^{149}Pm, with half-lives of 2.62 yr and 53.1 h, respectively, occur in isobaric decay chains of fission products. An interesting result from a recent analysis of the product yield curves for fission of various nuclei[14] is that the total yield for the chains involving promethium is approximately constant (about 2% for $A=147$, and 1% for $A=149$) for light transuranium elements. For heavier elements, up to the additional island of stability near $Z=114$, it is expected that the light mass peak in the mass distribution of fission fragments is shifted towards the heavy peak, and symmetric fission occurs. This enhances the yield of isobaric chains including promethium, and it may be even as high as 5%.

We thus see that promethium cannot be regarded as a rare fission product of the heavier fissioning nuclei. Provided large amounts of such nuclei were produced in the exploding primary component of the binary system[10] and then they were transferred to the surface of the recent Ap star, they are a continuous source of promethium. It is important to notice that all superheavy nuclides eventually decay to spontaneously fissioning nuclei, and thus a final result of their initial presence in any material is an enrichment of the fission products in that material. Thus Dakowski[15] proposed that the symmetric fission of superheavy nuclei may account for the anomalies in the isotopic compositions of Xe and Kr in meteorites. It cannot be excluded that the overabundances of certain rare earths in the Ap stars of the main group may be attributed to a similar reason.

It is important to know the initial abundance of superheavy nuclei in the surface matter of Ap stars. The synthesis of these elements in the r process is well understood. Schramm and Fowler[16] obtained recently an abundance of ~ 0.6 for the eight long-lived superheavy nuclei with half-lives exceeding 10^3 yr (on the scale with $\mathrm{Si}=10^6$). Thus if we know the promethium abundance in HR 465, it will be possible, in principle, to calculate from it the concentration of the superheavy nuclei in the atmosphere (which may exceed by many orders of magnitude the concentration of Pm).

An important conclusion of our reasoning is that promethium may also be present in other peculiar A stars, especially in those where uranium has already been found. Its abundance should be correlated to the occurrence of r-process elements. This should be confirmed by observation. It may also be possible that superheavy elements can be found in at least some of the peculiar A stars in which promethium will be present. With these elements regarded as a continuous source for promethium, we can hope that they still exist in observable amounts. It is only the lack of knowledge of their optical spectra that makes us unable, at present, to make a search for their lines in the spectra of peculiar A stars. It may be expected that in the future this can be overcome, but even now the observation of promethium seems to confirm the existence of superheavy elements suggested on the basis of other data by Marinov *et al.*[17] and by Bhandari *et al.*[18].

B. KUCHOWICZ

Department of Radiochemistry and Radiation Chemistry, University of Warsaw

Received June 24, 1971.

[1] Kuchowicz, B., *Nature*, **227**, 156 (1970).
[2] Kuchowicz, B., *Bull. Acad. Pol. Sci. Sér. Math. Astron. Phys.*, **18**, 673 (1970).
[3] Jaschek, M., and Malaroda, S., *Nature*, **225**, 246 (1970).
[4] Brandi, E., and Jaschek, M., *Publ. Astron. Soc. Pacific*, **82**, 847 (1970).
[5] Guthrie, B. N. G., *Observatory*, **89**, 224 (1969).
[6] Dworetsky, M. M., *Astrophys. J. Lett.*, **156**, L101 (1969).
[7] Aller, M. F., and Cowley, C. R., *Astrophys. J. Lett.*, **162**, L145 (1970).
[8] Aller, M. F., *Sky and Telescope*, **41**, 220 (1971).
[9] Van den Heuvel, E. P. J., *Bull. Astron. Inst. Netherlands*, **19**, 11 (1967).
[10] Guthrie, B. N. G., *Publ. Roy. Observatory Edinburgh*, **6**, 145 (1968).
[11] Kuchowicz, B., in *Proc. Intern. Conf. Heavy Ion Physics*, Dubna, February 1971 (Joint Inst. Nucl. Res., Dubna, in the press).
[12] Burbidge, G. R., and Burbidge, E. M., *Astrophys. J. Suppl.*, **1**, 431 (1955).
[13] Brancazio, P. J., and Cameron, A. G. W., *Canad. J. Phys.*, **45**, 3297 (1967).
[14] Wahl, A. C., in *Proceedings of the Symposium on Physics and Chemistry of Fission*, Salzburg, 1965, **1** (IAEA, Vienna).
[15] Dakowski, M., *Earth Planet. Sci. Lett.*, **6**, 152 (1969).
[16] Schramm, D. N., and Fowler, W. A., *Nature*, **231**, 103 (1971).
[17] Marinov, A., Batty, C. J., Kilvington, A. I., Newton, G. W. A., Robinson, V. J., and Hemingway, J. D., *Nature*, **229**, 464 (1971).
[18] Bhandari, N., Bhat, S. G., Lal, D., Rajagopalan, G., Tamhane, A. S., and Venkatavaradan, V. S., *Nature*, **230**, 219 (1971).

Tungsten-181 and Other Short Lived Fission Products in Ground Level Air in North Germany and North Norway

SAMPLES of atmospheric dust have been collected for several years at 1.5 m above ground in Brunswick (52° 18′ N, 10° 28′ E) as part of an environmental radioactivity surveillance programme. Before analysis, each filter, made from 'Microsorban' (Delbag, Berlin) is ashed at 450° C. The content of fresh fission products in the samples is then measured in a 10 cm by 10 cm NaI(Tl) well-type scintillation spectrometer. Later, the ashes of all filters exposed during half a month—containing dust from about 50,000 m³ of air—are combined and measured in a 45 cm³ well-type Ge(Li) detector with a multichannel analyser (ND 2200). The activity concentrations of various fission products and certain cosmogenic radionuclides are evaluated from the γ-spectra numerically. Since October 1970 a filter sample containing dust collected from about 300,000 m³ of air in Tromsö (Norway) is also analysed once a month. Within recent months fresh fission products were detected on three occasions.

First, 10 days after the eleventh nuclear test of the Chinese People's Republic (October 14, 1970) ^{131}I was found in a rain sample, dated October 24–25, 1970. The concentration was 2 pCi/l.; during the following days, dust samples both from Brunswick and Tromsö contained ^{140}Ba+^{140}La, ^{147}Nd and ^{237}U. In Brunswick the concentrations were 1.8, 1.2 and 3 fCi/m³ ± 20%, respectively. The activity concentrations of some other radionuclides are listed in Tables 1 and 2. The increasing ratio ^{103}Ru/^{106}Ru from 0.1 in October 1970 to 1.0 in late March 1971 (Brunswick) implies that most of these Chinese fission products reached the surface layer through the stratosphere during the spring peak.

Second, dust samples collected between December 24, 1970, and January 2, 1971, in Tromsö, and between December 30, 1970, and January 2, 1971, in Brunswick contained ^{131}I and, in very low concentrations, ^{132}Te and ^{140}Ba+^{140}La, probably produced by the United States underground nuclear test, Nevada, December 16, 1970.

Third, between April 2 and 6, 1971, ^{140}Ba+^{140}La was found in Brunswick; by Ge(Li) spectrometry of the combined ashes of the filters exposed between March 31, 1971, and April 16, 1971, in Brunswick, ^{181}W was also detected with a mean activity concentration of 0.27 fCi/m³ ± 20% (for quantitative evaluation the Ta K X-ray peaks were used); before and after this time interval, no ^{181}W was detectable. The filter exposed in Tromsö during the same month also contained ^{140}Ba+^{140}La as well as ^{181}W, the latter with a concentration of 0.38 fCi/m³ ± 20%.

Table 1 Activity Concentrations in Brunswick (fCi/m³)

Sampling period	^{95}Zr	^{95}Nb	^{103}Ru	^{106}Ru	^{137}Cs	^{141}Ce	^{144}Ce
30.10.70–12.11.70	—	—	2.3	7.5	1.2	1.8	12
30.12.70–1.2.71	3.2	5.0	2.4	5.1	1.1	1.2	8.8
1.2.71–1.3.71	9.5	15	6.5	8.4	1.8	3.6	16
1.3.71–15.3.71	15	26	10	13	2.5	5.1	23
15.3.71–31.3.71	21	36	13	13	1.9	6.5	23
31.3.71–16.4.71	30	55	16	17	2.4	8.7	35
16.4.71–30.4.71	70	130	33	46	6.6	15	81
30.4.71–17.5.71	63	120	28	44	5.9	12	78
17.5.71–1.6.71	46	90	18	35	4.5	8.1	69
1.6.71–11.6.71	100	200	38	84	11	15	150

Relative standard deviation = ±5%

Table 2 Activity Concentrations in Tromsö (fCi/m³)

Sampling period	^{95}Zr	^{95}Nb	^{103}Ru	^{106}Ru	^{137}Cs	^{141}Ce	^{144}Ce
2.1.71–1.2.71	2.0	3.4	1.2	3.9	0.84	0.72	7.2
1.2.71–16.2.71	3.8	6.3	2.6	4.7	0.96	1.5	8.8
16.2.71–1.3.71	8.5	15	5.6	10	2.1	3.0	18
1.3.71–16.3.71	11	19	7.2	10	2.1	3.7	19
16.3.71–1.4.71	15	26	9.0	11	1.9	4.6	20
1.4.71–30.4.71	32	59	16	21	3.3	7.6	39
30.4.71–1.6.71	46	88	19	33	4.7	7.8	59

Relative standard deviation = ±5%.

In December 1968 tungsten-181 was found in ground level air in Italy[1], Finland[2] and the United Kingdom[3], after an underground nuclear test carried out in Nevada on December 8, 1968. The origin of the small ^{181}W contamination detected in early April this year in North Germany and North Norway is still unknown.

Newspapers reported two USSR underground tests on March 22, 1971, in Siberia and on March 23, 1971, in the western Ural Mountains. At that time there was a prevailing westerly wind between Central Europe and the Ural region. If there was any venting into the atmosphere nuclear débris must have been transferred at first in easterly directions. Some days later a high pressure system arose over eastern Europe and led easterly winds to North Germany in early April, the time at which fresh fission products arrived at Brunswick. So it cannot be excluded with certainty that these fission products as well as ^{181}W stem from one of the above mentioned USSR underground tests.

W. Kolb

Physikalisch-Technische Bundesanstalt,
D-33 Braunschweig.

Received July 9, 1971.

[1] De Franceschi, L., Gentili, A., Gremigni, G., and Guidi, P., *Nature*, **224**, 571 (1969).
[2] Blomquist, L., and Asikainen, M., *Nature*, **225**, 59 (1970).
[3] Cambray, R. S., Fisher, E. M., Brooks, W. L., and Peirson, D. H., *AERE-R 6212.*

Composition of Airborne Lead Particles

Knowledge of the composition of airborne particles and the reactions that take place in the atmosphere is important to the understanding of the fate of these particles. Lead compounds are present in a concentration of about 1% in the particulate and information about their composition would be especially helpful to people conducting experimental studies on the health effects of lead in air.

Hirschler *et al.*[1] have shown that the chief lead products exhausted from automobiles are lead halides and ammonium lead halides; lead chlorobromide is the principal component. The automobile is the principal source of airborne lead[2]. The fate of these materials and the changes in chemical composition in the atmosphere have been poorly understood. Work done by the Stanford Research Institute, using electron and X-ray diffraction, showed that most of the lead compounds in the atmosphere were amorphous or present in such low concentrations that identification was nearly impossible[3]. Their work further showed that the mass median diameter of airborne lead particulate is about 0.25 µm (ref. 4).

In our study, airborne particulate lead compounds were physically identified by using an electron microprobe (an ARL type EMX modified to give an electron spot size of the order of 0.2 µm). Samples were taken from a controlled environment, near and at a distance from a busy highway, and in a rural area.

Airborne particulate was collected on 0.45 µm 'Millipore' membrane filters, which were dissolved in acetone and the particles were centrifuged out of the resulting solution. The particles were then mounted in a film of nitrocellulose on ultrapure polished beryllium substrates, coated with about 300 Å of carbon. Beryllium was chosen because it has very low white radiation and no line interference with the elements of interest. The thin film of carbon eliminated any reaction with lead bromides, but did not interfere with the analysis.

All the analytical work was based on analysis by ratios of elements rather than by absolute intensities. This was necessary both because of the small particle size and because of the time allotted for data acquisition. It was possible to pick out the various classes of compounds apparently present and then return to individual particles known to be this compound—or at least suspected to be this compound—and analyse them in more detail. Several particles from each class of compounds and each sample were analysed in detail in this way.

Samples of airborne particles were collected near to and about 400 yards from Eight Mile Road, a state highway on the northern edge of Detroit that carries about 100,000 cars each day. Samples were also taken in a rural area about 400 yards from a very lightly travelled roadway that is 15 miles from the nearest city (population 25,000). The samples taken near the highway and at 400 yards from the highway contained about 2.6% and 1.2% lead respectively. The rural sample contained a few tenths of a per cent lead.

Samples were also taken of automobile exhaust; these were collected in a black bag—a 4,000 foot^3 plastic container filled with clean dry air. For the study, 300 feet^3 of exhaust gas taken from an automobile operating on the seven-mode Federal Cycle was passed into the bag. The ten-fold dilution prevents condensation. Samples for analysis were taken immediately after automobile exhaust was discharged into the black bag and 18 h later. The particulate samples from the bag were about 25% lead. They had a size distribution similar to that found in atmospheric samples.

Table 1 Sample Composition by Electron Microprobe

	Percentage of total particles counted													
Sample	$PbCl_2$	$PbBr_2$	PbBrCl	Pb(OH) Cl	Pb(OH) Br	$(PbO)_2$ $PbCl_2$	$(PbO)_2$ $PbBr_2$	$(PbO)_2$ PbBrCl	$PbCO_3$	Pb_3 $(PO_4)_2$	PbO_x	$(PbO)_2$ $PbCO_3$	PbO · $PbSO_4$	$PbSO_4$
Black bag														
Zero time	10.4	5.5	32.0	7.7	2.2	5.2	1.1	31.4	1.2	—	2.2	1.0	—	0.1
18 h	8.3	0.5	12.0	7.2	0.1	5.6	0.1	1.6	13.8	—	21.2	29.6	0.1	—
Eight Mile Road														
Near road	11.2	4.0	4.4	4.0	2.0	2.8	0.7	2.0	15.6	0.2	12.0	37.9	1.0	2.2
400 yards	10.5	0.7	0.6	8.8	1.1	5.6	0.3	0.6	14.6	0.3	25.0	21.3	4.6	6.0
Rural site	5.4	0.1	1.6	4.0	—	1.5	—	1.0	30.2	—	20.5	27.5	5.0	3.2

Table 1 shows the electron microprobe data for the samples. Individual sizes from 0 to 0.2, 0.2 to 0.6, 0.6 to 2, and greater than 2 µm were examined. Because the composition distribution was so similar for all sizes, the sample data were averaged.

Examination of the data for exhaust gas from the black bag is very informative. At zero time, a significant percentage of the exhaust particles consists of $PbBr_2$, PbBrCl, Pb(OH)Br, $(PbO)_2PbBr_2$ and $(PbO)_2PbBrCl$. Some intermediate halides might also be present up to concentrations of 10%. After 18 h, about 75% of the bromide is gone. About 30 to 40% of the chloride compounds also appear to have been lost. The products formed are lead carbonates, oxycarbonate, and oxides. Basic lead carbonate may be the actual product rather than the oxycarbonate. As water is easily lost from these compounds in the electron beam, however, the product identified is the oxycarbonate. It is estimated that the particles examined contained less than 1% of cations other than lead.

The loss of bromine was confirmed using X-ray fluorescence to measure the ratio of bromine to lead in the particulates in the black bag. The ratio rapidly decreased with time, with most of the loss occurring during the first hour.

When the composition in the black bag at 18 h is compared with the samples taken from the atmosphere, striking similarities are apparent. The sample taken near Eight Mile Road still contains significant quantities of bromide, although not as much as in the black bag at zero time. On the other hand, the sample taken away from the road contains almost no bromide and is very similar in composition to the black bag sample at 18 h. As in the black bag, the chief reaction products are lead carbonates, oxycarbonate and oxides. Because of the presence of SO_3 in the atmosphere and the use of phosphorus additives in some fuels, small amounts of lead phosphate and lead sulphate are also found.

The sample from the rural area shows clearly that very little lead halide remains. The more insoluble lead salts make up the bulk of the lead particulates.

The results of this study make it unnecessary to postulate photolysis of the lead compounds[5] to account for the loss of halogen. Indeed the results from the black bag in the absence of light make this impossible. The clear parallel between the results from the black bag and the atmosphere strongly suggests that the mechanism is not photolytic.

In the black bag, the primary reaction appears to be a simple acid base reaction with carbonate or hydroxide ion displacing the bromide ion and, in some cases, the chloride ion from the lead halides initially exhausted. Oxidation, however, cannot be ruled out completely.

These results suggest that lead oxide, lead carbonate, basic lead carbonate or a mixture of these would be a good choice for experimental studies of the health effects of lead in the atmosphere.

GARY L. TER HAAR

Research and Development Department,
Ethyl Corporation,
Ferndale, Michigan

MICHAEL A. BAYARD

Research and Development Department,
Walter C. McCrone Associates, Inc.,
Chicago, Illinois

Received March 18; revised July 2, 1971.

[1] Hirschler, D. A., Gilbert, L. F., Lamb, F. W., and Niebylski, L. M., *Ind. Eng. Chem.*, 49, 1131 (1957).
[2] Engle, R. E., Hammer, D. I., Horton, R. J. M., Lane, N. M., and Plumlee, L. A., *Environmental Lead and Public Health* (Environmental Protection Agency, Air Pollution Control Office, 1971).
[3] Robinson, E., and Ludwig, E. L., *J. Air Poll. Control Assoc.*, 17, 644 (1967).
[4] Robinson, E., Ludwig, F. L., DeVries, J. E., and Hopkins, T. E., *Variations of Atmospheric Lead Concentration and Type with Particle Size* (Stanford Research Institute, 1963).
[5] Pierrard, J. M., *Environ. Sci. Tech.*, 3, 48 (1969).

BIOLOGICAL SCIENCES

Effect of Inclusion of Cellulose in an "Atherogenic" Diet on the Blood Lipids of Children

SUNDARAVALLI *et al.*[1] have reported that increased plasma and liver cholesterol induced by cholesterol feeding in the rat could be largely counteracted by the concurrent feeding of 20% cellulose in the diet. We now report the results of similar investigations on humans.

Ten girls (10–12 yr) who volunteered for the study received (1) control diet (2) control diet with cholesterol (4 g/day) and (3) control diet with cholesterol (4 g/day) and cellulose (100 g/day) in the same order; thus each subject acted as her own control. Each diet was fed for a period of 10 days and the faeces were collected for analysis during the last 3 days. Blood samples were taken at the end of each period, and total and free cholesterol were estimated according to Wootton[2], total lipids by the method of Sperry[3] and phospholipids by the method described by Oser[4].

The composition of the control diet is given in Table 1. The diet provided 1,880 kcalories/day, of which 72% was from carbohydrates, 18% from fat and 10% from protein. Various recipes were prepared from the ingredients shown in Table 1. The cholesterol supplement given during the second and third period was dissolved in the fat by gentle warming and this mixture was used for all the cooking. Cellulose (microcrystalline cellulose, 'Avicel-pH', marketed by FMC Corporation, Marcus Hook, Pennsylvania) given as supplement, formed 20% of the diet; this was mixed with wheat flour–corn starch blend and made into *chapathies* (unleavened bread) which were offered to the subjects. This cellulose is of very fine particle size (>300 mesh) and could be easily incorporated in the diet without affecting the taste and acceptability.

Table 1 Composition of Control Diet

Ingredients of control diet	Daily quantities (g)
Corn starch	150
Wheat flour (80% extraction)	150
Sucrose	40
Groundnut oil	35
Skim milk powder	50
Vegetables	50
Tea leaves	6
Salt	15
Condiments	18

Table 2 Serum Lipids and Faecal Bile Acids of Experimental Children

Diet	Control	Control with cholesterol	Control with cholesterol and cellulose
Cholesterol (mg/100 ml.)			
Total	137.5 (127.1–148.0)	226.3 (184.8–255.1)	170.3 (152.1–190.2)
Free	45.9 (40.3–52.2)	63.1 (51.0–72.1)	44.4 (36.5–57.5)
Total lipids (g/100 ml.)	0.61 (0.56–0.64)	0.81 (0.75–0.84)	0.66 (0.59–0.69)
Phospholipids (mg/100 ml.)	184.9 (170.1–192.1)	244.3 (212.0–266.0)	203.3 (185.3–213.2)
Faecal bile acids (mg/day)	78.5 (71.0–82.8)	146.9 (133.8–162.1)	212.7 (192.1–231.6)

Values for serum lipids and faecal bile acids of the children are given in Table 2. The concentration of serum cholesterol of subjects on the control diet was 137.5 mg/100 ml.; approximately 33% of this was in the free form. Feeding of cholesterol

(4 g/day) resulted in a significant increase in serum cholesterol to 226.3 mg/100 ml. There were concomittantly significant increases in serum lipids and phospholipids. Inclusion of cellulose to the extent of 20% of the diet resulted in a significant reduction in serum cholesterol, total lipids and phospholipids. The faecal excretion of bile acids (mg/day) increased from 78.5 to 146.9 when cholesterol was included in the diet. Inclusion of cellulose in the diet to the extent of 20% increased the bile acid excretion to 212.7.

Keys *et al.*[5] reported that cellulose in the diet did not exert any beneficial effect on lowering of cholesterol. The present studies, however, have shown that cellulose at higher levels (20% of the diet) exerts a beneficial effect in lowering serum cholesterol in children, receiving a hypercholesterolaemic diet. Sundaravalli and Narayana Rao[6] reported that inclusion of microcrystalline cellulose even to the extent of 20% did not significantly influence the utilization of nitrogen in young weanling rats. The paucity of information about the fibre content of human diets makes it difficult to relate the present studies to diets as eaten normally. A study by Hardinge, Chambers *et al.*[7] showed that the fibre intake could vary widely over a period of years without adverse effects on the digestive tract. The amount of cellulose used in these studies is certainly higher than commonly ingested in so called civilized diets.

The mechanism of the effect of cellulose is unknown, but it may result from increased excretion of bile acids in the faeces. Using tagged compounds, Portman and Murphy[8] reported that inclusion of 20% celluflour in a sucrose synthetic diet resulted in an increased excretion of cholate and degradation products of cholate.

We thank Dr H. A. B. Parpia for his interest.

K. S. SHURPALEKAR
T. R. DORAISWAMY
O. E. SUNDARAVALLI
M. NARAYANA RAO

Applied Nutrition and Dietetics Discipline,
Central Food Technological Research Institute,
Mysore-2a

Received March 19, 1971.

[1] Sundaravalli, O. E., Shurpalekar, K. S., and Narayana Rao, M., *J. Agric. Food Chem.*, **19**, 116 (1971).
[2] Wootton, I. D. P., *Micro-analysis in Medical Biochemistry*, fourth ed., 85 (Churchill, London, 1964).
[3] Sperry, W. M., *Methods of Biochemical Analysis*, **2** (edit. by Glick, D.), 83 (Wiley, New York, 1955).
[4] Oser, B. L., *Hawk's Physiological Chemistry*, fourteenth ed., 1060 (McGraw-Hill, New York, 1965).
[5] Keys, A., Grande, F., and Anderson, J. T., *Proc. Soc. Exp. Biol. Med.*, **106**, 555 (1961).
[6] Sundaravalli, O. E., and Narayana Rao, M., *J. Amer. Dietet. Assoc.*, **57**, 517 (1970).
[7] Hardinge, M. G., Chambers, A. C., Crooks, H., and Stare, F. J., *Amer. J. Clin. Nutrit.*, **6**, 523 (1958).
[8] Portman, O. W., and Murphy, P., *Arch. Biochem. Biophys.*, **76**, 367 (1958).

Cytological Evidence for the Association of the Short Arms of the *X* and *Y* Chromosomes in the Human Male

EVIDENCE for the association of the *X* and *Y* chromosomes during the first meiotic division in man has been documented[1,2]. Until the centromere positions of the meiotic chromosomes were located with certainty, no information about the arm relationship between the two sex chromosomes could be obtained. The identification of the positions of the centromere in human male meiotic chromosomes at the late diplotene stage has been reported[3]. This observation revealed that the short arm of the *Y* chromosome was in association with the short arm of the *X* chromosome. Partial support for this finding was presented by Pearson and Bobrow[4], who interpreted fluorescent staining of the distal end of the long arm of the *Y* chromosome of the *XY* bivalent in man as evidence that the short arm of the *Y* chromosome was associated with the *X* chromosome. Because the centromere position of the *X* chromosome could not be demonstrated in their material, it was not possible to determine which arm of the *X* chromosome was associated with the short arm of the *Y* chromosome. The purpose of this communication is to provide additional cytological evidence for the association of the *X* and *Y* chromosomes at their short arms.

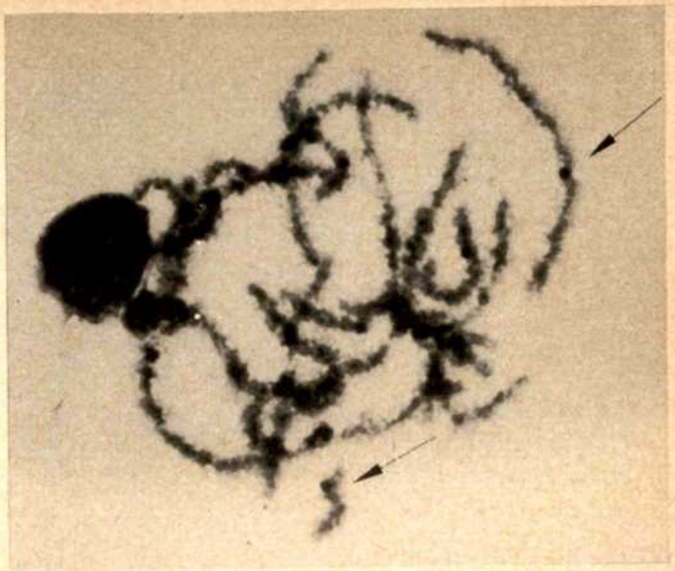


Fig. 1 Cell in pachytene. The broken arrow points to a *G* group bivalent and solid arrow to an *A* group bivalent, most possibly chromosome number 2.

The technique for the preparation of the meiotic material was a modification of that reported previously[5] and was as follows: after the outer membraneous capsule (tunica albuginea) of the tissue was removed, the specimen was teased apart in a Petri dish containing 1% sodium citrate solution by the use of teasing needles. The Petri dish was agitated gently from time to time to loosen the maximum number of cells from the tubules. The cell suspension was decanted into a centrifuge tube and centrifuged at 1,000 r.p.m. for 10 min after which the supernatant was removed and 1 ml. of freshly prepared fixative (3 : 1 methanol and glacial acetic acid) was added in a dropwise fashion. The material was refrigerated at 4° C for 5 min, the cell button was then resuspended in fixative and placed in the refrigerator for a further 25 min. The cells were centrifuged and then washed in a new fixative until a button and a clear supernatant were obtained. The button of cells was then placed on clean slides, flame-dried and stained with Giemsa (Azure Blend Type, Harleco).

The materials used for this report were obtained from the testicular tissue of three men treated for cancer of the prostate. The dark staining bodies which we have taken to indicate the centromere positions were observed not only at the late diplotene stage[3] but also at other stages of meiosis I. For example, some bivalents at pachytene could be readily identified on the basis of length and centromere positions (Fig. 1). It was still technically difficult, however, to identify all the individual bivalents at this stage, because they were elongated and intertwined. Convincing evidence that the dark staining region of the pachytene chromosomes was at the centromere position was presented by Fredga[6] who karyotyped the pachytene chromosomes of the Wallaby kangaroo.

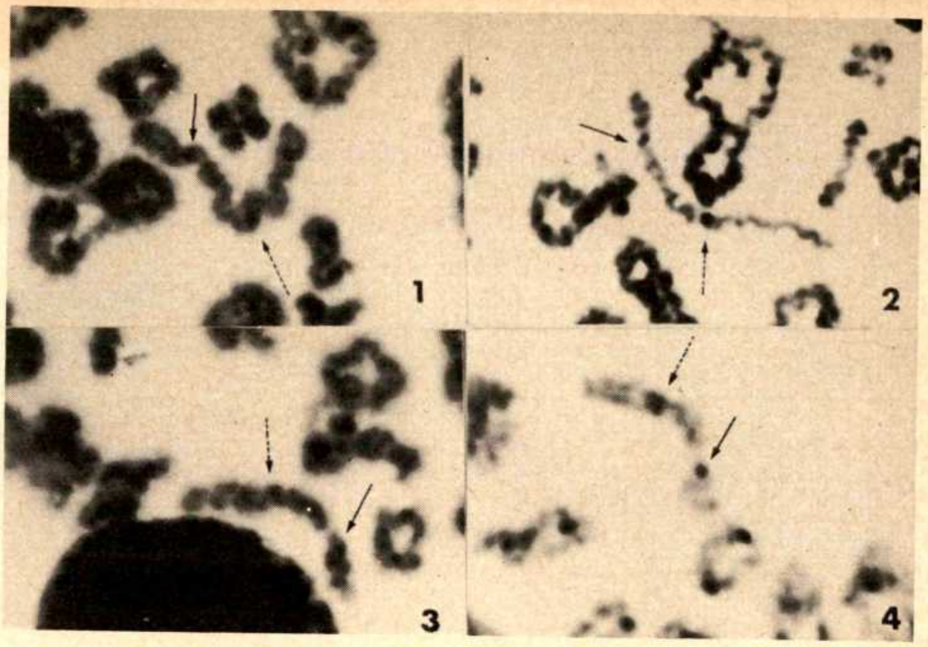


Fig. 2 Partial meiotic chromosome complement from four cells at or near diakinesis. The broken arrow points to the centromere position of the X chromosome and the solid arrow to that of the Y chromosome.

Although the arrangement of the X and Y chromosomes at pachytene could not be visualized because they were condensed into a heteropyknotic sex vesicle[7], they were observed in an end to end relationship at the late diplotene stage as well as at diakinesis. The long and short arms of the two sex chromosomes from ten cells at the latter two stages were measured using camera lucida drawings. The measurements revealed that the mean ratio between the long and short arms of the X chromosomes was 1.37 (s.e. = 0.05) and that of the Y chromosomes 3.57 (s.e. = 0.18). Furthermore, the data confirmed the short arm association of the X and Y chromosomes (Fig. 2).

This study was supported in part by a grant from the Research Corporation. We thank Dr C. E. Ford for helpful comments.

ANDREW T. L. CHEN
ARTHUR FALEK

Division of Human Genetics,
Georgia Mental Health Institute
and Department of Psychiatry,
Emory University,
Atlanta,
Georgia 30306

Received August 24; revised December 7, 1970.

[1] Ford, C. E., and Hamerton, J. L., *Nature*, **178**, 1020 (1956).
[2] Hulten, M., Lindsten, J., Ming, P. M. L., and Fraccaro, M., *Ann. Human Genet.*, **30**, 119 (1966).
[3] Chen, A. T. L., and Falek, A., *Science*, **166**, 1008 (1969).
[4] Pearson, P. L., and Bobrow, M., *Nature*, **226**, 959 (1970).
[5] Evans, E. P., Breckon, G., and Ford, C. E., *Cytogenetics*, **3**, 289 (1964).
[6] Fredga, K., *Exp. Cell Res.*, **36**, 696 (1964).
[7] Fraccaro, M., Hulten, M., and Lindsten, J., *Ann. NY Acad. Sci.*, **155**, 664 (1968).

Para-nucleolar Position of the Human Y Chromosome in Interphase Nuclei

INDIVIDUAL human chromosomes cannot be seen during interphase, and little is known of their intra-nuclear position during most of the cell cycle. The principal exception to this has been the X chromosomes: when there is more than one, they condense during interphase to form the sex chromatin, which is often located on the nuclear membrane. It has recently become possible to locate the major portion of the Y chromosome in interphase nuclei[1], and we have used this technique to demonstrate that the Y chromosome is spatially associated with the nucleolus.

Because of their relatively large size and favourable morphology, cultured fibroblast nuclei were fixed in either methanol or Carnoys fixative, stained with quinacrine[1], and viewed on a Leitz ortholux microscope fitted with Ploëm vertical illuminator. The use of incident ultraviolet light and transmitted white light facilitates fluorescent and phase contrast microscopic observations either simultaneously or in rapid succession. A comparison of photographs taken under phase contrast with the same nuclei after Giemsa staining confirmed that the nucleoli could be well defined under phase contrast.

Nuclei in which the Y body could be clearly identified were located, and then examined under phase contrast. Material was obtained from nine individuals of XY sex chromosome constitution, the mean number of cells scored per individual being 133 (range 59–256). The proportion of cells in which the fluorescent Y body was in contact with a nucleolus varied in different individuals from 37%–86%, the overall mean being 59.1%.

Ninety-four randomly selected cells were photographed, and the areas of nuclei and nucleoli measured by planimetry. The mean nucleolar area in different individuals ranged from a minimum of 8.92% (s.d. 3.7) to a maximum of 13.47% (s.d. 4.19) of the nuclear area. The overall mean for ninety-four cells was 10.7%. It is apparent that the proportion of cells in which the Y body lies in contact with a nucleolus considerably exceeds the expectation, had the Y body been randomly positioned within the nucleus. A similar association of the Y body with the nucleolus has also been observed in Sertoli cell nuclei, PHA-stimulated peripheral lymphocytes, and buccal mucosal cells, although phase-contrast observations are much more difficult to make on the latter cell types.

Fibroblast nuclei from a male of XYY sex chromosome constitution showed both Y bodies to be associated with the nucleolus in 36% of cells, while neither Y body was so associated in 24.6%. There was no tendency for both Ys to be associated with the same nucleolus. Nucleolar area was 11.98% (s.d. 2.95) of nuclear area.

In some species the number of nucleoli per nucleus can be related to the number of nucleolar-organizing regions present in the genome[2]. We therefore investigated the relationship of the number of *Y* chromosomes in the karyotype to the mean nucleolar count per cell. The within-individual variability of nucleolar counts made it difficult to get reproducible results on fibroblast nuclei, and this part of the investigation was therefore done on 72 h PHA-stimulated peripheral blood cultures. Preparations were stained with Giemsa, and the number of nucleoli counted in 100 consecutive cells. Two separate counts were made for each individual, and the results expressed as the mean number of nucleoli per nucleus (nucleolar index).

Table 1 Mean Number of Nucleoli per Cell (Nucleolar Index) in Cultured Lymphocytes of Different Chromosome Constitution

Group	Chromosome constitution	No. of individuals	Nucleolar index Mean	s.d.
1	46 *XY*	10	1.86	0.22
2	46 *XX*	10	1.68	0.29
3	47 *XYY*	4	1.77	0.21
4	47 *XXY*	3	1.74	0.16
5	45 *XO*	3	1.69	0.12
6	47 *XXX*	1	1.75	–
7	45 *XYt(DqGq)*	1	1.70	–
8	47 *XX* 13+	1	1.80	–
9	47 *XY* 21+	6	2.12	0.31
10	47 *XX* 21+	5	2.26	0.31

Data on forty-four individuals of different chromosome constitutions are summarized in Table 1. Analysis of variance on the whole data, and comparisons of group means with one another by *t* tests, demonstrated no significant effect of sex chromosome complement on the nucleolar index. Individuals with trisomy 21 had a significantly raised nucleolar index ($P<0.001$ for both sexes combined). The numbers available for examination in some of the groups, however, are so small that real differences could have gone undetected.

Information on the nucleolus-organizing capacity of human chromosomes is based largely on the study of "satellite associations" persisting into metaphase. Although the *Y* chromosome long arm does have a secondary constriction[3], and there have been suggestions that it may be involved in satellite association[4], the weight of evidence seems to be against this view[5]. Our demonstration of a spatial proximity of this chromosome to the nucleolus does not necessarily imply a functional relationship, but several features of the *Y* chromosome may be relevant to this question. (*a*) Intense quinacrine fluorescence is characteristically seen, apart from the *Y* long arm, on the short arms of the acrocentric *D* and *G* group chromosomes and next to the centromere of chromosome 3. The *D* and *G* group short arms are the known nucleolus-organizing regions of the human genome[6], and we have observed interphase fluorescence from a marker chromosome of this group associated with the nucleolus in a large proportion of nuclei. Whether the centromeric region of chromosome 3 is also related to the nucleolus is not yet known. (*b*) Recent techniques for demonstrating a form of constitutive heterochromatin stain the distal part of the *Y* long arms[7]. The procedure involves denaturing and reannealing DNA, and is thought to demonstrate highly repetitive DNA sequences. There is evidence that nucleolus-organizing regions consist of repetitive DNA[8]. (*c*) The intensely fluorescent part of the *Y* chromosome is known to vary considerably in size, and may even be completely absent, without apparent clinical effect[9,10]. This is compatible with the concept that it is composed of genetic sequences also present elsewhere in the genome. (*d*) The only well proven function of the *Y* is in sex determination, and these genes are probably localized in the non-fluorescent area[11]. (*e*) The human *Y* chromosome is relatively large compared with those of other primates, which lack intensely fluorescent chromatin[12]. This again suggests a genetic content over and above that necessary for male sex determination.

These arguments are all indirect, but it seems possible that the distal part of the long arm of the *Y* chromosome contains a specialized form of genetic material related to nucleolar function. Further investigation may throw light, not only on the function of the *Y* chromosome, but on the nature of the histochemical differentiation recently revealed within the human chromosome complement.

M. Bobrow
P. L. Pearson
H. E. A. C. Collacott

MRC Population Genetics Unit,
Old Road,
Headington,
Oxford

Received April 16, 1971.

[1] Pearson, P. L., Bobrow, M., and Vosa, C. G., *Nature*, **226**, 78 (1970).
[2] Busch, H., and Smetana, K., *The Nucleolus* (Academic Press, New York, 1970).
[3] Court Brown, W. M., *Human Population Cytogenetics* (North-Holland, Amsterdam, 1967).
[4] Gripenberg, U., *Chromosoma*, **15**, 618 (1964).
[5] Ferguson-Smith, M. A., and Handmaker, S. D., *Ann. Hum. Genet.*, **27**, 143 (1963).
[6] Ferguson-Smith, M. A., *Cytogenetics*, **3**, 124 (1964).
[7] Arrighi, F. E., and Hsu, T. C., *Cytogenetics* (in the press).
[8] Hsu, T. C., Brinkley, B. R., and Arrighi, F. E., *Chromosoma*, **23**, 137 (1967).
[9] Bobrow, M., Pearson, P. L., Pike, M. C., and El-Alfi, O. S., *Cytogenetics* (in the press).
[10] Borgaonkar, D. S., and Hollander, D. H., *Nature*, **230**, 52 (1971).
[11] Ferguson-Smith, M. A., *Bull. Europ. Soc. Hum. Genet.*, **4**, 39 (1970).
[12] Pearson, P. L., Bobrow, M., Vosa, C. G., and Barlow, P. W., *Nature*, **231**, 326 (1971).

Evidence for a Genetic Factor in the Transmission of Spontaneous Lymphosarcoma (Leukaemia) of Young Pigs

A histological survey of lymphosarcoma[1] (leukaemia) in 1968 in abattoirs throughout Great Britain showed an incidence of twenty-five cases per million pigs slaughtered, that is, ninety-two cases from 3.7 million pigs. There was multicentric lymphosarcoma in fifty-seven pigs and thymic lymphosarcoma in thirty-five pigs, and there was no indication of lymphosarcoma in other pigs on the farms concerned or even that it had occurred there previously. It is therefore of some interest that we have now found lymphosarcoma to have occurred several times in a single herd.

A minimal disease (MD) piggery established in 1963 was stocked with colostrum-deprived hand reared Large White piglets obtained by caesarian section from nine unrelated sows. The herd has since been maintained as a closed community with fourteen to seventeen breeding sows and one or two boars. The only contact with other pigs has been the occasional use of semen from outside boars for artificial insemination.

In August 1966, lymphosarcoma was diagnosed in two 3 month old sibling piglets. These were the offspring of a boar (D) mated to his sister (362). The boar died before the mating could be repeated but the sister subsequently had litters of nine and four offspring respectively to another brother (138). None of these developed lymphosarcoma although four were kept for more than a year. No further cases were detected in the herd until September 1969, since when nineteen have occurred, all confirmed at necropsy as multicentric lymphosarcoma. Although present in many of these pigs, the thymus was not involved in the disease.

The age at which the disease could first be diagnosed with any certainty conformed to a more or less normal distribution between 6 and 18 weeks, apart from one case in which diagnosis was obscured for several weeks by intercurrent disease.

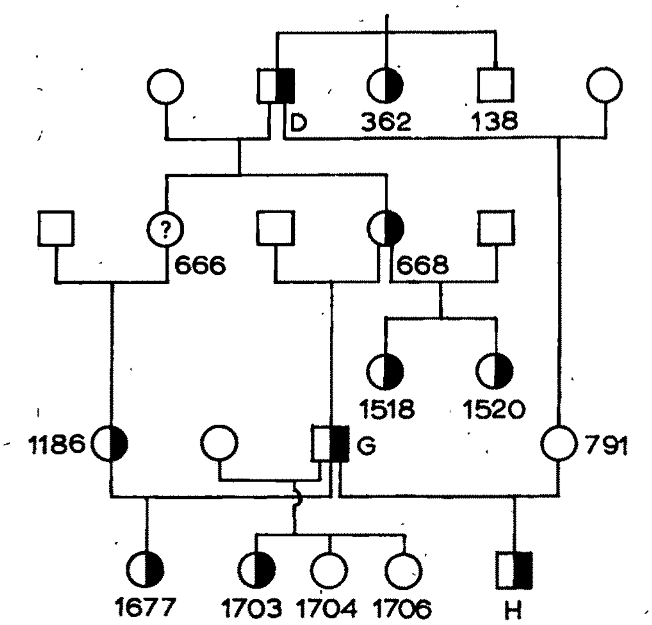


Fig. 1 Relationship between parents of lymphosarcoma cases; Square, male; circle, female; half filled symbol, parent of L/S; open symbol, not parent of L/S; ?, status doubtful, not suitably test-mated.

Most animals reared in the piggery are slaughtered for bacon at 5–6 months of age but to date eighty-two animals have been kept for breeding, most for 2 or 3 yr, some for 6 or 7 yr. No signs of lymphosarcoma have been detected in any of these which include all the parents of all known lymphosarcoma cases. The entity we are reporting is thus essentially a disease of young pigs. Occasionally we have found lymphosarcoma in older pigs in other herds but such cases are sporadic. This is in keeping with other reports[1,3–5].

Eleven of our cases were males (including five castrates) and ten were females. All resulted from matings with a degree of consanguinity of four or less between three boars and seven sows which had a common ancestry. The ways in which they were mated and the distribution of lymphosarcoma cases in the resultant litters are detailed in Table 1.

Table 1 Matings Producing Lymphosarcoma and Distribution of Cases among Litters

Matings			Composition of litters		
			No lymphosarcoma		
Boar	Sow	Degree of consanguinity	Died before 6 weeks	At bacon weight (5–6 months)	L/S cases
D	362	2	2	7	2
G	668	1	3	7	1
			7	3	2
	1,186	4	1	9	1
			2	8	0
			6	6	2
	1,518	2	1	5	1
			1	9	1
	1,520	2	5	4	2
			4	9	2
	1,677	1	4	0	2
H	1,703	2	2	5	5

A number of piglets died before 6 weeks of age and are not included in our assessment of incidence of lymphosarcoma. This averaged 22.6% which is not significantly different from the 25% to be anticipated if an autosomal recessive gene were involved ($\chi^2 = 0.03$, 1 d.f.). This hypothesis is supported by the facts that when any of the ten parents of lymphosarcoma cases was mated to other pigs no cases were found among 478 offspring in fifty-seven litters, nor were any cases encountered in 753 offspring in ninety-nine litters arising from matings which excluded the ten, even though in both groups about a quarter of the matings had a degree of consanguinity of four or less. A proportion of animals in the affected strain would be expected to be non-carriers of the disease. We have found four such animals (138, 791, 1,704, and 1,706). It has so far not proved possible to use affected animals in test matings which might help to confirm the hypothesis because they have all remained stunted and sexually immature even when living to be more than 1 yr old.

Evidence from Russia[6] suggests that in cattle susceptibility to the disease is inherited through a recessive homozygous factor but the situation differs from ours in that the disease in cattle tends to appear later in life when they have already bred successfully and in the Russian herd every affected offspring had an affected or suspect parent.

We thank Professor Sir Alexander Robertson for encouragement and facilities.

H. S. McTaggart
K. W. Head
A. H. Laing

Royal (Dick) School of Veterinary Studies,
University of Edinburgh,
Easter Bush,
Roslin,
Midlothian

Received April 5; revised May 17, 1971.

[1] Anderson, L. J., and Jarrett, W. F. H., *Cancer*, **22**, 398 (1968).
[2] McTaggart, H. S., and Rowntree, P. G. M., *Brit. Vet. J.*, **125**, 240 (1969).
[3] Meier, H., *Adv. Vet. Sci.*, **7**, 43 (1962).
[4] Cotchin, E., *Vet. Rec.*, **72**, 816 (1960).
[5] Monlux, A. W., Anderson, W. A., and Davis, C. L., *Amer. J. Vet. Res.*, **17**, 646 (1956).
[6] Emeljjanov, A. S., Smolina, K. A., and Smetanina, G. K., *Zhivotnovodstvo, Mosk.*, **28**, 53 (1966).

Cell Division in Medium lacking Serum Growth Factor: Comparison of Lines transformed by Different Agents

Cells of the permanent lines derived from mouse embryo tissue, 3T3 and Balb/c 3T3, exhibit density dependent inhibition of cell division[1,2] anchorage dependence[3,4], and also require a factor in serum to initiate DNA synthesis[5–8], but these growth controls[4,6,9–11] are lost in transformed cells. It has been suggested that loss of density dependent inhibition is a consequence of the loss of a requirement for serum factor[6], in which case it would be expected that transformed lines derived from 3T3 would lack both growth controls. We have therefore tested various transformed lines of Balb/c 3T3 lacking the density dependent inhibition for the ability to grow without this serum factor or factors. Both virus-transformed lines of Balb/c 3T3 and independent isolates of Balb/c 3T12 (see ref. 2) grow well in medium lacking the serum growth factor(s), but most non-virus-transformed variants of Balb/c 3T3 grew poorly if at all. These observations suggest that the loss of density dependent inhibition of growth does not necessarily depend on the loss of the need for serum growth factor.

Balb/c 3T3, clone A31, was separately transformed by the small plaque mutant of SV40, the Kirstein and Moloney strains of murine sarcoma virus (MSV) and the Bratislava strain of Rous sarcoma virus (RSV). Most of the other transformed clones were isolated from the occasional cells that spontaneously grew out of the Balb/c 3T3 monolayer. We also used one transformed line induced by X-irradiation (1,500 rads) and another induced by ultraviolet light (2,000 ergs mm^{-2}). All the transformed lines had saturation densities ranging from 3.5–7 × 10^5 cells cm^2. The preparation of factor-free medium which permits growth of SV40-transformed Balb/c 3T3 but not of normal cells has been described[12].

Exponentially growing cultures were trypsinized and planted in factor-free medium and medium containing 10% whole calf serum (complete medium) at cell concentrations ranging from 100–6,000 cells per 50 mm Petri dish (Falcon). After 2–3 weeks the plates were fixed, stained[12] and the colonies scored. In case the lines did not grow in factor-free medium because they were unable to settle onto the plastic dish, all lines with little or no growth were also planted in complete medium and changed to factor-free medium 1 day later.

Fig. 1 gives the relative plating efficiencies of cells in factor-free medium (colonies growing in factor-free medium × 100/colonies growing in complete medium). All SV40-transformed clonal lines had efficiencies, in factor-free medium, of 20% to 60%, as did clonal lines transformed by sarcoma virus. The latter included a line transformed by the Moloney strain of MSV with associated leukaemia virus (G), two lines without associated leukaemia virus (one transformed by Moloney MSV (*H*) and one by Kirstein MSV (*I*)[13]) and one line transformed by RSV (*J*) without associated leukaemia virus. All the Balb 3T12 lines had high relative plating efficiencies in factor-free medium, and included a line (*N*) infected with murine leukaemia virus and three (*K*, *L*, *M*) with no demonstrable leukaemia virus[14]. In these conditions, the plating efficiency of Balb/c 3T3 was $<0.1\%$ (data not shown).

The virus-transformed and non-virus-transformed Balb/c 3T3 lines differed in their ability to grow in factor-free medium. Only three-eighths of the non-virus-transformants grew well; the remainder grew poorly, if at all. There was no correlation between the saturation densities of these lines (which ranged from $3.5\text{–}6.5 \times 10^5$ cells cm^{-2}) and their ability to grow in the absence of serum growth factor(s). Planting cells directly into factor-free medium, or shifting cells from complete to factor-free medium, gave similar relative plating efficiencies for all the lines except (*P*) which had an efficiency of only 1% after the cells were planted directly in factor-free medium although it increased to 125% after being planted first in complete medium. This line may require a serum factor lacking in factor-free medium to settle and flatten out[15].

Our experiments confirm and extend the observation that all virus-transformed lines of Balb/c 3T3, as well as Balb/c 3T12, can grow in medium depleted of the serum growth factor(s). Furthermore, because most non-virus-transformed cells grow poorly, if at all, it is clear that loss of density dependent inhibition does not always result from a lowered requirement for these factors.

In virus transformations, there are two indications that loss of density dependent inhibition is not identical to decreased requirement for serum growth factor(s). First, the two properties are acquired at different times after SV40 infection: immediately after infection, Balb/c 3T3 cells become able to grow in factor-free medium[12] although several generations are necessary before the cells lose density dependent inhibition[16]. Second, there are SV40-transformed lines which have only one of the growth properties associated with virus-transformation, that is, the ability to grow in factor-free medium. Most of these lines may be the result of a secondary alteration as they were selected from a fully transformed line by FUdR[17], but because there was no secondary selection involved in the development of two lines with these properties (ref. 13 and our unpublished observation) it is likely that the virus induced only the ability to grow in factor-free medium.

The simplest hypothesis consistant with all the data is that the two growth properties, that is loss of density dependent inhibition and loss of requirement for serum factor, are independent changes and that viruses usually induce both. Alternatively, there may be more than one way for a cell to lose density dependent inhibition. In virus-transformed lines and possibly in those non-virus-transformed lines that also grow in factor-free medium, the loss of requirement for serum factor may be necessary, but not sufficient, for the loss of density dependent inhibition. In other cases (that is most non-virus-transformed lines) a mechanism not dependent on the loss of serum factor requirement could also cause the loss of density dependent inhibition. Whichever hypothesis is correct, it is clear that these two growth properties are not identical.

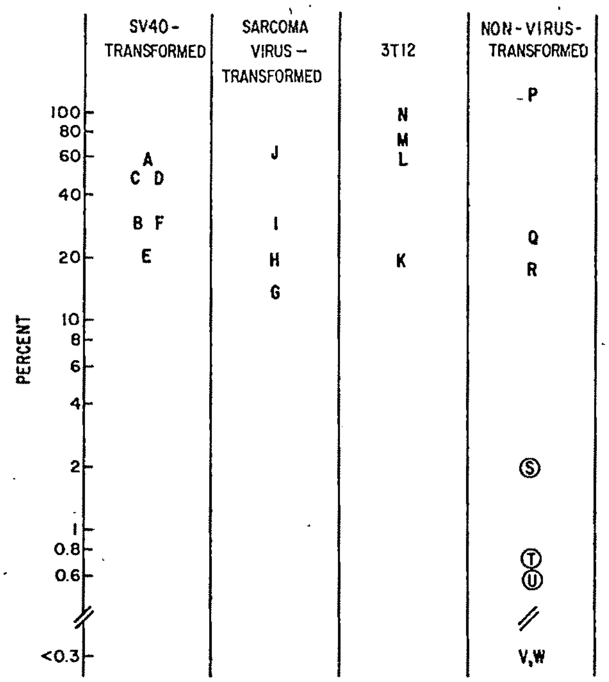


Fig. 1 Relative plating efficiency of transformed lines in medium lacking serum growth factors. Data are expressed as colonies growing in factor-free medium ×100/colonies growing in complete medium. Colonies were scored 2 weeks after planting. The circled letters indicate colonies that grew more slowly in factor-free medium and were scored, 3–4 weeks after planting. SV40-transformed lines: *A*, SV$_1$; *B*, SV$_3$; *C*, SV$_4$; *D*, SV$_5$; *E*, SV$_6$; *F*, SVT$_2$. Sarcoma virus transformed lines: *G*, MS24; *H*, 85–2; *I*, KA31CL$_{234}$; *J*, B77. Balb/c 3T12 lines: *K*, 3T12/1; *L*, 3T12/2; *N*, 3T12/3; *N*, 3T12/P13. Non-virus transformed lines: *P*, IIC12; *Q*, R-4; *R*, TA31; *S*, εA31; *T*, A31A1C11; *U*, 17C11; *V*, STA31, *W*, IC16.

We thank Dr S. Aaronson for many transformed lines, Miss K. Reynolds and Mrs K. Gottesman for technical assistance, and Dr G. Todaro for discussions and the hospitality of his laboratory. This work was supported by a contract from the National Cancer Institute.

HELENE S. SMITH *
CHARLES D. SCHER

National Cancer Institute,
Bethesda, Maryland, and
Mel-Labs Inc.,
Springfield, Virginia

Received November 10, 1970.

* Present address: Cell Culture Laboratory, School of Public Health, University of California, Berkeley, California.

[1] Todaro, G. J., and Green, H., *J. Cell. Biol.*, **17**, 299 (1963).
[2] Aaronson, S. A., and Todaro, G. J., *J. Cell. Physiol.*, **72**, 141 (1968).
[3] Macpherson, J. A., and Montagnier, L., *Virology*, **23**, 291 (1964).
[4] Black, P. H., *Virology*, **28**, 760 (1966).
[5] Todaro, G. J., Matsuya, Y., Bloom, S., Robbins, A., and Green, H., in *Growth Regulating Substances for Animal Cells in Culture* (edit. by Defendi, V., and Stoker, M.), 87 (Wistar Institute Press, Philadelphia, 1967).
[6] Holley, R. W., and Kiernan, J. A., *Proc. US Nat. Acad. Sci.*, **60**, 300 (1968).
[7] Dulbecco, R., *Nature*, **227**, 802 (1970).
[8] Clark, G. D., Stoker, M. G. P., Ludlow, A., and Thornton, M., *Nature*, **227**, 798 (1970).
[9] Jainchill, J., and Todaro, G. J., *Expt. Cell Res.*, **59**, 137 (1970).

[10] Temin, H. M., in *Growth Regulating Substances for Animal Cells in Culture* (edit. by Defendi, V., and Stoker, M.) (Wistar Institute Press, Philadelphia, 1967).
[11] Burk, R. R., *Nature*, **212**, 1261 (1966).
[12] Smith, H. S., Scher, C. D., and Todaro, G. J., *Virology*, **44**, 359 (1971).
[13] Aaronson, S. A., and Rowe, W. P., *Virology*, **42**, 9 (1970).
[14] Aaronson, S. A., Hartley, J. W., and Todaro, G. J., *Proc. US Nat. Acad. Sci.*, **64**, 87 (1969).
[15] Fisher, H. W., Puck, T. T., and Sato, G., *Proc. US Nat. Acad. Sci.*, **44**, 4 (1958).
[16] Todaro, G. J., and Green, H., *Proc. US Nat. Acad. Sci.*, **55**, 302 (1966).
[17] Pollack, R. E., Green, H., and Todaro, G. J., *Proc. US Nat. Acad. Sci.*, **60**, 126 (1968).

Incidence of Mycoplasma Infection in Guinea-pigs

THE isolation of a new mycoplasma species, *Mycoplasma caviae*, was recorded recently[1]. This species has been isolated from the nasopharynx and vagina of 10% of 232 guinea-pigs and is apparently non-pathogenic to these and other common laboratory animals. During this survey, no other known species of mycoplasma were isolated. (A strain of *M. caviae* G. 122 has been lodged in the National Collection of Type Cultures and has been given the reference number NC/TC 10126.)

Although we have failed to isolate other known species of mycoplasma from 232 guinea-pigs, it has been claimed that in Germany all guinea-pigs carry *M. pulmonis* in the vagina[2]. It is interesting to speculate why the incidence of infection in Germany should be so different to that in the United Kingdom. Further information on the incidence of mycoplasma infection of guinea-pigs in different countries would be most interesting.

AURIOL HILL

MRC Laboratory Animals Centre,
Medical Research Council Laboratories,
Woodmansterne Road, Carshalton, Surrey

Received June 17, 1971.

[1] Hill, A., *J. Gen. Microbiol.*, **65**, 109 (1971).
[2] Juhr, von, N. C., and Obi, S., *Z. Versuchstierk.*, **12**, 383 (1970).

Transplacental Transmission of Endogenous Interferon in Pregnant Mice inoculated with Influenza or Newcastle Disease Viruses

THE possibility of intrauterine infection transmitted from affected mother to foetus has been established by both clinical[7,11] and experimental[3,6,9,10] investigations. Inoculation of pregnant animals with pathogenic viruses often brings about foetal infection resulting in impaired intrauterine development. When non-pathogenic strains are used, however, pregnancy may proceed normally and result in the birth of healthy offspring[4]. It has been demonstrated repeatedly that viruses with low pathogenicity for animals are effective producers of endogenous interferon. Preliminary administration of such interferon inducers has been shown to promote non-specific protection in animals against inoculation of lethal viruses[5,8].

In our investigation we used influenza A1/3711 virus and Newcastle disease virus (NDV) which were non-pathogenic for mice for interferon induction. These viruses are known to stimulate active production of endogenous interferon in mice, resulting in development of short term insusceptibility to subsequent inoculation with lethal influenza infection[2]. The aim of our study was to test the possibility of transplacental transmission of endogenous interferon from mother to foetus with resulting activation in the offspring of non-specific immunity to infection with various viruses.

Production of endogenous interferon was induced on days 17–21 of pregnancy by intranasal instillation of 0.2 ml. of allantoic fluid containing influenza A1/3711 virus, or by intravenous administration of 1.0 ml. of NDV. Both preparations contained 10^6ID$_{50}$/0.2 ml.

At different intervals after A1/3711 or NDV inoculation, sera and suspensions of washed lung, placenta and foetal tissues were tested for virus and interferon content. Serum and lungs of newborn mice were tested for the same. Interferon was titrated on mouse fibroblast tissue cultures in terms of suppression of cytopathogenic action of vesicular stomatitis virus. Influenza A1 virus or NDV were inactivated in these specimens by preliminary treatment in acid medium (*p*H 2.0).

Newborn mice from experimental or control (uninoculated) mothers were challenged on different postnatal days intranasally (0.06 ml.) with mouse-pathogenic allantoic influenza AO/PR8 virus of 10^4ID$_{50}$/0.2 ml. activity. The inoculated sucklings were observed for 1 month.

Inoculation of influenza A1/3711 virus or NDV 3–4 days before birth did not affect the course of pregnancy, and the offspring survived the observation period.

Intranasal inoculation of pregnant mice with influenza A1 virus resulted in subclinical infection, accompanied by significant accumulation of interferon in the lungs from the first day after inoculation. Maximal values of interferon activity were obtained on days 3–4, when the level in the lungs was two to four times higher than that in bloodstream, uterus, placenta or foetuses (Table 1).

Table 1 Interferon Levels in Serum and Organs of Mice inoculated with Influenza A1/3711 or Newcastle Disease Viruses on Days 17–18 of Pregnancy

Interferon induced with virus	Specimens tested	Time of interferon titration (h after inoculation)				
		6–8	24	48	72	96
Influenza A1/3711	Serum*	—	2	8	32	16
	Lungs†	—	4	8	64	64
	Uterus	—	2	4	8	4
	Placenta	—	2	8	8	8
	Foetus	—	2	4	8	8
Newcastle disease	Serum	512	128	32	16	4
	Uterus	64	16	4	—	—
	Placenta	64	32	4	2	—
	Foetus	64	24	4	2	—

* Activity of interferon in U/ml. of undiluted serum.
† Activity of interferon was tested in 50% suspension of washed organs and then was calculated on wet weight.

In contrast to influenza virus, NDV induced production of maximal interferon levels in circulating blood as early as 6–8 h after inoculation. At this time, high levels were found in uterus, placenta and foetuses (64 U/ml.).

The results of this investigation demonstrate the possibility of antenatal (intrauterine) transmission of non-specific resistance from mother to foetus. A positive correlation was found between intensity of interferon production in pregnant mice by term and the grade of resistance of their offsprings against lethal influenza infection. Mice born of experimental mothers were fully protected against fatal doses of influenza AO virus if the time of birth corresponded to peak interferon production in the mother. When birth occurred during a period of minimal interferon production in the mother, the extent to which the offspring were protected against influenza AO/PR8 virus decreased considerably (Table 2).

Influenza virus AO/PR8 was 100% fatal in the offspring of uninoculated (control) mice, at any postnatal age. But the offspring of females subjected to interferon induction during pregnancy and challenged on the first, third or fifth days after birth with the same fatal doses of influenza AO virus, survived the whole observation period. Complete protection of off-

Table 2 Non-specific Protection of Newborn Mice Against Fatal Influenza Infection Due to Antepartum Interferon Induction in Pregnant Mice with Influenza A1/3711 Virus and Newcastle Disease Virus

Interferon induced with virus	Day of pregnancy when interferon production induced	Sucklings challenged on postnatal day	Interferon level in sucklings	No. of sucklings	No. dying	Death rate (%)	Protection factor* (%)
Influenza A1/3711	17–18	1	1 : 8	12	0	0	100
		3	1 : 2	15	0	0	100
		5	—	17	0	0	100
		7	—	14	4	29.0	71
		10	—	10	4	40.0	60
		13	—	12	9	75.0	25
		16	—	9	9	100	0
	20–21	1	1 : 1	15	1	7.0	93
		3	—	13	4	30.0	70
		5	—	9	5	55.0	45
		7	—	11	11	100	0
Newcastle disease	17–18	1	1 : 1	17	3	17.0	83
		2	—	18	7	39.0	61
		4	—	16	10	63.0	37
	20–21	1	1 : 16	18	0	0	100
		3	1 : 2	17	0	0	100
		5	—	19	6	31.0	69
		7	—	12	5	42.0	58
		13	—	15	13	87.0	13

* Compared with groups of control sucklings in which influenza AO virus caused 100% death, at any age after birth.

spring was demonstrated in animals born at the time of peak interferon production induced in mothers with A1/3711 virus or NDV.

When older sucklings (7–13 days after birth) were challenged, their protection against influenza virus AO/PR8 was found to have diminished, but still persisted at the 50–70% level, while death was delayed (for 3–4 days), compared with control sucklings.

Protection of newborn mice may be effected by relatively low concentrations of interferon, which cannot be detected by current methods of titration. This is confirmed by the results of titration of influenza AO/PR8 virus on the 3 day old offspring of mothers in whom interferon had been induced with NDV 1 day before term. Animals which received maternal interferon before birth proved to be fully resistant to AO virus. Interferon circulating in their bloodstream (1 : 2 titre) neutralized 10^5 infective doses of the challenging virus (Table 3). This is supported by tissue culture experiments[1].

Table 3 Titration of Influenza AO/PR8 Virus on 3 Day Old Offspring of Mice exposed to Interferon Induction with Newcastle Disease Virus inoculated on Days 18–19 of Pregnancy

Groups of sucklings	Challenged with dilutions of influenza AO/PR8 virus						
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Born of mothers exposed to interferon induction	18	17	14	10	8	6	
Died when resistance challenged	7	2	0	0	0	0	
Death rate (%)	39.0	12.0	0	0	0	0	
Born of control mothers	4	8	7	9	14	13	6
Died	4	8	7	9	10	4	0
Death rate (%)	100	100	100	100	71.0	31.0	0

These studies confirm the possibility of protecting mice antenatally against postnatal fatal influenza infection. Here, the leading part belongs to endogenous interferon, which penetrates the placenta and creates in the foetus a state of resistance against virus infection. The significance of maternal interferon in protection of newborn mice is supported by experiments in which normal newborn sucklings were inoculated with influenza A1/3711 virus at different postnatal days. No interferon could be detected in the lungs of mice inoculated within the fifth to seventh postnatal days. With age, however, the production of interferon increased.

B. M. KORSANTIYA
AL. A. SMORODINTSEV

All-Union Research Institute of Influenza,
Leningrad, and
Research Institute of Hygiene,
Tbilissi

Received August 4; revised November 23, 1970.

[1] Korsantiya, B., Gvozdilova, D., and Smorodintsev, Al., *Tsitologia*, **9**, 1304 (1967).
[2] Smorodintsev, Al., Gvozdilova, D., and Aksenov, O., in *Interferon*, 93 (1970).
[3] Soloviev, V., Khesin, Y., and Gutman, N., *Vopr. Virus.*, **1**, 60 (1970).
[4] Ferm, V., and Low, R., *J. Pathol. Bact.*, **89**, 295 (1965).
[5] Isaacs, A., and Hitchcook, G., *Lancet*, ii, 7141, 69 (1960).
[6] Middelkamp, I., Reed, C., and Patrizi, G., *Proc. Soc. Exp. Biol. Med.*, **125**, 757 (1967).
[7] Mitchell, I., and McCall, F., *Amer. J. Dis. Child.*, **106**, 207 (1963).
[8] Rytel, M., *J. Gen. Virol.*, **3**, 429 (1969).
[9] Soike, K., *J. Inf. Dis.*, **117**, 203 (1967).
[10] Takeyama, T., *Tohoku J. Exp. Med.*, **89**, 321 (1965).
[11] Witzleben, C., and Driskoll, S., *Pediatrics*, **36**, 192 (1965).

Genetic Recombination in Malaria Parasites

THE complexity of the life cycle of malaria parasites and the lack of suitable strain characters which could be used as genetic markers have made genetic studies of these organisms difficult. We have now available, however, a number of strains of *Plasmodium berghei* isolated from wild rodents and mosquitoes in Africa, which can be differentiated from one another by starch-gel electrophoresis of certain enzymes[1]. We have also derived parasite lines resistant to the drug pyrimethamine from some of these strains, and such resistant lines remain stable even in the absence of the drug. By making crosses between lines which differ in both enzyme type and drug sensitivity, we have been able to obtain parasites exhibiting recombinant characters.

The parasite lines selected for use are described here as lines A and C. Line A was derived from strain 17X of *P.b. yoelii* and is pyrimethamine resistant. The resistance was produced in a single step[2] by treating mice infected with strain 17X with pyrimethamine at doses of 50 mg/kg daily for 4 days. Line A, like strain 17X, contains the electrophoretic form of the enzyme glucose phosphate isomerase denoted GPI-1[1]. Line C was derived from strain 33X of *P.b. yoelii*, and is pyrimethamine-sensitive. In the 4 day suppressive test[3], it is more than fifty times as sensitive to pyrimethamine as line A. Line C contains the enzyme form GPI-2[1].

The sequence in which this work was conducted is presented in Fig. 1. Equal amounts of blood (0.3 ml.) were taken from two tree rats (*Grammomys surdaster*), one of which was infected with line A and the other with line C. The blood from each animal was mixed and injected intravenously into an uninfected tree rat. This produced an immediate patent parasitaemia in the recipient (113 A+C in Fig. 1). Mosquitoes (*Anopheles stephensi*) were fed immediately on this animal in the hope that gametes of each strain would mix. The resulting sporozoites were used to infect further tree rats from which ten C57 black mice (127 A+C in Fig. 1) were subsequently infected. Five of these mice were treated with subcutaneous doses of pyrimethamine[4] at 15 mg/kg daily for 4 days. All ten mice became infected and examination of the parasites on starch gels (Fig. 2) revealed the presence of both GPI-1 and GPI-2 in all animals.

To confirm that the GPI-2 parasites in the treated mice were drug-resistant, infected blood from these mice was diluted in serum Ringer[5] into aliquots containing an average of one parasitized cell. The aliquots were inoculated into individual mice, from which blood infections were established. Two GPI-1 and two GPI-2 lines were derived in this way, each of which was found to grow equally well when subjected to pyrimethamine treatment.

As controls the parent lines A and C were passaged through mosquitoes, tree rats and mice in the same way as the mixture. Line A parasites survived drug treatment, and line C parasites were eradicated. The work has been repeated several times with similar results; line C has been exposed to pyrimethamine at doses of 15 mg/kg in fifty mice, none of which has remained infected. We think it very unlikely, therefore, that mutation could account for the appearance of resistant GPI-2 parasites in the mixed infections.

Two further controls were set up to determine whether the recombinant parasites obtained were due to the phenomenon described by Yoeli *et al.*[4] as "synpholia". In synpholia, it is suggested that pyrimethamine resistance can be transferred from one strain to another when they grow up together in blood cells of the same host. In the first control, tree rat 113A+C (inoculated with equal numbers of blood parasites of each line) was kept alive for 7 days after mosquitoes had fed on it. Blood parasites from this animal were then used to infect ten mice, five of which were treated with pyrimethamine. In these mice, only GPI-1 parasites survived, but in the untreated mice both GPI-1 and GPI-2 parasites were present. In the second control, sporozoites of each parent line were pooled and inoculated into tree rats. After five days, blood parasites from these animals were used to infect ten mice, five of which were given pyrimethamine. In untreated animals, both GPI-1 and GPI-2 parasites were detected, while the animals given pyrimethamine contained only GPI-1 parasites after treatment.

We have been unable, therefore, to produce parasites exhibiting recombinant characters by simply mixing blood parasites or sporozoites of the parent lines. This does not disprove the phenomenon of synpholia, which may occur as a rare event detectable only when double selection pressure is applied[4], but suggests that it cannot account for our results.

We consider that these results are important in two respects. First, they show that a conventional genetic analysis of malaria parasites is possible. This should enable us to obtain much more precise information than has been available hitherto on the biological basis of such important characters as drug resistance and virulence. Second, they indicate that genetic recombination is continually taking place in natural populations of malaria parasites, which enables the rapid evolution of new strains and their spread in populations of animals and man. We propose to exploit the technique outlined here to investigate the nature of other types of drug resistance as well as enzyme polymorphism and virulence.

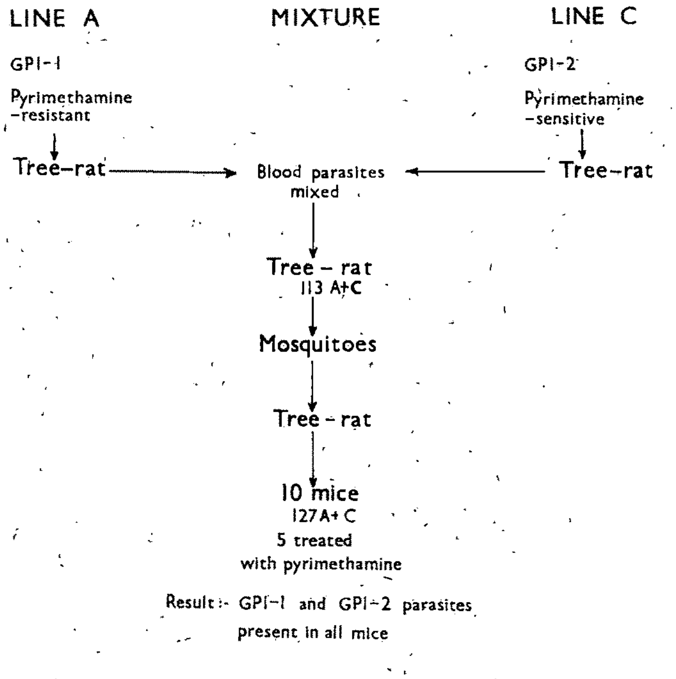


Fig. 1 Procedure adopted in producing recombinant parasites.

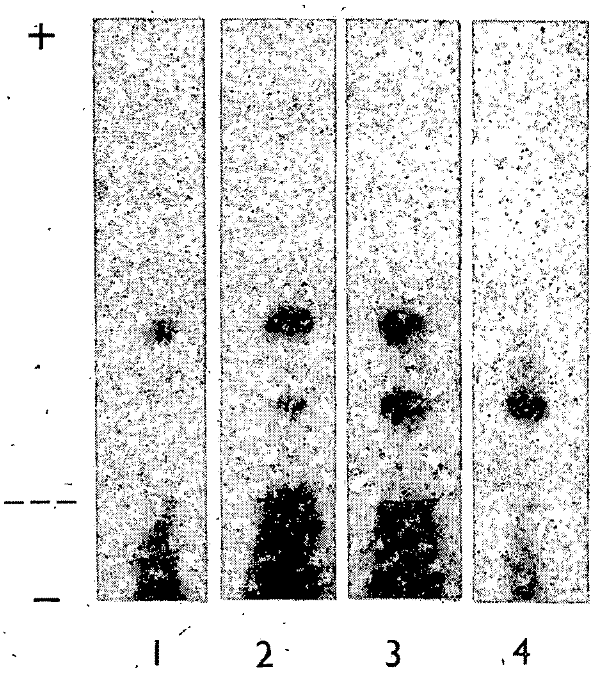


Fig. 2 Starch gel zymograms of glucose isomerase (GPI) in line A, line C, and mixtures of lines A and C. 1, Line A (GPI-1); 2, mixture following pyrimethamine treatment of mice 127A+C (showing GPI-1 and GPI-2); 3, mixture in untreated mice 127A+C (showing GPI-1 and GPI-2); 4, line C (GPI-2).

We thank Mr A. Sanderson for technical assistance. This work is supported by a grant from the Medical Research Council to Professor G. H. Beale.

D. WALLIKER
R. CARTER
S. MORGAN

Protozoan Genetics Unit,
Institute of Animal Genetics,
University of Edinburgh,
Edinburgh EH9 3JN

Received April 21; revised May 10, 1971.

[1] Carter, R., *Trans. Roy. Soc. Trop. Med. Hyg.*, **64**, 401 (1970).

[2] Diggens, S. M., Gutteridge, W. E., and Trigg, P. I., *Nature*, **228**, 579 (1970).

[3] Peters, W., *Exp. Parasitol.*, **17**, 80 (1965).

[4] Yoeli, M., Upmanis, R. S., and Most, H., *Parasitology*, **59**, 429 (1969).

[5] Warhurst, D. C., and Folwell, R. O., *Ann. Trop. Med. Parasitol.*, **62**, 349 (1968).

Frequency Analysis of the Thirst Control System

DRINKING is essential for the homeostasis of body fluids, and a theoretical structure which makes the principles and details of homeostasis explicit is provided by control theory. A control model of thirst has been described by Oatley[1,2] and simulated on a digital computer[3].

Responses to sinusoidally varying stimuli can give quantitative information about the structure of biological control systems[4,5], and we present here an analysis of drinking by rats in response to sinusoidal salt injections and compare it with the simulated drinking response of the model[3]. We show how frequency analysis applied to a system which has non-linear components (for example, a threshold), can indicate the relative importance of linear and non-linear components.

Sinusoidal infusions of sodium chloride were given at frequencies of 1, 2, 4 and 8 cycles per hour (c.p.h.) to sixteen albino rats chronically implanted with jugular cannulae made of silicone rubber tubing. The mean body weight of the rats was 387 g, and this value was also used for the simulation. The injection waveform was controlled by pulsing an infusion pump at intervals varying sinusoidally with time. The flow rate of hypertonic salt solution into the rat produced in this way approximated the equation:

$$A + A\cos\omega t \qquad (1)$$

where A is the mean rate, 0.0544 mequiv. of Na/min. The infusion varied between zero and $2A$ mequiv./min. The concentration of the salt was 3 M, and injections were continued until a sufficient number of cycles (a minimum of 4 at 1 c.p.h. and a maximum of 24 at 8 c.p.h.) had been accomplished. All rats drank water in response to at least one infusion frequency (each rat had one frequency per day) but clotting of cannulae sometimes occurred so that some rats had to be discarded. They were also relieved of further duty if signs of discomfort appeared during injection. Although we collected between eight and ten readings at each frequency, only three of the rats ran satisfactorily through all four.

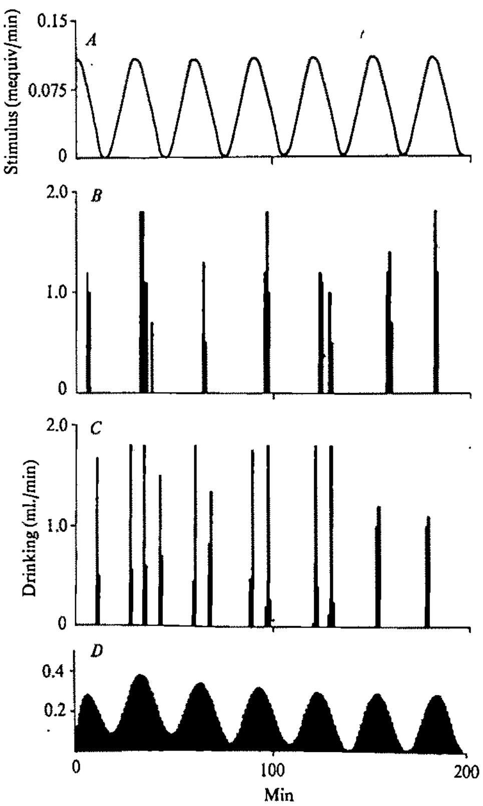


Fig. 1 Sinusoidal input infusion at 2 c.p.h., and histograms of drinking responses to it: (*A*) input infusion defined by equation (1); (*B*) typical experimental result showing one rat's drinking response; (*C*) computer simulation response run with threshold; (*D*) computer simulation response run without threshold. The histograms (*B*, *C* and *D*) represent drinking rate, with the height of each vertical bar showing the volume of water drunk in each 1 min period of the 200 min test. Note that the vertical scale for histogram (*D*) is exactly twice that of histograms (*B* and *C*).

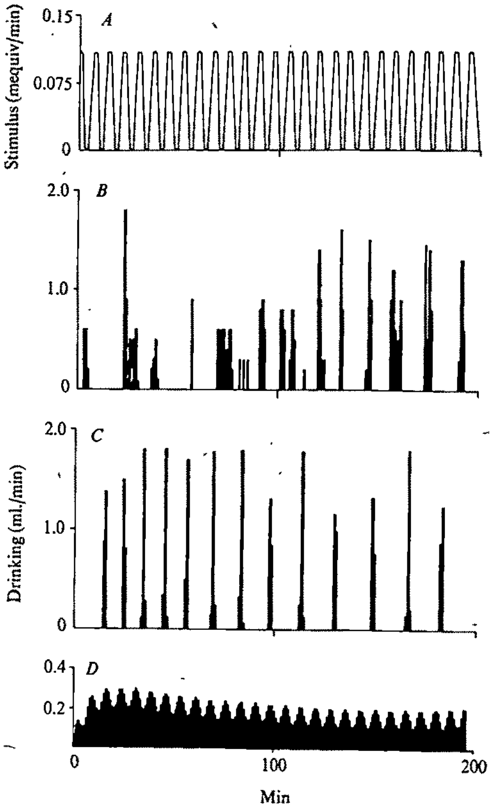


Fig. 2 Sinusoidal input infusion at 8 c.p.h. and histograms of drinking responses to it. Except for the higher frequency the description is as for Fig. 1.

Fig. 1*b* shows a typical response to a 2 c.p.h. stimulus, and Fig. 1*c* shows the computer simulation of this experiment. At 1 and 2 c.p.h., responses consistently showed the same dominant frequency as the input stimulus. In the 4 and 8 c.p.h. results, the pseudo-linear following of the input frequency that occurred at the lower frequencies failed to occur. Instead of transmitting the 4 and 8 c.p.h. signals, animals and simulation responded by an oscillation of drinking at a lower frequency (Fig. 2).

The principal non-linear component in the system is a threshold, which the deficit of body water must reach for drinking to be initiated. Toates and Oatley[3] reviewed experiments in which the value of the threshold has been measured, and took 0.7 ml./100 g body weight as a typical value, which also fitted experiments reported in that article, as well as the data reported here. When the threshold was removed from the simulation, the computer followed input frequencies throughout the range tested, that is, 0.16 to 30 c.p.h. This response, without the threshold, was totally different from that of rats or of the complete simulation (Figs. 1*d* and 2*d*).

Frequency response methods are most useful for linear systems, but an output from non-linear systems can be approximated by a sinusoid at the input frequency and of an appropriate amplitude and phase. A computer program was written to perform the fitting procedure. It searched each combination of amplitude and phase to find the best sinusoidal fit to the drinking data, using as a criterion the minimum value of the square of the error at each point summed over the time observed. Functions were fitted after discarding the first 30 min of the drinking response, to exclude the major part of transient effects.

The open circles in Fig. 3 show the gain and phase of the sinusoids that best fitted the response of the model without the threshold, measured at several frequencies. This response approached a maximum phase shift of −90°, characteristic of a

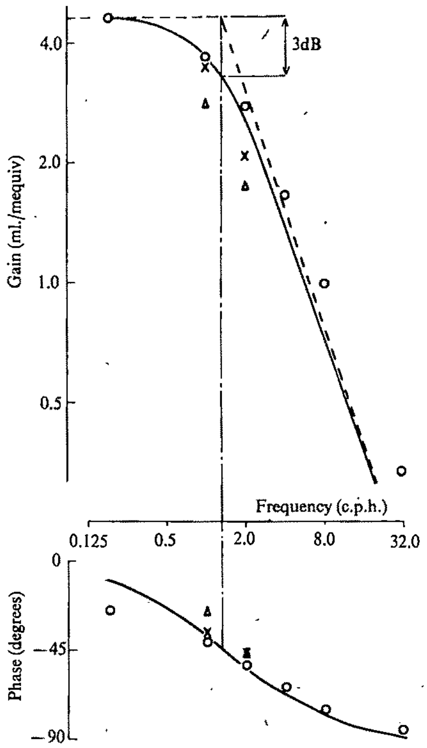


Fig. 3 Gain (expressed as drinking rate in ml./min, divided by input infusion rate in mequiv. of Na/min) and phase plots of frequency responses. The solid lines represent the function $K/1+\tau s$, with $\tau=0.12$ h, the lines - - - are the low and high frequency asymptotes to this function, and the line —.— indicates their intersection at a frequency equal to $1/2\pi\tau$. Best fitting sinusoidal frequencies for experimental data and computer runs are shown by: (△) mean experimental results: (×) computer simulation, with threshold; (○) computer simulation without threshold.

first order system described by the transfer function

$$H(s) = \frac{K}{1+\tau s} \qquad (2)$$

where K is the gain constant, τ the time constant, and s the Laplace operator.

McFarland and Budgell[6] found a transfer function for key pecking by thirsty birds in response to modulations of ambient temperature which was of the same kind as this drinking response to salt injections, though it had a slightly smaller time constant.

The phase lag of a first order system is given by: arctan $\tau\omega$, where ω is the radian frequency per unit time. The mean value of τ calculated from the phase results of the simulation without the threshold was 0.12 h (7.2 min) and with this value, the gain and phase given by equation (2) are shown as solid lines in Fig. 3.

Though the simulation contains non-linearities other than the threshold, Fig. 3 shows the gain of the simulation without threshold to be well fitted by this first order characteristic; and the phase also, except at the lowest frequencies. The best fitting sinusoidal responses for the experimental results and for the simulation with threshold at 1 and 2 c.p.h. are also plotted on Fig. 3 and both gain and phase approach that of the first order approximation. Since frequency following was abolished at 4 and 8 c.p.h. no points could be plotted for these frequencies.

An overall transfer function such as equation (2), can give some idea of the basic structure of a biological system. In this case the first order function arises because rates of flow into and out of body fluid compartments (hypertonic saline causes flow of water out of the cellular compartment) are integrated, and drinking acts as a feedback signal, restoring deficits. An integrator with feedback around it constitutes just such a first order system as described by equation (2), but frequency analysis applied to the real system and to the model[3] indicates that characterization of the system as first order with $\tau=0.12$ h is only a superficial description of the system. It gives no clue as to how physiological processes make up the total system. In comparison with the three components (summing junction, integrator and constant coefficient) required to represent the first order transfer function (2), the block diagram of our simulation[3] which contains a minimal representation of the physiological events that control thirst and body water, has eighty-seven such elements. Clearly there are limits to the adequacy with which transfer functions derived from overall linear frequency analysis can represent what is going on in biological control systems.

In this analysis, however, frequency methods have first confirmed the adequacy of the model[3] (built on a physiological understanding of the component processes and with no arbitrary components or *ad hoc* alteration of parameters) to represent the real system and to generate an overall drinking response comparable with that of a real animal; and, second, these methods establish the relative importance of linear and non-linear features of the system. The time constant (0.12 h) of the system estimated from this small signal analysis differs from the estimate of 0.5 h, in response to a single large transient injection of 2.73 mequiv. of sodium[3]. Thus even the pseudo-linear behaviour at lower frequencies provides evidence for non-linearity, which for large signals is due to non-linear inhibition of drinking by large volumes of water in the stomach.

The claim by Corbit[6] that there is no threshold for drinking in response to hypertonic NaCl seems to be refuted, for in these experiments the threshold dominated the form of the response. Apart from making drinking intermittent at all frequencies (see Figs. 1 and 2, *b* and *c*) a threshold with hysteresis[3] was able to account for the fact that drinking of neither the model nor the rat was able to follow the input at higher frequencies. At 4 and 8 c.p.h. the integral of the rate of salt injected in any one cycle was not sufficient to exceed the threshold. Instead drinking in response to these frequencies was similar to that for a steady infusion at the same mean rate; it exhibited a relaxation oscillation as a function of the mean infusion rate and the threshold value. For the simulation with the threshold the frequency of the relaxation oscillation was approximately 3.5 c.p.h. (Fig. 2). Thus, although with infusions of 1 and 2 c.p.h. no rat ever missed drinking in response to any cycle of the input, at 4 and 8 c.p.h., cycles were missed. It is not easy to say exactly at what frequency the drinking response oscillates for these inputs (Fig. 2*b*); it was less than the driving frequency, and of the order of 3.5 c.p.h. as shown by the model.

The role of the threshold for drinking may be as part of a mechanism which allows the animal to distribute its time between drinking and other systems that can control behaviour.

We thank D. A. Tonge for help in implanting cannulae, and the MRC for making this assistance possible.

KEITH OATLEY
F. M. TOATES

Laboratory of Experimental Psychology,
University of Sussex,
Brighton

Received January 4; revised April 5, 1971.

[1] Oatley, K., *Med. Biol. Eng.*, **5**, 225 (1967).
[2] Oatley, K., *Nature*, **225**, 797 (1970).
[3] Toates, F. M., and Oatley, K., *Med. Biol. Eng.*, **8**, 71 (1970).
[4] Milsum, J. H., *Biological Control Systems Analysis* (McGraw-Hill, New York, 1966).
[5] Stark, L., *Neurological Control Systems* (Plenum Press, New York, 1968).
[6] McFarland, D. J., and Budgell, P. W., *Nature*, **226**, 966 (1970).
[7] Corbit, J. D., *J. Comp. Physiol. Psychol.*, **67**, 3 (1969).

One, Two or Three Connecting Links between Triassic and Jurassic Ammonoids?

It has long been recognized that the Jurassic and Cretaceous ammonoids are very different from those of the Triassic and it is generally concluded that the whole order Ammonoidea nearly became extinct at the Triassic–Jurassic boundary. More than a century ago Suess[1] recognized that one group, the phylloceratids, was common to both systems. Most authors[2–5] have accepted this view and have further concluded that the phylloceratids provide the only link between the faunas of the Triassic and the Jurassic. In taxonomic terms this is expressed by Arkell *et al.*[5] as follows: Jurassic and Cretaceous ammonoids comprise suborders Phylloceratina, Lytoceratina and Ammonitina. Of these only Phylloceratina extend back into the Triassic. In the Lower Triassic, Phylloceratina merge with Ceratitina, the dominant Triassic group, which became extinct at the end of the Triassic. It was the opinion of Arkell *et al.* that both Lytoceratina and Ammonitina were derived from Phylloceratina.

Wiedmann[6] has now brought forward a very different interpretation, indicating that both Lytoceratina and Ammonitina lived in Triassic times and that the faunal break is much less profound than supposed by all previous workers, with three groups (Phylloceratina, Ammonitina and Lytoceratina) crossing the boundary. Schindewolf, Shevyrev and Teichert, as mentioned below, also agree that the Lytoceratina extend back to the Triassic. Wiedmann's recognition of Ammonitina in the Triassic is wholly novel.

I suggest that there is still no satisfactory evidence that Lytoceratina and Ammonitina are represented in the Triassic faunas and that Wiedmann's conclusions are not justified.

The recognition of Lytoceratina in the Triassic is based on one specimen, named *Trachyphyllites costatus* by Arthaber[7]. To Arthaber, *Trachyphyllites* was a subgenus of *Monophyllites* (Phylloceratina). He noted resemblances between *Trachyphyllites* and Lower Jurassic Lytoceratina but attributed them to convergence. Later workers[2,8–12] have considered these resemblances to be of phylogenetic significance. There is now general agreement, which I do not dispute, that *Trachyphyllites* is a representative of Lytoceratina, but is it a Triassic ammonoid? N. J. Silberling has drawn my attention to good reasons for suggesting that it may be Jurassic, not Triassic. The unique specimen of *Trachyphyllites costatus* was collected by a Dutch expedition from a limestone block in Oë Bihati, about 2 km east of Baun in Indonesian Timor. Collections were made from numerous blocks in this river. Most were Triassic ammonoids but some of Jurassic age were also obtained. Individual blocks were designated by numbers and Greek letters. Their exact location in the river bed is shown on a map published by Burck[13]. Arthaber[7] gives Bihati lower case theta (ϑ) as the locality for *T. costatus* in his text, but as lower case delta (δ) on the plate description. With the exception of *Rhacophyllites fallax* Arthaber, which has the same ambiguous locality data, no other ammonoids have been recorded from block δ. Arthaber[7] wrote that Diener noted the occurrence of both Triassic and Jurassic ammonoids from Bihati δ. But in Diener's report[14] a mixing of Triassic and Jurassic ammonoids was recorded only from Bihati upper case theta (Θ). Diener makes no reference to Bihati δ. Burck's map shows both block δ and block Θ, but no ϑ. This suggests that both Θ and ϑ refer to the same block. There seems to be at least an even chance, therefore, that *Trachyphyllites costatus* came from a block (Θ = ϑ) which contained both Triassic and Jurassic ammonoids. The exact age of *Trachyphyllites* can only be considered uncertain. Nothing like *Trachyphyllites* has been found in Triassic rocks elsewhere, but comparable ammonites occur in the Lower Jurassic according to Spath[2] and Basse[8]. Extending the range of Lytoceratina into the Triassic on this occurrence alone seems unwarranted. *Trachyphyllites* is probably what it looks like, a Jurassic ammonite.

The alleged extension of Ammonitina back into the Triassic must now be considered. Wiedmann[6] describes a new genus, *Phyllytoceras*, based on Upper Triassic specimens from Iran and Austria. *Phyllytoceras* is classified as a member of a new family (Phyllytoceratidae) of Psilocerataceae (Ammonitina) by Wiedmann. He regards *Phyllytoceras* as the ancestor of *Psiloceras*, of the basal Jurassic Hettangian Stage; and possibly also the ancestor of Lytoceratina. *Phyllytoceras intermedium* Wiedmann, the only species known, is a smooth serpenticone. The suture line is ammonitic, with two lateral lobes, a dependent suspensive lobe, a non-lituid internal lobe, and a deep incision on the outer side of the external saddle. I submit that all these characters are found in the Gymnitidae, a family of Triassic Ceratitina. Many Gymnitidae are smooth serpenticones. They have ammonitic suture lines, with two lateral lobes, a dependent suspensive lobe and commonly a deep incision on the outer side of the external saddle[12]. Gymnitidae attain a large size and have a more deeply incised suture line than *Phyllytoceras*. *Phyllytoceras* is known only from small specimens, however, the largest being 4.5 mm in diameter, so the differences in degree of incision are probably not significant. The internal lobe of *Japonites* (generally considered a gymnitid) is known to be non-lituid[12], as in *Phyllytoceras*. Non-lituid internal lobes have also been illustrated for *Tropigymnites planorbis* (Hauer)[15] and *Arctogymnites sonini* Popov[16]. These two, if not Gymnitidae, are not far removed from members of the family. Wiedmann seems to be unaware that Gymnitidae have non-lituid internal lobes because he indicates that a lituid internal lobe is diagnostic of Ceratitina[17]. Also, he makes no comparison between the suture lines of *Phyllytoceras* and Gymnitidae. The non-lituid internal lobe of *Phyllytoceras* evidently influenced Wiedman in his decision to regard it as a representative of Ammonitina, but clearly, this character is not decisive. Wiedmann[6] has exposed the primary suture line of *Phyllytoceras*, showing it to have four lobes. The primary suture line of *Gymnites* and allied genera has not yet been described so the significance of this character of *Phyllytoceras* cannot be assessed at present. Most Triassic Ceratitina, however, have four-lobed primary suture lines[17]. Psilocerataceae, and most, if not all, other Ammonitina have five-lobed primary suture lines[17]. The four-lobed primary suture line of *Phyllytoceras*, therefore, in no way supports its assignment to Ammonitina. There seems to be no doubt that *Phyllytoceras* is a member of Gymnitidae, possibly an immature representative of *Gymnites* itself. Suture lines of Psilocerataceae[5,6,18] do not closely resemble those of *Phyllytoceras* and *Gymnites*. All have dependent suspensive lobes but the Psilocerataceae lack the deep incision in the external saddle that gives character to the suture lines of *Gymnites* and *Phyllytoceras*.

I conclude that there is no good evidence that Lytoceratina and Ammonitina are represented in the Triassic ammonoid fauna. The Triassic–Jurassic faunal break is as profound as believed by Arkell *et al.*[5] and there is still only one group, the phylloceratids, known to straddle the boundary.

E. T. TOZER

Geological Survey of Canada,
Department of Energy, Mines and Resources,
601 Booth Street,
Ottawa, Ontario

Received March 22, 1971.

[1] Suess, E., *Sitzungber. Akad. Wiss. Math.-Naturwiss. Kl.*, **52** (I–II), 1, 71 (1865).
[2] Spath, L. F., *Cat. Fossil Cephalopoda Brit. Mus.* (*NH*), Part IV (1934).
[3] Arkell, W. J., *J. Paleontol.*, **24**, 354 (1950).
[4] Donovan, D. T., *Biol. Rev. Cambridge Phil. Soc.*, **39**, 259 (1964).
[5] Arkell, W. J., *et al.*, *Treatise on Invertebrate Paleontology*, Part L (Geol. Soc. Amer., and Univ. Kansas Press, 1957).
[6] Wiedmann, J., *Eclogae Geol. Helv.*, **63**, 923 (1970).

[7] Arthaber, G. v., *Jb. Mijnw. Ned. Oost.-Ind.*, **55**, 1 (1927).
[8] Basse, E., in *Traité de Paléontologie* (edit. by Piveteau, J.), 2, 461 (Masson, Paris, 1952).
[9] Schindewolf, O. H., *Akad. Wiss. Lit. Mainz, Abh. Math.- Naturwiss. Kl.*, 1960, 635 (1961).
[10] Teichert, C., *Univ. Kansas Dept. Geol. Spec. Publ.* 2, 162 (1967).
[11] Wiedmann, J., *Neues Jb. Geol. Paläontol. Abh.*, **125**, 49 (1966).
[12] Shevyrev, A. A., *Tr. Paleont. Inst. Akad. Nauk. SSSR*, **119** (1968).
[13] Burck, H. D. M., *Jb. Mijnw. Ned. Oost.-Ind.*, **49**, 1 (1923).
[14] Diener, C., *Jaarb. Mijnw. Ned. Oost.-Ind.*, **49**, 73 (1923).
[15] Hauer, F. v., *Denkschr. Akad. Wiss. Wien*, **63**, 237 (1896).
[16] Popov, Yu. N., *Tr. Nauch.-Issled. Inst. Geol. Arktiki*, **79** (1961).
[17] Kullmann, J., and Wiedmann, J., *Univ. Kansas Paleontol. Contr.*, 47 (1970).
[18] Frebold, H., *Geol. Survey Canada Bull.*, **158** (1967).

Cranium of *Plesiadapis*

THE reconstruction by Szalay of the only known skull (CR 125, Muséum National d'Histoire Naturelle, Paris) of the Palaeocene primate *Plesiadapis* was presented[1] as differing from previous reconstructions[2-4] in the occlusal relationship of the enlarged procumbent incisors and in the relationship of the bones of the snout.

Szalay's principal contribution was his supposedly new location posteriorly of the point of occlusion of the lower incisors with the uppers. The incisor relationship illustrated by Szalay is not new, however; it is precisely that illustrated in the original reconstruction of this skull by Simons[2] (Fig. 1). Szalay refers to this 1960 reconstruction but does not discuss it, preferring to address his comments to a derived illustration which accompanied a popular article written later by Simons[3].

Reconstruction of the snout of this animal by Russell[4] was prefaced by the remark "L'écrasement subi a rendu difficile la détermination de la limite postérieure et dorsale des prémaxillaires". This remains true. Russell's discussion, together with Szalay's Fig. 2, support the original interpretation of bone relationships of the snout by Russell[4,5], not that newly presented by Szalay. A premaxillary-frontal contact is present in living mammals with procumbent *Plesiadapsis*-like incisors (compare the marsupial *Dactylopsila*, the primate *Daubentonia*, rodents and lagomorphs) and is probably related to the function of such incisors. Until better preserved material is discovered, Russell's original interpretation that the premaxilla makes contact with the frontal in *Plesiadapis* remains the most reasonable alternative.

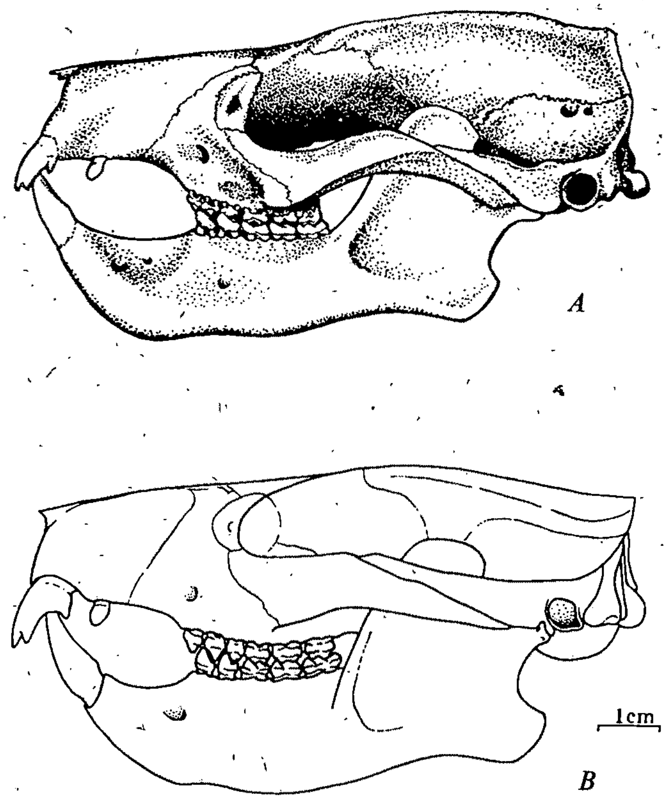


Fig. 1 Reconstructions of lateral view of skull of *Plesiadapis tricuspidens*. *A*, Original reconstruction by Simons (ref. 2); *B*, reconstruction by Szalay (ref. 1).

Szalay also criticizes the statement by Simons and Russell[6] that "only in Cheirogaleinae, among living and fossil Lemuriformes, is the os planum present" and cites Forsyth Major[7] as evidence for disagreeing with them. In fact, what Forsyth Major said was: "Amongst the Malagasy Lemurs a fairly large os planum is present in all the species of *Microcebus*. In the other genera the planum becomes fused with the palatal at a very early date". Thus Forsyth Major's evidence agrees with Simons and Russell's statement, not with Szalay's. A distinct os planum is present in mature skulls of Lemuriformes only in the Cheirogaleinae (including *Microcebus*) (see ref. 8 for a more recent discussion).

Szalay objects to the use of the presence of an os planum in the orbital wall as a diagnostic feature of the order Primates. As Le Gros Clark outlined[9], and Martin restated[10], any definition of primates must be based on a suite of characters, a "total morphological pattern" or a "total functional pattern", not all of which will be present in all forms included in the order. Absence of an os planum in certain early Tertiary primates and some living lemurs does not preclude using the presence of an os planum as one of a suite of characters defining the order primates; an os planum is present in most primates.

In short, Szalay's recent article contributes no addition to knowledge of the cranial or dental anatomy of *Plesiadapis*.

PHILIP D. GINGERICH

Department of Geology and Geophysics, and
Peabody Museum of Natural History,
Yale University,
New Haven, Connecticut 06520

Received April 29, 1971.

[1] Szalay, F. S., *Nature*, **230**, 324 (1971).
[2] Simons, E. L., *Amer. Sci.*, **48**, 179 (1960).
[3] Simons, E. L., *Sci. Amer.*, **211**, 51 (1964).
[4] Russell, D. E., *Mém. Mus. Nat. Hist. Nat.*, C, **13**, 1 (1964).
[5] Russell, D. E., *Bull. Soc. Géol. Fr.*, ser. 7, **1**, 312 (1959).
[6] Simons, E. L., and Russell, D. E., *Breviora*, **127**, 1 (1960).
[7] Forsyth Major, C. I., *Proc. Zool. Soc. Lond.*, **1**, 129 (1901).
[8] Charles-Dominique, P., and Martin, R. D., *Nature*, **227**, 257 (1970).
[9] Le Gros Clark, W. E., *The Antecedents of Man* (Edinburgh University Press, 1962).
[10] Martin, R. D., *Man*, **3**, 377 (1968).

Ethmoid Component in the Orbit of Primates

SZALAY[1] has attributed to Forsyth Major[2] the statement that the ethmoid bone appears in the medial orbital wall of many Madagascar prosimians. But in fact, an orbital exposure (os planum) of the ethmoid is known only in *Cheirogaleus* and *Microcebus* among Lemuriformes. As Jones[3] and Kollman[4] have demonstrated, the element which Forsyth Major mistook for an os planum is the pre-nasopalatine part of the palatine bone.

The ethmoid of mammals is a cartilage-replacement bone which forms in the cartilaginous walls of the embryonic nasal chamber. In most non-primates, the ethmoid is covered by membrane bones laid down by the periorbita that lines the eye socket. If the orbits are very large or very close together, the periorbita is pressed against the nasal capsule in the

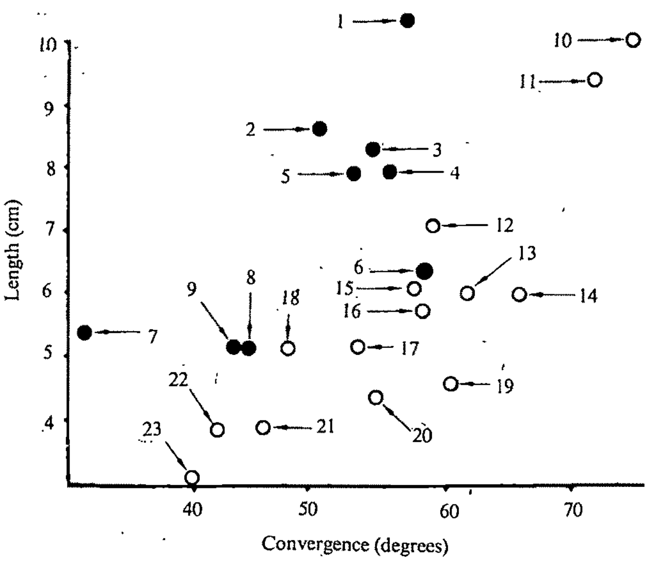


Fig. 1 Sample means of orbital convergence and prosthion-inion length in anthropoid and prosimian primates. "Convergence" is defined as the dihedral angle between the mid-sagittal plane and the plane of the medial orbital margin. Open circles represent species with an os planum in the medial orbital wall. Numerical designations (and sample sizes) of sampled species: 1, *Indri indri* (n=6); 2, *Lemur macaco* (6); 3, *Lemur catta* (6); 4, *Propithecus verrauxi* (6); 5, *Daubentonia madagascariensis* (2); 6, *Hapalemur griseus* (8); 7, *Phaner furcifer* (2); 8, *Lepilemur mustelinus* (6); 9, *Avahi laniger* (4); 10, *Cercopithecus nictitans* (6); 11, *Cebus apella* (6); 12, *Galago crassicaudatus* (6); 13, *Aotus trivirgatus* (6); 14, *Callicebus moloch* (24), 15, *Perodicticus potto* (6); 16, *Nycticebus coucang* (7); 17, *Nycticebus pygmaeus* (5); 18, *Cheirogaleus major* (4); 19, *Loris tardigradus* (6); 20, *Callithrix jacchus* (6); 21, *Tarsius syrichta* (6); 22, *Galago senegalensis* (6); 23, *Microcebus murinus* (6). Data from ref. 7.

embryo, and the ethmoid appears in the medial orbital wall of the adult.

Primates with an os planum are either small animals with relatively large eyes (as a result of negative allometry), or animals with pronounced orbital convergence, or both (Fig. 1). With increase in body size and consequent decrease in relative eye size, orbit and nasal fossa "compete" less for the available cranial volume, and the ethmoid may disappear again beneath laminae of the periorbital membrane bones. This sometimes happens in large gorillas[5], and may have occurred in the evolution of larger Madagascar primates from *Microcebus*-sized ancestors. If the last common ancestor of the extant primates was a small animal with large and convergent orbits, it probably had an ethmoid component in the medial orbital wall. It seems as likely that *Daubentonia*, *Phaner*, the lemurines and the indriids have lost the os planum independently, as that *Microcebus*, *Cheirogaleus*, *Tarsius*, the Lorisiformes and the anthropoids have acquired it independently. Although *Necrolemur* is said to lack an os planum, the medial orbital wall of *N. antiquus* as reconstructed by Simons and Russell[6] strongly resembles that of an adult *Microcebus* after the disappearance of the frontoethmoidal suture[4]; a distinct os planum may well have been present in juvenile *Necrolemur*.

MATT CARTMILL

Departments of Anatomy and Sociology–Anthropology,
Duke University,
Durham, North Carolina 27706

[1] Szalay, F. S., *Nature*, 230, 324 (1971).

[2] Forsyth Major, C. I., *Proc. Zool. Soc. Lond.*, 1, 9 (1901).

[3] Jones, F. W., *Proc. Zool. Soc. Lond.*, 1, 323 (1917).

[4] Kollman, M., *Mém. Soc. Linn. Normandie* (Zool.), NS 1, 1 (1925).

[5] Bolk, L., *Verh. K. Akad. Wet.*, Amsterdam, 2 sectie, **20** (5), 1 (1919).

[6] Simons, E. L., and Russell, D. E., *Breviora*, 127 (1960).

[7] Cartmill, M., thesis, Univ. Chicago (1970).

Is Vertesszöllös II an Occipital of European *Homo erectus*?

THE taxonomy of the two human fossils discovered at the Intermindel occupation site of Vertesszöllös in western Hungary has been a topic of considerable interest. The fragments of a deciduous tooth representing the first individual (V1) were judged by Thoma to be indistinguishable from deciduous teeth from Choukoutien[1], but he later concluded that the second individual (V2), represented by an occipital fragment, is a member of the taxon *Homo sapiens*[2,3]. He places V2 on the lineage leading to Swanscombe, in preference to separate lineage leading to the neandertals.

The "chopper-chopping tool" industries at Vertesszöllös and at Choukoutien are quite similar[4], which leads to the somewhat bizarre conclusion that two different hominid species are associated with a chopper-chopping tool industry at Choukoutien (*Homo erectus*) and at Vertesszöllös (*Homo sapiens*), and the same two species are associated with a hand axe industry at Ternifine (*Homo erectus*) and Swanscombe (*Homo sapiens*). Because the industry at Vertesszöllös is, for all intents and purposes, the same as that at Choukoutien where *Homo erectus* material from the same time period has been found[5], and because Thoma convincingly demonstrated that V1 cannot be distinguished from this *Homo erectus* sample[1], it is of some interest to examine his basis for placing V2 in *Homo sapiens*[2,6].

In his most recent analysis, Thoma concludes[2] that "the majority of gross morphological features of Vsz. 2 is Archanthropic in character". Most of these features are characteristic of *Homo erectus*[7,8], and measurements of thickness are completely within the *Homo erectus* range, usually falling between the values for the material from Peking and Java. The inion angle of the occipital is quite low. The nuchal torus is heavy, and extends laterally across the entire bone in the fashion of almost every other *Homo erectus* specimen, and unlike australopithecines, neandertals, modern men, and the other early European specimens—Swanscombe and Steinheim. The torus is quite high. The inion is considerably higher than the internal occipital protuberance which indicates the extent of the nuchal musculature, and suggests that the individual was large and probably male. The total morphological pattern is far more similar to a large *Homo erectus* than it is to a large neandertal. The two other early European crania are later, and are possibly female. Comparisons are therefore misleading.

In contrast, there are few reasons for placing the occipital in *Homo sapiens*, Thoma's *Paläanthropus* grade. Thoma claims only two: a high and fairly curved profile of the upper part of the occipital squama projected in the sagittal plane, and a cranial capacity significantly greater than *Homo erectus*. The sagittal curvature of the upper part of the squama is indicated by the index of the lambda-inion arc to the lambda-inion chord. In V2 this index is 108.2; a sample of nine *Homo erectus* specimens average 105 and range from 102.3 to 108.4 (s.d. = 1.8).

The high profile of the upper part of the squama is misleading, for the angle at the inion is not particularly great. Its value, 103°, is equal to or less than those of almost every occipital in the Peking group, and is considerably smaller than the angle in Swanscombe (118°), a more recent European specimen. The upper squama matches Peking crania such as 10 quite well in sagittal profile, except for the fact that it extends more anteriorly, and consequently is higher. There are, however, wormian bones in the vicinity of lambda which make it impossible to determine exactly where lambda is. Thoma chose the most anterior point, whereas Martin suggests[9] the point where lines smoothly approximating the course of the lamboidal suture cross each other. This point is more posterior, and consequently lower, in the V2 occipital. Use of the proper point would diminish the two chords which now fall outside the *Homo erectus* range by being too large, lambda-inion and lambda-opisthion.

Table 1 Comparison of Regression Parameters, Fit and Correlation for Lambda-Opisthion Chord and Cranial Capacity

		Slope	Intercept	Correlation	Percentage error of regression fit	Sample size
Homo erectus	(1)	23.77	−1,052.4	0.95	3.6	5
	(2)	22.30	−910.7	0.89	4.1	7
Neandertals		30.36	−1,344.3	0.82	6.4	14
Homo sapiens		25.71	−1,047.7	0.83	5.8	200

Table 2 Calculation of *Homo erectus* Cranial Capacities by Regression with the Lambda-Opisthion

Specimen		Lambda-opisthion chord (mm)	Actual cranial capacity (cm^3)	Calculated cranial capacity by regression		
				Wolpoff (n=5)	Wolpoff (n=7)	Thoma
Java	(1)	78*	850		822	877
	2	75	755	731	753	797
	4	78	750	802	822	877
	6	81*	975		891	957
Peking	3	84	915	944	960	1,037
	11	86	1,015	992	1,006	1,090
	12	87	1,030	1,016	1,029	1,117
Average absolute difference				32	37	68
Percentage difference				4%	4%	8%
Calculated Vertesszöllös capacity				1,325	1,328	1,463

* Estimated.

Thoma estimates the cranial capacity of V2 by means of a regression equation[2,6] which relates the lambda-opisthion chord to cranial capacity. Of the twelve specimens used by him, only five were *Homo erectus*, and with these he mixed five Solo crania and the Swanscombe and Broken Hill specimens. He conservatively uses the lower 95% confidence limit and concludes that the capacity must be greater than 1,400 cm^3, using his lambda-opisthion measure of 102 mm, or greater than 1,350 cm, using Tobias's measure of 100 mm.

I have calculated separate regressions for *Homo erectus*, neandertals, and 200 *Homo sapiens* specimens chosen at random from publications in *Biometrika*. Of the seven available specimens of *Homo erectus*, the positions of opisthion were uncertain in Java 1 and 6 and have been estimated in the literature. Regressions were calculated for the *Homo erectus* sample both with and without these specimens. The equations and correlations are given in Table 1.

The correlation for the regression using the small (unestimated) *Homo erectus* sample alone is greater than Thoma's correlation of 0.93 for his entire sample. The correlation value for the neandertals is lower, but this calculation used all of the published neandertal measurements (n=14), instead of only a few (n=6).

Table 2 indicates a test of the regressions by predicting the known cranial capacities in the *Homo erectus* sample. The percentage error for *Homo erectus* both with and without the two questionable specimens is close to 4%, but the percentage error of Thoma's equation when used to predict capacities only for *Homo erectus* is twice as great. The percentage error of Thoma's equation used to predict only the five specimens for which opisthion is known is greater still; almost 10%.

The archaeological evidence, the morphology of VI from the same site and by Thoma's admission most features of V2 suggest that the specimen is *Homo erectus*. It thus seems reasonable to use a regression based on *Homo erectus* specimens to estimate its cranial capacity. The regression for either sample gives almost identical estimations for V2, using Tobias's value of 100 mm for the lambda-opisthion chord. Because the actual value of the chord may be less, the estimation of about 1,325 cm^3 is a maximum.

Is the capacity of V2 large enough to be outside of the expected range of *Homo erectus* variation? The 95% range cannot be used because it is too large to be meaningful (±345 cm^3). The estimate is only 100 cm greater than the Peking 10 capacity. A *t* test comparing V2 with a sample of cranial capacities[10] of twelve *Homo erectus*, excluding the V2 value itself, indicates that V2 is not significantly different from *Homo erectus* on the 5% level. Along with all of the Peking crania, the capacity is, of course, within the range of variation for *Homo sapiens*.

In sum, it seems likely that V2 is the occipital of a *Homo erectus*. V2s similarity to the Swanscombe material is not surprising in view of the strong possibility that the English cranium is a member of the same lineage somewhat later in time.

M. H. WOLPOFF

Department of Anthropology,
University of Michigan,
Ann Arbor,
Michigan 48104

Received March 15; revised May 4, 1971.

[1] Thoma, A., *Z. Morphol. Anthropol.*, **58**, 152 (1967).
[2] Thoma, A., *Z. Morphol. Anthropol.*, **60**, 229 (1969).
[3] Thoma, A., *Yb. Phys. Anthropol.*, **13**, 137 (1965).
[4] Kretzoi, M., and Vertes, L., *Curr. Anthropol.*, **6**, 74 (1965).
[5] Howells, W. W., *Sci. Amer.*, **215** (5), 46 (1966).
[6] Thoma, A., *L'Anthropologie, Paris*, **70**, 495 (1966).
[7] Weidenreich, F., *Bull. Geol. Soc. China*, **19**, 479 (1940).
[8] Weidenreich, F., *Anthrop. Pap. Amer. Mus. Nat. Hist.*, 40 (1945).
[9] Martin, R., *Lehrbuch der Anthropologie*, vol. 2, *Krainologie, Osteology*, 614 (Fisher, Stuttgart, 1928).
[10] Tobias, P. V., *Olduvai Gorge*, **2**, *The Cranium and Maxillary Dentition of Australopithecus (Zinjanthropus) boisei* (Cambridge University Press, 1967).

New Carbon-14 Evidence for Six Hundred Years Old *Canna compacta* Seed

THE longevity of a viable seed of *Canna* considered to be about 550 years old[1–3] has been questioned by Godwin[4]. The specimen, enclosed in a *Juglans australis* nutshell forming part of a rattle necklace was collected from tomb 92 in Santa Rosa de

Tastil, Argentina. New carbon-14 dates presented here, on the nutshell and a trash heap, confirm this to be the oldest viable seed known.

Santa Rosa de Tastil is the first archaeological town dated in the Argentine north-west (24° 25′ S, 65° 50′ W, 2,800 m altitude). In the site were excavated remains of a conglomerate of about 400 dwellings with an evolved network of streets and squares. About 100 tombs and several contemporaneous trash heaps show that occupation was continuous.

The first radiocarbon analysis, on wood charcoal[3] collected in heap 1 together with cameloid bones, dated the site as 530±30 yr BP (years before present defined[5,6] as before AD 1950). Sivori *et al.*[1,2] reported in 1968 the germination and growth of one of the *Canna* seeds found inside the nutshells. The plant has since been growing healthily[7,8] and has reached a height of about 2 m. It flowered and was identified as *Canna compacta* Rosc., by H. Fabris at the University of La Plata. The flowers were self fertilized and produced seeds, which were sown. These germinated with fewer geotropic irregularities than their archaeological ancestor[1,2,7,8].

The kind of evidence about the age of the seed demanded by Godwin and Willis[4,9] is the most desirable if reliable dates are to be found for viable seeds. It involves assaying the carbon-14 of germinating seed before the seedling begins to photosynthesize[4,9]. This is, however, a destructive analysis, requiring early interruption of growth and combustion of the seedling. This was not feasible in the present case: (1) because of the interest in observing the plant growth and eventually determining its species; (2) the organic chemicals used to stimulate germination might have altered the radiocarbon content of the seed by assimilation or by isotopic exchange, and (3) the amount of material—several grammes of carbon—necessary to provide a precise date was not available. The weight of each of the three *Canna* seeds found was about 200 mg. With a yield of carbon only a fraction of the total weight, dating could have been possible only in a "mini-counter"[10]. This, however, would have not been useful because of the large standard deviation of such a measurement (1σ=several hundred years; see Fig. 2 of ref. 10).

Fortunately there were two indirect ways of radiocarbon dating the seed: by analysing the nutshell and the charcoal remains. These data could be interpreted on the basis of the archaeological evidence now being documented[8] by Sivori *et al.*[1,2], and summarized here. (1) The ceramics and other remains, such as the bone artefacts and textiles found in tomb 92 associated with the rattle necklace, are of the same style as those found in heap 1; (2) there are no signs of any intrusions or other perturbations in the material accumulated in the trash heap; (3) the seed has to be contemporaneous or older than the *Juglans* nutshell, because the seed could only have been introduced into the nutshell as long as the nut was still unripe. This was done through a slit cut into it, which after hardening contributes to the characteristic sound of the rattle. Similar rattles have been described by Casanova and von Rosen[11,12], for pre-Inca sites.

By measuring the carbon-13 content of the samples[3,19] all radiocarbon analyses were corrected for isotopic fractionation[13] to avoid errors which could be as much as 250 yr[14–16,3,17]. The carbon-14 dates obtained in BP were transformed to true, historical or calendar years AD. This conversion was done by means of a calibration curve based on measurements of carbon-14 in tree rings. The ambiguity[4,18] between the scales BP and AD caused by the fluctuations of the atmospheric content of the isotope can thus be removed. As the curve of Lerman *et al.*[19] based on Argentine samples does not extend further back than 550 yr BP, we used the curve of Stuiver and Suess[20] for the Northern Hemisphere, modified to account for the observation that Southern Hemisphere samples seem to be about 40 yr older[19,21].

The nutshell was dated as 620±60 BP (GrN–5818)*, converted to AD 1362±73. Heap 1 was divided in seven stratigraphic layers of 20 cm thickness each. Charcoal from layers 1, 4 and 7 was radiocarbon dated: 580±35 BP (GrN–5807), 650±45 BP (GrN–5808), and 635±30 BP (GrN–5809), respectively. These dates and that previously reported[3] (530±30 BP (GrN–5147)) also dates layer 1. The converted dates are: AD 1418±19 for layer 1 (weighted average of the two samples); AD 1336±50 for layer 4; AD 1349±41 for layer 7. Our statistical analysis suggests that the accumulation of the whole trash heap took about 90 yr[22], and that the longest accumulation period, with a confidence level of 95% (2σ interval), was AD 1282 to AD 1467 (ref. 22). As we have shown, the rattle as well as the seed were contemporaneous with the trash heap. The antiquity of the nut ranges from AD 1216 to AD 1508 (2σ interval). The nutshell and the trash heap dates are thus consistent.

The carbon-14 analyses also agree with the dating based on the archaeological and historical chronologies for the region: the excavated potsherds belong to the Late Ceramic Period which dates between AD 1000 and AD 1450, and the absence of Inca objects determines as *terminus ante quem* AD 1450 to AD 1550.

We conclude therefore that the seed from Santa Rosa de Tastil was about 600 yr old when germinated. Compared with documented lists[4,9,23,24] this is the oldest known viable seed. The resistance of *Canna* seeds to hard treatment has been reported[25] (for example, germination of seeds threaded forming a rosary, and of seeds kept about 30 yr in a herbarium).

We are indebted to the Groningen Physics Laboratory for the isotope analyses. This work was begun while J. C. L. was on the staff of that laboratory.

J. C. LERMAN

Physikalisches Institut,
Universität Bern,
3000 Bern, Switzerland

E. M. CIGLIANO

Facultad de Ciencias Naturales y Museo,
Universidad Nacional de La Plata,
La Plata, Argentina

Received October 26, 1970.

[1] Sivori, E., Nakayama, F., and Cigliano, E. M., *Nature*, **219**, 1926 (1968).
[2] Nakayama, F., and Sivori, E., *Rev. Fac. Agron. Univ. Nac. La Plata*, **44** (1), (1968).
[3] Vogel, J. C., and Lerman, J. C., *Radiocarbon*, **11**, 351 (1969).
[4] Godwin, H., *Nature*, **220**, 708 (1968).
[5] Deevey, E. S., Flint, R. F., and Rouse, I., *Radiocarbon*, **5**, v (1963).
[6] Olsson, I. U., in *Radiocarbon Variations and Absolute Chronology* (*Proc. Twelfth Nobel Symp.*) (Almqvist, Stockholm; Wiley, New York, 1970).
[7] Cigliano, E. M., Lerman, J. C., and Sivori, F., *Actas Thirty-ninth Cong. Intern. Amer. Lima 1970* (in the press).
[8] Sivori, F., and Nakayama, F., in *Santa Rosa de Tastil: una Comunidad Prehispánica* (edit. by Cigliano, E. M.) (in the press).
[9] Godwin, H., and Willis, E. H., *Radiocarbon*, **6**, 132 (1964).
[10] Oeschger, H., Alder, B., Loosli, H., and Langway, C. C., *Earth. Planet. Sci. Lett.*, **1**, 49 (1966).
[11] Casanova, E., *Relac. Soc. Argent. Antrop.*, **3** (1942).
[12] von Rosen, E., in *Un mundo que se va*, 123 (1957).
[13] Craig, H., *J. Geol.*, **62**, 115 (1954).
[14] Lerman, J. C., in *Radiocarbon Variations and Absolute Chronology* (*Proc. Twelfth Nobel Symp.*) (edit. by Olsson, I. U.), 104 (Almqvist, Stockholm; Wiley, New York, 1970).
[15] Oeschger, H., Riesen, T., and Lerman, J. C., *Radiocarbon*, **12**, 358 (1970).
[16] Oeschger, H., and Lerman, J. C., *Bull. Schweiz. Verein. Atomenerg. Beil.*, No. 12, 1 (1970).
[17] Goodwin, H., *Amer. J. Bot.*, **57**, 723 (1969).
[18] *Radiocarbon Variations and Absolute Chronology* (*Proc. Twelfth Nobel Symp.*) (edit. by Olsson, I. U.) (Almqvist, Stockholm; Wiley, New York, 1970).
[19] Lerman, J. C., Mook, W. G., and Vogel, J. C., in *Radiocarbon Variations and Absolute Chronology* (*Proc. Twelfth Nobel Symp.*) (edit. by Olsson, I. U.), 275 (Almqvist, Stockholm; Wiley, New York, 1970).

---

* Laboratory numbers are given in parentheses.

[20] Stuiver, M., and Suess, H. E., *Radiocarbon*, **8**, 534 (1966).

[21] Lerman, J. C., Mook, W. G., Vogel, J. C., and de Waard, H., *Science*, **165**, 1123 (1969).

[22] Lerman, J. C., in *Santa Rosa de Tastil: Una comunidad pre-hispánica* (edit. by Cigliano, E. M.) (in the press).

[23] Godwin, H., and Willis, E. H., *New Phytol.*, **63**, 410 (1964).

[24] *Sci. Progrès—La Nature*, No. 3395, 95 (1968).

[25] Kränzlin, F., in *Das Pflänzenreich* (edit. by Engler, A.), 4, No. 47, 16 (Engelmann, Leipzig, 1912).

Isozyme Variation under Selection in *Zea mays*

THE extent of polymorphism in genetically determined isozyme variants (allozyme variation) in natural populations raises the issue of its adaptive significance. It was originally thought that this variation is neutral with respect to fitness[1], but recent evidence supports the assertion that its maintenance involves a selective component[2]. If an allelic substitution yields an enzyme with altered properties[3], maintenance by selection in natural populations may result in a distinct geographic distribution of allele frequencies. Such a pattern has been described[4] in the case of a serum esterase polymorphism in the freshwater fish *Catostomus clarkii*.

Isozyme gene frequencies were examined in a symmetrical set of lines after prolonged selection for and against a specific metabolic property. We did this by assaying enzyme polymorphism in the highly selected strains of maize from the breeding experiment established by Hopkins[5]. From 163 open-pollinated ears of the local maize variety Burr White, he set up four strains (HO, LO, HP and LP) which were selected for high and low seed levels of oil and protein respectively. The long term responses to selection in these Illinois strains are now considered classic examples in both evolutionary theory[6] and plant breeding[7]. Reverse selection[5] has displayed considerable genetic variance persisting after forty-eight generations, and variance analysis[8] after sixty-five generations. Thus, a comparison of electrophoretic variants in these strains may provide a test of whether isozyme variants are selectively neutral.

Seedlings of the four directional strains (HO, LO, HP and LP) and Leng's four reversed strains (RHO, RLO, RHP and RLP) were assayed for E_1, E_3, E_4 and E_{12} (esterases), alcohol dehydrogenase (*Adh*) and peroxidase (*Px*) by techniques described before[9]. Twenty generations of selection have elapsed since the four reverse lines were isolated[5] in 1948. Sample size exceeded fifty seedlings except for LO of which there were only thirty because it germinated poorly. The gene frequency estimates (Table 1) show that each of the eight strains is genetically distinct. More than two strains are polymorphic for each locus: the *S* and *N* alleles at the E_1 and *Px* loci and the *S* and *F* alleles at the E_3, E_{12}, *Adh* and E_4 loci, while the low protein (LP) strain was polymorphic for the *S* and *N* alleles at the E_4 locus.

Table 1 Gene Frequency Estimates of the Slow (S) Allele for Six Isozyme Loci in Eight Selected Strains of Maize

Selected strain	E_1	E_3	E_4	E_{12}	*Adh*	*Px*
Oil content						
High (HO)	0.93	1.00	0.00	0.29	0.33	0.00
Low (LO)	0.57	0.00	1.00	0.83	0.04	1.00
Reverse high (RHO)	0.14	0.76	0.50	0.15	0.29	0.51
Reverse low (RLO)	0.14	0.00	0.34	1.00	0.00	0.23
Protein content						
High (HP)	0.00	0.49	1.00	0.00	0.00	0.50
Low (LP)	1.00	0.00	0.30*	0.00	0.00	0.00
Reverse high (RHP)	0.00	0.18	1.00	0.00	0.74	0.81
Reverse low (RLP)	0.28	0.00	1.00	0.00	0.15	0.12

* Polymorphic for the *S* and *N* alleles.

The mating design of the experiment[5] has enforced outcrossing between selected ears within a strain. To test for the Hardy–Weinberg genotypic distribution, estimates of the fixation index were made for each variable locus in each strain. The fixation index (*F*) is a measure of the proportionate deficiency of heterozygotes at census, and absolute values of *F* greater than $1.96/N^{\frac{1}{2}}$ ($=0.28$) are required to reject the null hypothesis[10] that $F=0$. The values shown in Table 2 conform to the Hardy–Weinberg distribution.

Table 2 Fixation Index Estimates for Six Isozyme Loci in Eight Selected Strains of Maize

Selected strain	E_1	E_3	E_4	E_{12}	*Adh*	*Px*
Oil content						
High HO	−0.08	—	—	0.10	−0.07	—
Low LO	−0.24	—	—	−0.21	−0.05	—
Reverse high RHO	−0.03	0.18	0.06	−0.02	−0.09	0.03
Reverse low RLO	−0.17	—	−0.11	—	—	−0.02
Protein content						
High HP	—	−0.21	—	—	—	0.06
Low LP	—	—	−0.03	—	—	—
Reverse high RHP	—	−0.08	—	—	−0.07	−0.11
Reverse low RLP	0.13	—	—	—	−0.18	−0.14

Selection of twelve ears per strain in this design reduces the effective population size (N_e) to no less than thirty individuals. Assuming that by 1948 the rate of neutral fixation of loci had reached the steady state ($1-1/2N_e$) of variable loci would remain unfixed after each generation. Therefore $(59/60)^{20} \simeq 0.7$ of the variable neutral loci in 1948 would remain unfixed after twenty generations.

Consider the hypothesis that all the allozymic differentiation among the strains is attributable to genetic drift. The following points argue in favour of this hypothesis. First, half of the twelve comparable results at specific loci in Table 1 do not directly correspond to directionally consistent changes. For example, the $E_1{}^s$ allele is less frequent in the reverse low oil (RLO) strain than in the low oil (LO) strain, whereas it is most frequent in the high oil (HO) strain. Second, when reverse selection began, there were at least eighteen loci still variable because eighteen of the twenty-four paired comparisons between the current forward and reverse strains are dissimilar. Of these, twenty-four out of thirty-six remain unfixed in the current generation, and this proportion agrees well with that predicted by drift.

Yet other observations suggest that a selective component may be superimposed. First, of the twelve fixations occurring in the last twenty generations, nine were fixed under forward and only three under reverse selection (one-tail probability of 0.07). Second, the assumption of the steady state and the minimal estimate of N_e have probably inflated the estimate of the effect of drift in these strains. And Table 1 shows that while on average the reverse selection strains vary at more loci than the forward strains (a mean of 3.75 c.f. 2.25), the same difference occurs between the oil strains as a group and the protein strains. Such biases could arise from selective rather than neutral fixation.

These results are acceptable on the hypothesis of purely neutral drift but the deviations suggest that isozyme variants are not entirely neutral to selection.

I thank Drs J. W. Dudley and D. E. Alexander for seeds and Dr J. R. G. Turner and Professor M. H. Williamson for discussion.

ANTHONY H. D. BROWN

Department of Biology,
University of York

Received March 9; revised May 10, 1971.

[1] Shaw, C. R., *Science*, **149**, 936 (1965).

[2] Richmond, R. C., *Nature*, **225**, 1025 (1970).

[3] Gibson, J., *Nature*, **227**, 959 (1970).
[4] Koehn, R. K., *Science*, **163**, 943 (1969).
[5] Leng, E. R., *Z. Pflanzenzucht*, **47**, 67 (1962).
[6] Stebbins, *Processes of Organic Evolution* (Prentice Hall, New Jersey, 1966).
[7] Allard, R. W., *Principles of Plant Breeding* (Wiley, New York, 1963).
[8] Dudley, J. W., and Lambert, R. J., *Crop. Sci.*, **9**, 179 (1969).
[9] Brown, A. H. D., and Allard, R. W., *Crop. Sci.*, **9**, 72, 634 (1969).
[10] Brown, A. H. D., *Genetica*, **41**, 399 (1970).

Shrub Ecotypes in a Salt Desert

A SALINITY gradient extends from the highly saline beaches of the Great Salt Lake of Utah to the surrounding non-halomorphic soils, with a correlated vegetation gradient in which a succession of plant communities grows in concentric zones. The chief communities, in decreasing order of soil salinity, are dominated by (1) *Salicornia–Allenrolfea;* (2) *Sarcobatus vermiculatus;* (3) *Atriplex–Eurotia;* and (4) *Artemisia tridentata*[1]. Within these communities are perplexing local distributions of co-dominant species. Typical communities within the *Atriplex–Eurotia* zone include pure *Atriplex confertifolia, Atriplex–Eurotia,* pure *Eurotia lanata, A. confertifolia Artemisia spinescens–Eurotia* grass, and pure *Atriplex nuttallii.* Although these communities are separated by sharp ecotones, local soil variations are insufficient to explain the distribution of the different plant communities[2].

We have investigated first, the possibility that genetic differences between local populations of *A. confertifolia* and *E. lanata* explain their distribution in or near the five communities within the *Atriplex–Eurotia* zone. Second, we investigated the contribution of genetic differences to the wide tolerance to salinity shown by *A. nuttallii,* which occurred locally in pure stands along the whole salt gradient. Populations of *A. nuttallii* were sampled from the *Salicornia–Allenrolfea* zone, from two communities in the *Sarcobatus* zone, and from the *Atriplex–Eurotia* zone in Curlew Valley, north of the Great Salt Lake.

Sample plants of *A. confertifolia* and *E. lanata* from within the *Atriplex–Eurotia* zone, and of *A. nuttallii* along the salt gradient were removed in blocks of native silt with minimal root damage, and were grown for at least 6 weeks in a glasshouse. Initial soil conductivities were recorded (Table 1). The plants were then grown at 27° C with a 16 h photoperiod in nutrient culture[3] with high levels of salt (10 mM) and boron (0.1 mM) to simulate the average soil condition. Concentrated sodium nitrate solution (16.5 mM) was added to increase plant growth rate. These cultures had conductivities of approximately 50 mmho.

Three measurements were made on six plants from each site in November 1970. These were (*a*) mean shoot dry weight increase in 3 weeks ($\overline{W}_S$); (*b*) relative leaf growth rate[4] ($\overline{R}_L$), and (*c*) nitrate reductase activity ($\overline{A}_N$)[3]. These were chosen as representing different levels of expression of plant activity.

Plants of *E. lanata* from the *Atriplex-Eurotia* zone had a large yield and a very active nitrate reductase system, but variability between populations in these parameters was small (Table 1). On the other hand, populations of *A. confertifolia* demonstrated marginally significant ($P=0.05$) differences in both yield and nitrate reductase activity between sites. The pure stand (P) plants had the lowest dry weight increase, whereas the *A. nuttallii* site (N) plants had lowest nitrate reductase activity. This implies that genetic differences did exist between the different populations of *A. confertifolia.* This species is dioecious with vegetatively distinct sexes, and it was also found that female plants yielded more than males ($P<0.05$).

Populations of *A. nuttallii* from along the salt gradient demonstrated significant differences ($0.05>P>0.001$) in all parameters (Table 1). Further, plants of *A. nuttallii* from the *Atriplex–Eurotia* zone had distinctly smaller leaves than the other populations. In the other sites, R, K and S (Table 1) *A. nuttallii* appeared to be morphologically uniform. Several subspecies, or possibly true species, have been described within the *A. nuttallii* complex[5], so that the distinct morphology and physiology of the plants in the *Atriplex–Eurotia* zone may indicate that they should be accorded subspecific status.

In spite of the large variation in soil conductivity within the *Sarcobatus* zone (Table 1), its two *A. nuttallii* populations (R and K) did not differ significantly. The *A. nuttallii* population from the *Salicornia–Allenrolfea* zone, however, was lower in yield, leaf growth rate and nitrate reductase activity than in the *Sarcobatus* zone plants. Three distinct populations of *A. nuttallii* therefore existed along the salt gradient, which may account for the wide salt tolerance of this species.

Table 1 Soil and Yield Characters of Three Salt Desert Species

	E. lanata				*A. confertifolia*			
Site	Soil con-ductivity	$\overline{W}_S$	$\overline{R}_L$	$\overline{A}_N$	Soil con-ductivity	$\overline{W}_S$	$\overline{R}_L$	$\overline{A}_N$
Pure stand (P)	0.8	18	0.35	235	10	8	0.15	35
Atriplex–Eurotia (M)	0.8	15	0.25	260	0.8	12	0.35	48
Near *A. nuttallii* (N)	1.4	16	0.15	278	1.4	16	0.37	11
Atriplex–A. spinescens–Eurotia grass (G)	1.0	18	0.18	272	1.0	12	0.37	82
SE	—	3.4	0.08	43	—	1.8	0.10	22

		A. nuttallii		
Site	Soil conduc-tivity	$\overline{W}_S$	$\overline{R}_L$	$\overline{A}_N$
Atriplex–Eurotia zone (N)	1.4	10	0.39	29
Sarcobatus zone (R)	5.2	17	0.60	106
Sarcobatus–Suada (K)	96	21	0.60	173
Salicornia–Allenrolfea zone (S)	121	14	0.42	9
SE		3.2	0.05	24

Soil conductivity is given in mmhos; $\overline{W}_S$, dry weight (mg) per shoot; $\overline{R}_L$, relative leaf growth rate; $\overline{A}_N$, nitrate reductase activity (mM $NaNO_2$/g fresh weight/h).

The variation in nitrate reductase activity as well as in yield and leaf growth rate in *Atriplex* but not in *Eurotia* suggests that in *Atriplex,* as in the Gramineae[6], nitrate reductase activity may be inherently variable. All of the three parameters were sufficiently variable to indicate considerable genetic variation in the *Atriplex* species studied. This contrasts with the finding of Hannon and Bradshaw[7] who reported that two co-existing maritime grasses were in a similar state of evolutionary adaptation to salinity. Our work suggests that these three desert chenopods, *A. confertifolia, E. lanata* and *A. nuttallii,* differ considerably in their state of evolutionary diversification. Indeed, the observation of interspecific hybridization in *A. nuttallii*[5] indicates that this species is in a very active state of evolution, and this probably accounts for its wide distribution.

Investigations are continuing with clones of the different populations growing in different environments (particularly different salinities) to test the adaptive nature of the population differences. This work and the possibility of genetical experiments in the future, however, is limited by the slow growth of woody *Atriplex* clones.

This investigation was carried out at Utah State University with the aid of grants from the Ecology Center, the Utah

Pure Che istry Librar

Agricultural Experiment Station, the US/IBP Desert Biome and the British Agricultural Research Council.

P. J. GOODMAN

Welsh Plant Breeding Station,
Plas Gogerddan,
Aberystwyth

M. M. CALDWELL

Range Science Department and
The Ecology Center,
Utah State University,
Logan,
Utah 84321

Received February 8; revised May 18, 1971.

[1] Flowers, S., and Evans, F. R., in *Salinity and Aridity* (edit. by Boyko, H.), 367 (W. Junk, the Hague, 1966).
[2] Gates, D. H., Stoddart, L. A., and Cook, C. W., *Ecol. Monog.*, **26**, 155 (1956).
[3] Bowerman, A. E., and Goodman, P. J., *Ann. Bot.* (in the press).
[4] Watson, D. J., *Ann. Bot.*, **22**, 37 (1956).
[5] Hanson, C. A., thesis, Brigham Young University (1962).
[6] Beevers, L., and Hageman, R. H., *Ann. Rev. Plant Physiol.*, **20**, 495 (1969).
[7] Hannon, N., and Bradshaw, A. D., *Nature*, **220**, 1342 (1968).

Flow of Red Blood Cells stopped by Ultrasound

ULTRASOUND has applications in physiotherapy in the treatment of musculoskeletal disorders[1], and recent findings indicate a potential value as a local anabolic agent[2-4]. It is used to detect tumours[5] and foetuses[6], while a further application lies in measuring the rate of blood flow in terms of corpuscular velocity[7,8]. The increasing use of ultrasound on human tissues has led to concern for possible hazards. It is essential that safety limits are determined accurately and that the interactions of ultrasound with living tissues are investigated so that they can be predicted when ultrasound is used within these limits. Largely in this connexion, we are investigating the effects of low intensity ultrasound on blood circulation in living tissues and wish to report a striking finding: the aggregation of red blood cells into stationary clumps in the radiation field (Fig. 1).

A chick embryo at the 3.5 day stage of incubation, together with its extra-embryonal membranes, was dissected free from the yolk in such a way as to keep the sinus terminalis intact, and placed in a saline-filled irradiation chamber[9] maintained at 37° C by a heated microscope stage, and consisting of a shallow rectangular metal tray fitted with a strip transducer at one of the shorter ends. The wall at the end opposite the transducer could be faced with either a reflector or with various combinations of absorbing materials. There was a central viewing area of thin glass over which the embryo was placed with its attached extra-embryonal membranes lightly stretched. The heart beat continued apparently normally for several hours in this preparation. The flow of red cells through the vessels of the chick and its associated membranes were viewed with a light transmission microscope. The ultrasonic field was monitored by a small, sensitized, thermistor probe which could be positioned at any point in the radiation field by means of a micromanipulator attached to the irradiation chamber.

We found that when particulate matter was suspended in the saline it aggregated into clumps arranged in vertical planes at right angles to the direction of propagation of the ultrasound. These aggregates were spaced at half wavelengths throughout the field. When the embryo was placed in the chamber, we found that above certain threshold intensities the blood cells in the developing vessels aggregated in a similar manner, and in some cases circulation was arrested. Red cell stasis was temporary, for the clumps broke down when irradiation ceased.

So far we have used only continuous 3 MHz irradiation. Threshold intensities for red cell aggregation are apparently determined by the nature of the vessel. When the ease of aggregation is compared in adjacent arteries and veins of similar bore, the threshold intensity is significantly lower in the vein than in the artery ($d = -1.22$, $N = 9$, $t = -5.8568$, $P < 0.0005$). Red cell stasis occurred more readily in large bore veins than in those of small bore. In arteries near the heart the situation was more complex because the red cell clumps are affected by pressure changes at each heart beat.

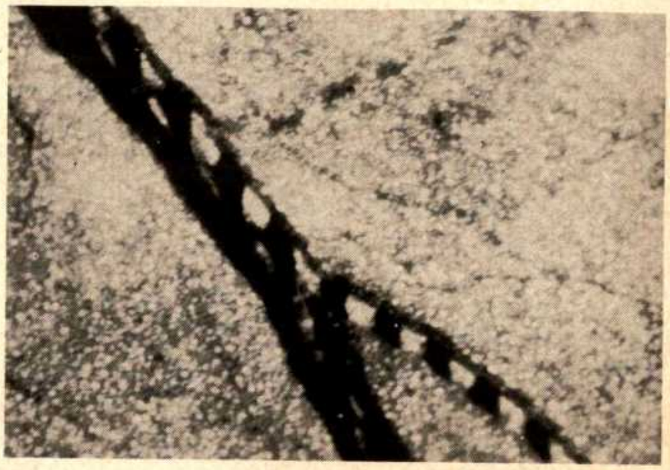


Fig. 1 Surface view of the area vasculosa of a chick embryo at the 3.5 day stage of incubation demonstrating ultrasonically induced red cell stasis (× *c.* 26).

When stasis was complete red cell aggregates were sharp-edged (Fig. 1). In conditions producing incomplete stasis, red cells could be seen breaking away from the downstream edges of the bands only to be trapped at the upstream edge of the next band. When irradiation ceased each band assumed the parabolic profile typical of Poiseuille flow before dissolution was complete (Fig. 2).

The fact that the aggregates were sharp-edged suggests that the red cells were not held together simply as a result of standing wave formation. We consider that Bernoulli attractions may be involved. If reflexion played a more important role in aggregate formation than simply determining the position of the aggregates in the radiation field, then one would expect a continuous gradation in red cell density between adjacent aggregates rather than the sharp limits that we have found. Other evidence also suggests that reflexion is a minor component of the forces producing aggregation. Masking the terminal reflector, the wall of the chamber opposite the transducer, by putting an absorber in front of it, did little to affect the threshold intensities required to produce aggregation. When the chamber was replaced by a tank lined by absorbers in which reflexion was minimal, linear aggregation of the red cells in the blood vessels still occurred. These results are interesting because they indicate that a strong reflected wave, such as might be obtained in medical ultrasonic usage at the interface between a soft tissue and air or bone, is not essential for the production of red cell stasis. It seems that only a minor change in acoustic impedance between one tissue and another is sufficient to trigger off the response.

The direction of the blood vessel, relative to the direction of propagation of the ultrasound, is also important in determining the effect on red cell motion. If the vessel is aligned parallel to the ultrasonic field stasis is readily evoked. If the vessel is at right angles to it then the red cells continue to flow within the cell aggregates.

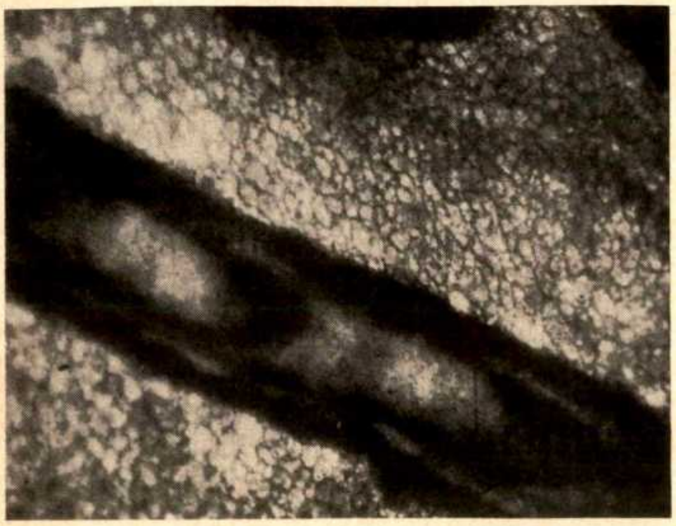


Fig. 2 Parabolic profile of dissociating red cell aggregates in a vein (× *c.* 186).

One serious effect of red cell stasis in arteries and arterioles is that the tissues supplied by them will be exposed to conditions of reduced oxygen availability. We suggest that this can be minimized by moving the treatment head during ultrasonic irradiation so that the direction of the vessels relative to the direction of propagation of the ultrasound is continuously varied. When this is not possible then the dose parameters should be kept below those producing red cell stasis in the major vessels. We also suggest that the possibility of interference with red cell flow be considered when Doppler probes are used to monitor the circulation of the blood.

We thank Mr Kevin Fitzpatrick and the Department of Medical Photography for assistance with photography, the Medical Research Council and the Wellcome Trust for financial assistance, and Professor R. Warwick for advice and encouragement.

MARY DYSON
B. WOODWARD
J. B. POND

Anatomy Department,
Guy's Hospital Medical School,
*London Bridge SE*1

Received April 13; revised June 7, 1971.

[1] Summer, W., and Patrick, M. K., *Ultrasonic Therapy, a Textbook for Physiotherapists* (Elsevier, London, 1964).
[2] Dyson, M., Pond, J. B., Joseph, J., and Warwick, R., *Clin. Sci.*, **35**, 273 (1968).
[3] Dyson, M., and Pond, J. B., *Physiotherapy*, **56**, 136 (1970).
[4] Dyson, M., Pond, J. B., Joseph, J., and Warwick, R., *IEEE Trans. Sonics and Ultrasonics*, SU-17, 133 (1970).
[5] Holmes, J. H., *Prog. Clin. Cancer*, **3**, 135 (1967).
[6] Donald, I., *Brit. Med. Bull.*, **24**, 71 (1968).
[7] Rushmer, R. F., Baker, D. W., and Stegall, H. F., *J. Appl. Physiol.*, **21**, 554 (1966).
[8] Gosling, R. G., King, D. H., Newman, L., and Woodcock, J. P., *Ultrasonics for Industry, Conference Papers*, 16 (Iliffe, Guildford, 1969).
[9] Pond, J. B., Woodward, B., and Dyson, M., *Physics Med. Biol.* **16**, 521 (1971).

Defence and Territorial Behaviour dissociated by Hypothalamic Lesions in the Rat

IT has been pointed out that much of the literature on brain mechanisms of aggression fails to distinguish between different kinds of aggressive behaviour, and that this may account for some of the apparent contradictions in different publications[1]. In the experiments reported here the effects of hypothalamic lesions were measured on two different types of aggressive behaviour: defence behaviour, which consists of a defensive upright posture and boxing in the shock box; and territorial behaviour, which consists of the offensive side posture and the full attack posture with biting and kicking by a socially isolated home rat against an intruder. These postures associated with social interactions are quite stereotyped in the rat. They are also present in modified form in other rodents and have been illustrated and catalogued previously[2].

We used adult male hooded rats, raised in the laboratory. Fighting in response to foot-shock (defence behaviour) was elicited by placing a pair of rats in a 'Plexiglas' cage 20 × 15 cm with an electrified grid floor. Each trial consisted of a series of twenty 60 cycle a.c. shocks lasting 0.5 s and delivered once every second. A grid scrambling device was used to guarantee that the rats could not escape the shock by standing on certain pairs of grids. Fighting threshold was determined by the titration method; that is, shock intensity was raised in a trial after one in which the rats did not fight and was lowered after one in which they did fight. Fighting was defined as defensive upright posture and boxing by both rats in the pair. Among normal rats these were the predominant responses seen, although at high shock intensities the rats sometimes bit at each other or leapt and rolled over together biting and kicking. Behaviour similar to the offensive side posture and full attack posture was not exhibited by normal rats in the shock box.

Territorial behaviour was obtained from rats that had been socially isolated for at least 1 week in a large home cage (1.4 m^2 of space on six levels with connecting ramps). Fighting, defined as offensive side posture and/or the full attack posture with biting, was exhibited against a male intruder placed in the home cage. The behaviour was quantified by determining the number of minutes in which fighting occurred during the 10 min period after the first occurrence. If no fighting occurred after 30 min, the trial was terminated.

All pairs of rats tested in the shock box showed rearing and boxing. Some rats did not fight off an intruder in the territorial experiment, however, and they were not used in the study.

Testing a rat in the shock box inhibited it from showing territorial fighting characteristics in the home cage. The rat seemed to become conditioned in the shock box to expect pain when approached by another rat. For this reason, the experimental animals were not tested pre-operatively in the shock box, but only after the initial post-operative tests had been carried out in the territorial situation. Post-operative results in the shock box were compared only with results in normal controls.

Lesions were placed in the hypothalamus by stereotaxic procedure using a radio frequency lesion maker. Surgery was performed aseptically on animals anaesthetized with 'Nembutal' (60 mg kg^{-1}) and the rats were allowed 3 days for recovery before behavioural testing. Lesions involving the lateral hypothalamus abolished spontaneous feeding, in which case the animals were maintained by stomach tube feeding. Lesions were assessed on cresyl violet stained serial frozen sections.

Only rats with symmetrical lesions were included in the study. The experimental results (Fig. 1) depended on whether the lesion substantially destroyed the medial hypothalamus (six rats), the lateral hypothalamus (six rats) or both (six rats). The lesions were not confined to one particular structure, but lateral lesions always interrupted most of the medial forebrain bundle bilaterally. Medial lesions always destroyed the posterior hypothalamus and made substantial invasions at least into the dorsomedial hypothalamus and the posterior portion of the ventromedial nucleus.

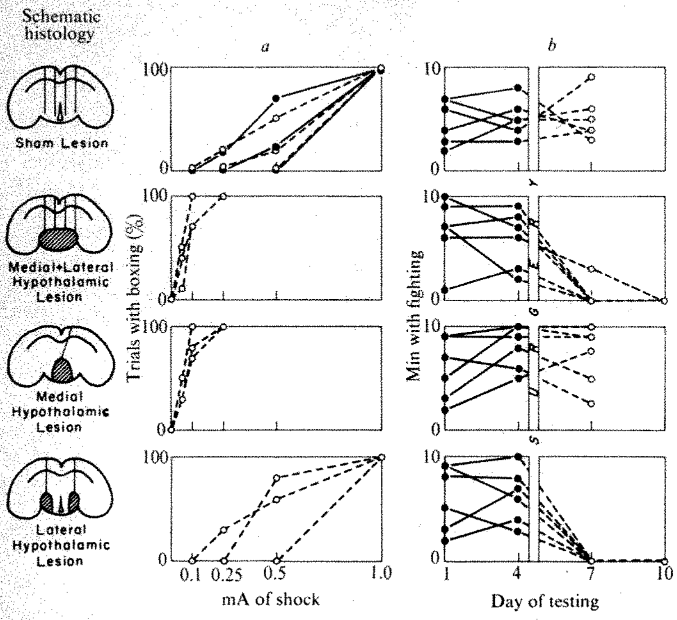


Fig. 1 Effects of hypothalamic lesions in hooded rats on threshold of defence behaviour in the shock box (*a*) and the persistence of territorial behaviour in home cage (*b*). Defence behaviour was defined as defensive upright posture and boxing by both members of pairs of lesioned rats. Territorial fighting was defined as the presence of offensive side posture or full attack posture with biting, and it was quantified by the number of minutes during a test session in which one or the other behaviour was exhibited by the rat in his home cage against an intruder. Pre-operative data were not obtained in the shock box because of an interaction between the shock box and territorial testing procedures. One set of data was obtained from normal animals and is shown on the top line, together with post-operative data from sham-lesion rats. ●—●, Pre-operative testing; ○ - - - ○, post-operative testing.

Lesions involving both lateral and medial hypothalamus on both sides abolished territorial behaviour and simultaneously enhanced defence behaviour in the shock box, both effects being very strong. Rats which had previously attacked an intruder male within 1 min of the intrusion and persisted with biting and offensive side posture for most of the succeeding 10 min were rendered totally docile in the same situation post-operatively. The intruder might sniff at the rat, wander around him, or even lie down on him, but the rat with the lesion would ignore the intruder or sniff at him and turn away. The same animal, on the other hand, would show defence upright posture and boxing in the shock box at thresholds only one-tenth of the intensity of normal thresholds. Whereas normal rats usually fought only during presentation of the shocks, the rats with lesions usually continued to fight after the shock was discontinued and often fought spontaneously between trials.

By confining lesions to the medial and lateral hypothalamus it was possible to separate the two effects. The enhancement of defence behaviour in the shock box was caused by lesions of the medial hypothalamus; a lesion confined to the lateral hypothalamus on both sides had no effect. The abolition of territorial behaviour was caused by lesions of the lateral hypothalamus; a lesion confined to the medial hypothalamus had no effect.

This work was supported by a USPHS Biological Sciences training grant.

DAVID B. ADAMS

Department of Psychology,
Wesleyan University,
Middletown,
Connecticut 06457

Received November 3, 1970; revised March 22, 1971.

[1] Moyer, K., *Comm. Behav. Biol.*, **2**, 65 (1968).
[2] Grant, E., and Mackintosh, J., *Behaviour*, **21**, 246 (1963); Barnett, S., in *The Rat: A Study in Behaviour*, 81 (1963).

Visual Sensations Produced by Cosmic Ray Muons

IN 1961, D'Arcy and Porter[1] demonstrated that when single cosmic ray muons passed through the eyes of supine, dark-adapted observers, a small proportion produced a detectable visual sensation. The effect was ascribed either to the production of Cherenkov light in the eye media by the relativistic, singly charged particles or to some form of direct stimulation of the retina. Interest in the possible visual effects produced by fast charged particles has revived recently, following reports by the Apollo astronauts that they saw "flashes of light" while in translunar flight; these flashes may have been caused by the action of cosmic ray nuclei on the eyes[2].

We now present the preliminary results of a study which confirms and extends the observations of D'Arcy and Porter. Cosmic ray muons travelling downwards within roughly 15° of the vertical were selected by a counter telescope consisting of one or two 2.5 cm diameter disks of plastic scintillator and a 58 cm diameter tank of liquid scintillator placed approximately 90 cm below. The observers lay with their heads between the scintillators comprising the telescope. Four body positions were used, to allow study of the effects of muons passing in different directions through the eyes and head. For the prone or supine positions two small scintillators were used, placed in close proximity to each eye. Alternatively, the observers lay on their sides with their visual axes horizontal and a single small scintillator positioned close to the head to select muons passing laterally through either both eyes or through the visual cortex. In each position, a muon detected by the counter telescope actuated a scaler and a loudspeaker to give a click audible to the observer. A higher rate of muons was detected by a second control telescope some distance from the observer; this triggered a second scaler and gave an identical click on the loudspeaker. The observer was simply required to state "yes" or "no" according to whether he saw a flash of light of any character occurring in coincidence with a loudspeaker click. His replies were recorded by an operator who also read the scalers and thus knew whether the muons had passed through the "genuine" or "control" telescopes. The experiment thus consisted of determining whether a higher proportion of affirmative responses was given for those muons which passed through the eyes or cortex as compared with those that passed through the control telescope.

Ten observers each underwent four recording sessions in each of the four body positions; a recording session consisted of 30 min of dark-adaptation followed by 30 min of responses. Our observers, like those of D'Arcy and Porter, differed considerably in their ratios of affirmative replies and for brevity we present in Table 1 the summed responses for all the observers, that is for a total of 20 h of actual observation in each position. *P* represents the probability by a χ^2 test that the yes/total ratios for the genuine and control events came from the same population.

Table 1 Responses of Observers

Position	Genuine counts Total	Genuine counts Yes	Genuine counts Yes/total	Control counts Total	Control counts Yes	Control counts Yes/total	P
Prone (face down)	3,878	558	0.144	9,404	1,050	0.112	<0.001
Supine (face up)	4,682	763	0.163	6.421	887	0.138	<0.001
Side (eye)	3,411	487	0.143	6,448	740	0.115	<0.001
Side (cortex)	3,549	456	0.128	6,256	806	0.129	0.94
Supine (D'Arcy and Porter[1])	274	65	0.237	1,613	247	0.153	<0.001

It is clear that, for the three cases when the muons passed through the eye, visual effects were observed; these were absent when the particles passed through the cortex. Although the Cherenkov angle in the eye is quite large (~41°) and the retina extends over rather more than a hemisphere, it would be expected that the coupling to the retinal receptors of Cherenkov light produced by muons travelling parallel to the visual axis and entering the front of the eye would be more efficient than that for similar particles entering from the rear of the eye. The resultant marked improvement in the detection efficiency for the supine over the prone position does not seem to exist. It has been shown[2] that it is unlikely that a singly charged fast particle could produce enough visible Cherenkov light to constitute a supra-threshold signal. Our results therefore seem to favour a direct-excitation phenomenon at the retinal level, probably related to that occurring with X-rays[3] and with knock-on protons produced by fast neutrons[4-6].

W. N. CHARMAN
CHRISTINA M. ROWLANDS

*Department of Ophthalmic Optics,
University of Manchester Institute of
Science and Technology*

Received May 5, 1971.

[1] D'Arcy, F. J., and Porter, N. A., *Nature*, **196**, 1013 (1962).
[2] Fazio, G. G., Jelley, J. V., and Charman, W. N., *Nature*, **228**, 260 (1970).
[3] Kimeldorf, D. J., and Hunt, E. L., *Ionising Radiation—Neural Function and Behaviour*, 131 (Academic Press, London, 1965).
[4] Fremlin, J. H., *New Sci.*, **47**, 42 (1970).
[5] Tobias, C. A., Budinger, T. F., and Lyman, J. T., *University of California Radiation Laboratory Report*, No. UCRL 19868 (1970).
[6] Charman, W. N., Dennis, J. A., Fazio, G. G., and Jelley, J. V., *Nature*, **230**, 522 (1971).

The Absolute Configuration and Crystal Structure of the Anticholinergic Drug Dexbenzetimide

THE pharmacological activity of (±) benzetimide (Fig. 1) ((±)-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine HCl), a potent and persistent anticholinergic agent, acting in both the peripheral and the central nervous system, is due entirely to the (+)-isomer dexbenzetimide[1]. The drug is used to abolish Parkinson symptoms sometimes evoked in psychiatric patients being treated with neuroleptic drugs[2]. The anticholinergic properties are due to the unaltered dexbenzetimide and not to its metabolites[3]. As dexbenzetimide is specifically taken up and retained by small nerve endings of the caudate nucleus of the rat[3], it is tempting to assume that the binding sites are at or near an acetylcholine receptor.

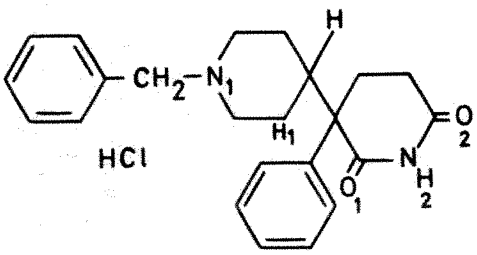


Fig. 1 (±) Benzetimide.

A Stuart–Briegleb model[4,5] of benzetimide indicates that except at the benzyl moiety, the molecule is rigid with a flat hydrophilic bottom and a lipophilic top. A footprint in plaster-of-paris was made of the hydrophilic side of the benzetimide model showing the imprints of N(1), H(1), O(1), H(2) and O(2). A model of the transconformation of acetylcholine, which is thought to be responsible for the muscarinic activity of the molecule[6], fits this footprint perfectly. The footprint was tested with a number of S-B models of optically active

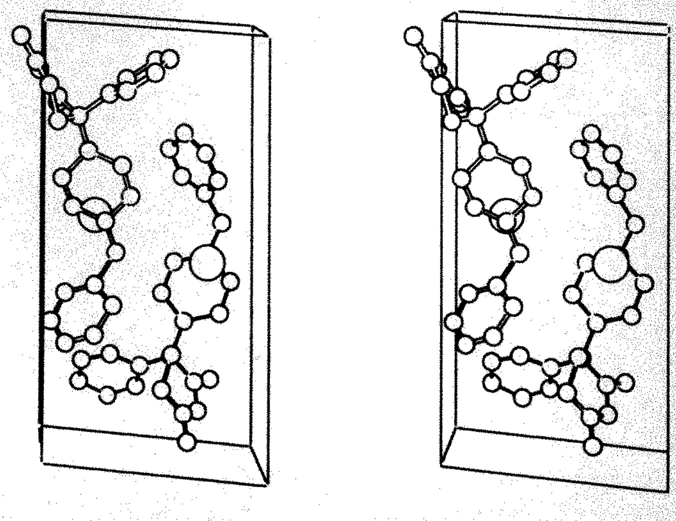


Fig. 2 Stereoscopic plot of the unit cell-content of the dexbenzetimide-HBr crystals. The larger circles represent Br and the small circles represent C, N or O.

agonists and antagonists with known absolute configuration of the pharmacologically active antipode, but only the models of the active antipode fitted the footprint as the acetylcholine model did. This strongly suggests that the model of benzetimide used in making the footprint is the model of the pharmacologically active antipode, which means that this dextrorotating antipode must have the S configuration.

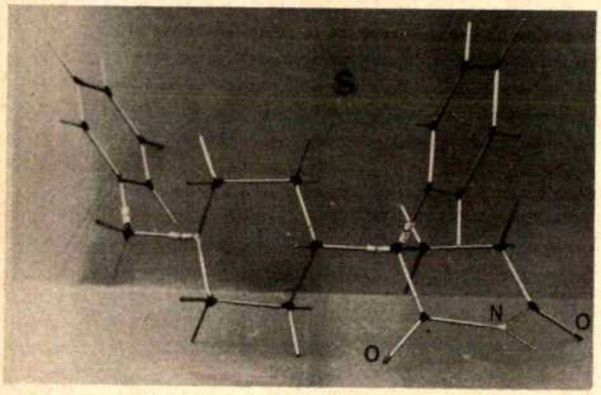


Fig. 3 Dreiding model of dexbenzetimide of one of the conformations in the unit cell.

On the strength of this we predicted the absolute configuration of the pharmacologically active antipode of 1-trimethylammonium-2-acetoxy cyclopropane[4,5] which was confirmed by X-ray diffraction[7]. To justify this method we determined the absolute configuration of (+) benzetimide (dexbenzetimide) by X-ray diffraction. The structure of the tricline crystals of dexbenzetimide-HBr was solved by using a new symbolic phasing procedure, the details of which will be published later (data available on request). The pertinent data are: space group P_1, cell dimensions $a = 7.319$ Å, $b = 9.014$ Å, $c = 17.49$ Å, $\alpha = 94.9°$, $\beta = 99.1°$, $\gamma = 80.3°$, two formula units in the unit cell, 56 non-hydrogen atoms in the cell. The absolute configuration, S as shown in Fig. 2, was determined by anomalous isotropic refinement of both enantiomorphs, using 2,604 reflexions. It resulted in

$$R_2 \equiv \left(\frac{\Sigma(F_0 - F_c)^2}{\Sigma F_0{}^2}\right)^{1/2}$$

values of 18.5% and 18.7% and there is therefore a significant difference between the fit of the observed structure factors and that of the calculated structure factors for each enantiomorph, fixing the absolute configuration to the predicted one, S. A Dreiding model of one of the molecules in the unit cell is shown in Fig. 3.

A. L. Spek
A. F. Peerdeman

Laboratory for Crystal Chemistry,
University of Utrecht,
Catharynesingel 51,
Utrecht

I. van Wijngaarden
W. Soudijn

Research Laboratories,
Janssen Pharmaceutica,
Beerse

Received March 11, 1971.

1 Janssen, P. A. J., Niemegeers, C. J. E., Schellekens, K. H. L., Demoen, P., Lenaerts, F. M., Van Nueten, J. M., van Wijngaarden, I., and Brugmans, J., *Arzneimittel-Forsch.*(in the press).
2 De Smedt, R., Rodrigus, E., Debandt, R., and Brugmans, J., *J. Clin. Pharmacol.*, **10**, 207 (1970).
3 van Wijngaarden, I., *Life Sci.*, **1**, **9**, 489 (1970).
4 van Wijngaarden, I., thesis, Univ. Leyden (1970).
5 van Wijngaarden, I., Soudijn, W., and Van der Eycken, C., *Life Sci.*, **9**, 1289 (1970).
6 Archer, S., Lands, A. M., and Lewis, T. R., *J. Med. Pharmacol. Chem.*, **5**, 423 (1962).
7 Chothia, C., and Pauling, P., *Nature*, **226**, 541 (1970).

Fracture of Bone Materials in Compression at Temperatures between −200° C and + 200° C

Recent studies[1,2] of bone materials have suggested that the fracture behaviour is sensitively dependent on specimen orientation, microstructure, the presence of external notches, temperature and the deformation rate imposed. The deformation properties of bone have been likened to those expected of composite materials[2,3]. This report provides further information about the strength of bone and helps to define the criteria which must be satisfied by theoretical models of the mechanical properties.

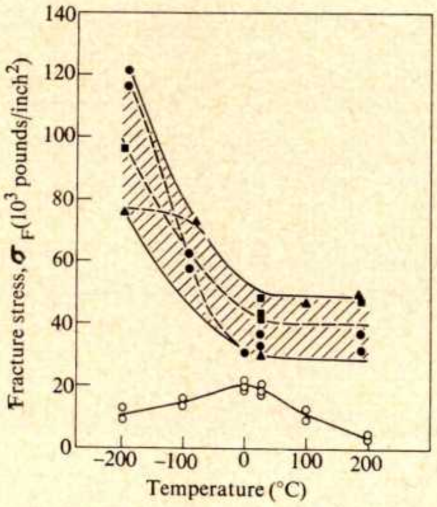


Fig. 1 Compressive and tensile fracture stress of bone at various temperatures. Compression tests: ●, aged, 25° C; ■, as received; ▲, annealed, 110° C. ○, Tensile results of Bonfield and Li[1].

Specimens for compression tests were made from two different bone materials. Ten cylindrical specimens 0.472 cm in diameter and 1.27 cm in height were made from the section of a steer femur which had been thoroughly dried by ageing for 2 yr in normal atmospheric conditions after the immediate removal of the centre marrow. The specimen axes were essentially parallel to the length of the bone. The specimens showed no macroscopic porosity and were machined on a lathe with a maximum layer removal of 0.025 cm per pass. Another set of ten tetragonal specimens 1.01 cm in height and square cross-section of 0.5 cm per side were produced from wet bovine femur material within two weeks of death. Specimen surfaces were ground flat and parallel to within 0.0025 cm by layer removal per pass equal to or less than 0.025 cm. Five of these specimens were annealed in a vacuum of 10^{-5} mm Hg at 110° C for 170 h. The specimens were tested within a specially constructed cylindrical compression chamber mounted on a floor model Instron testing machine so that the entire chamber could be immersed in liquid baths at various temperatures[4]. A strain rate of $\sim 10^{-3}$ s^{-1} was used.

The fracture stresses required at various test temperatures are shown in Fig. 1 along with the tensile fracture stress results of bovine femur material[1]. At room temperature, the measured compressive strengths lie between 29,000 and 48,000 pounds/inch^2. Within this range, the compressive strength does not change appreciably at temperatures above 0° C, but does increase strongly at temperatures below 0° C. The ratio of the fracture stresses in compression and tension varies from 8.0 to 12.0 at the highest and lowest test temperatures. At room temperature the ratio is between 1.5 and 2.5. The upper limiting values of this ratio may be explained on the basis that a single critical flaw governs the fracture strength according to a Griffith energy release hypothesis[5]. The lower limiting value of this ratio may be associated with brittle fracture behaviour in normally ductile polycrystalline materials whereby the yield stress, as measured in compression, is equated to the brittle (cleavage) fracture stress, as measured in tension[6].

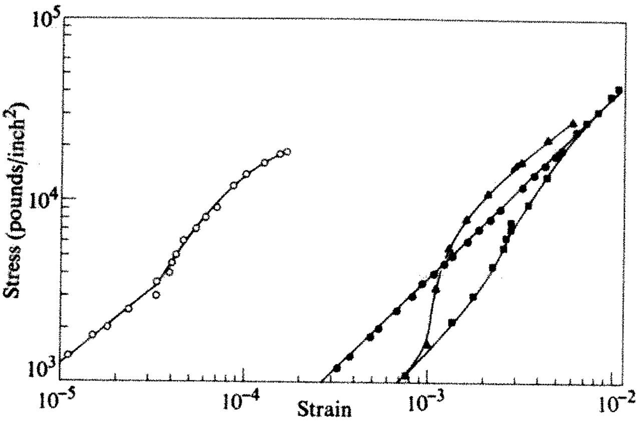


Fig. 2 Stress-strain behaviour of bone in compression or tension at 25° C. ■ and ▲, as in Fig. 1. Tensile results[1]: ○, plastic strain only; ●, total strain.

The compressive stress-strain behaviour of two specimens deformed at room temperature is shown in Fig. 2 versus the tensile stress-strain behaviour previously reported on a similar material[1]. From the original compression specimen, a linear curve was obtained at large strains that was essentially identical to the extended stress-strain behaviour predicted for the tensile specimen, both specimens giving an elastic modulus of 3.8×10^6 pounds/inch^2. But this compression specimen showed a yield point drop and an attendant elastic strain decrease at 7,600 pounds/inch^2. The better fabricated and aligned compression specimen in the annealed condition showed a linear stress-strain behaviour after smaller strains and an increased elastic modulus value of 5.0×10^6 pounds/inch^2, as expected. This comparison indicates that at room temperature the tensile and compressive stress-strain behaviour of bone material is similar except that fracture occurs in tension at smaller strain values.

The results show that the compressive strength of bone at various temperatures shows a distinctive behaviour which, when compared with tensile results on similar material, produces some new conditions to be explained by any theory proposed to encompass the total deformation and fracture behaviour.

We thank Dr S. B. Mohanty for bone materials and Dr W. L. Phillips for assistance with the measurements. This research has been supported by the Advanced Research Projects Agency through the Center of Materials Research and by the Office of Naval Research.

R. W. ARMSTRONG
B. ARKAYIN
G. HADDAD

Engineering Materials Group and
Mechanical Engineering Department,
University of Maryland,
College Park, Maryland 20742

Received January 22; revised April 5, 1971.

[1] Bonfield, W., and Li, C. H., *J. Appl. Phys.*, **37**, 869 (1966).
[2] Piekarski, K., *J. Appl. Phys.*, **41**, 215 (1970).
[3] Currey, J. D., *Biorheology*, **2**, 1 (1964).
[4] Wessel, E. T., *Bull. Amer. Soc. Testing Materials*, **211**, 40 (1956).
[5] Orowan, E., *Rep. Prog. Phys.*, **12**, 185 (1948).
[6] Armstrong, R. W., *Phil. Mag.*, **9**, 1063 (1964).
[7] Armstrong, R. W., *Fracture*, 314 (Chapman and Hall, London, 1969).

Synthesis of Cystine in Simulated Primitive Conditions

IN an attempt to utilize the very abundant long wavelength ultraviolet solar radiation for prebiological organic chemistry, a series of experiments was performed in which H_2S was introduced as the long wavelength photon acceptor. These experiments are described in detail elsewhere[1], but in a typical experiment a spherical reaction vessel was filled with methane, ethane, ammonia, water vapour and H_2S and the gases were irradiated in cylindrical geometry with the 2537 Å and/or 1849 Å resonance emission lines of mercury. The gases and their photolysis products were then circulated over a liquid water bath by a greaseless solenoid pump and returned to the reaction vessel (Fig. 1).

In one experiment, the initial mixture contained 612 cm^3 of CH_4, 2,024 cm^3 of C_2H_6, 1,004 cm^3 of NH_3, 542 cm^3 of H_2S and 16 cm^3 of liquid H_2O. The mixture was irradiated with 2537 Å radiation for 30 h at a gas temperature which ranged from 215° to 360° C. A deep brown viscid liquid was formed and examined by ion exchange chromatography in a Beckman model 120C amino-acid analyser. The identity of the peak which corresponded to cysteine was confirmed by adding a known standard chromatographically pure solution of cysteine to our brown liquid sample and noting that the appropriate peak increased in amplitude, with no shoulder introduced. The cysteine detected was present at a concentration of approximately 5×10^{-3} µmol/cm^3.

In a second experiment, 591 cm^3 of CH_4, 2,010 cm^3 of C_2H_6, 1,000 cm^3 of NH_3, 549 cm^3 of H_2S and 15 cm^3 of liquid H_2O were introduced into the reaction vessel and irradiated with 2537 Å and 1849 Å radiation for 2 days, with 2537 Å for 1/2 days and again with 2537 Å and 1849 Å for 9 days. The average gas temperature during this 12 day experiment was 400° C. The deep brown viscid liquid solution produced in this experiment was sequestered for 3 months. When examined with a Beckman model 120C amino-acid analyser, cystine was found but no cysteine; the amount of cystine produced was approximately 0.042 µmol/cm^3. The presence of cystine was confirmed by the absence of a shoulder when a cystine standard was performed on a Hitachi model 'KLA-3B' amino-acid analyser (Fig. 2). In this experiment 2 mCi of $^{14}CH_4$ and 1 mCi of $^{14}CH_3{}^{12}CH_3$ were also introduced into the reaction mixture at the beginning of the experiment and the liquid products were examined by two dimensional autoradiographic paper chromatography (Whatman No. 1 paper). The two solvents were 10 methanol : 10 n-butanol : 5 water : 2 ammonia, and 10 acetone : 10 n-butanol : 5 water : 2 ammonia. The chromatograms were then placed against Kodak medical X-ray film NS–54T and developed by a 1 month exposure. A spot corresponding to cystine was found. The presence of cystine in this experiment can be regarded as confirmation of the results of the previous experiment because cysteine has a tendency to oxidize to cystine.

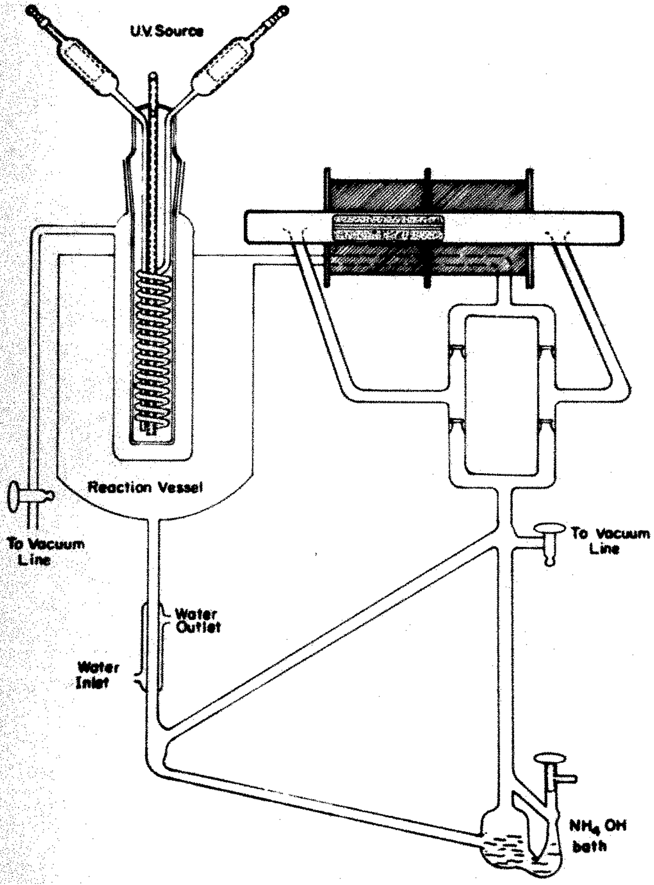


Fig. 1 Schematic diagram of experimental apparatus. Gases irradiated in the reaction vessel are circulated by the Watson pump, upper right, through an ammonia solution (*p*H 12), lower right.

In a third experiment, 1,638 cm^3 of CH_4, 6,055 cm^3 of C_2H_6, 3,035 cm^3 of NH_3, 1,678 cm^3 of H_2S and 50 cm^3 of liquid H_2O were introduced into the reaction vessel and irradiated with 2537 Å radiation for 25 days at a temperature of 50° C. Half of the 50 cm^3 of coloured liquid product was taken from the aqueous bath, dried under nitrogen and hydrolysed for 22 h with 5.7 M HCl. Although no free cystine was found, a trace was detected with the amino-acid analyser in the acid hydrolysate.

In all three experiments, the closely related α-amino-acid serine was also found. Control experiments—in which the gas was heated to 300–400° C for 11 days but not irradiated, or in which the gas was irradiated at 2537 Å at 20° C for 14 h but in the absence of ethane—gave negative results for amino-acids[1]. In all three experiments, we obtained preliminary column chromatographic evidence for the presence of cysteic acid.

In an earlier experiment in which a gaseous mixture of methane, ammonia, water and H_2S was irradiated with 10^9 rads from an electron beam accelerator, cysteic acid, taurine and cystamine were found[2]. And in another, an aqueous solution of ammonium thiocyanate was irradiated with 2537 Å; the sulphur-containing amino-acid methionine was identified by autoradiographic paper chromatography with three different solvent systems. In our experiments we found a spot which appeared to have the position and shape of the methionine paper chromatography spot. A known sample of methionine was run on Whatman No. 1 chromatographic paper, eluted and analysed on an AEI model 'MS–902' mass spectrometer. A similar analysis of the unknown spot gave a mass spectrum quite different to that of methionine. Similarly, the addition of a methionine standard to our solution showed the unknown to be the shoulder of the methionine standard (compare Fig. 2).

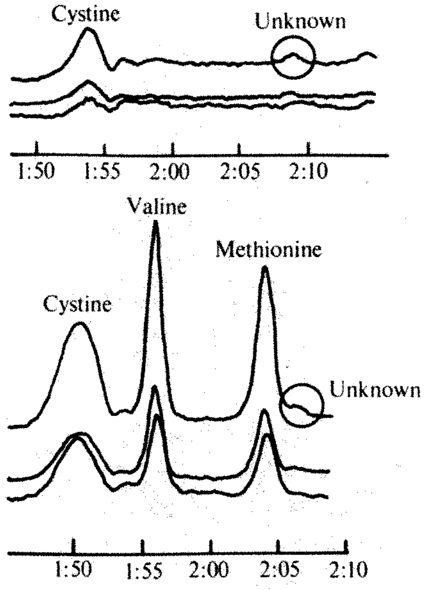


Fig. 2 Ion exchange chromatograms of experimental samples (above) and experimental samples plus chromatographically pure cystine, valine and methionine standards (below) produced by a Hitachi model KLA-3B amino-acid analyser. The coincidence of peaks near elution time 1 : 50 and the non-coincidence near 2 : 07 is evidence for the presence of cystine but not of methionine. The elution time phase shift in the two chromatograms, obtained in slightly different conditions, is expected.

The yield of cystine in our experiments was probably considerably larger than we detected. Both cystine and cysteine are known to decompose readily in alkaline solution to pyruvic acid, H_2S, NH_3 and sulphur[4]. Because NH_3 was present in our system, the conditions in aqueous solution were quite alkaline (*p*H 12). Sulphur polymers up to S_8 were detected by mass spectrometry in the aqueous phase of our system, but these are mostly direct products of H_2S photolysis. The alkaline hydrolysis of cystine regenerates the ammonia and H_2S involved in its photolytic production and leads to a considerable accumulation of pyruvic acid, a substance of particular interest in the context of the Horowitz hypothesis for the reverse evolution of enzymatic reaction chains[5]. Pyruvic acid is the end product of the anaerobic glycolytic pathway ubiquitous in terrestrial organisms. In addition Forbes and Savige[6,7] have found that visible and long wavelength ultraviolet light incident on alkaline or neutral solutions of cystine in the presence of air leads to a rapid conversion of cystine to alaninethiosulphonic acid and alaninethiosulphuric acid, together with smaller amounts of alaninesulphuric acid, cysteic acid, H_2S and two unidentified amino-acids. Thus, because both alkaline hydrolytic and photolytic destruction of cystine are known to occur in our experimental or post-experimental conditions, these experiments suggest a plausible mechanism for the high yield production of cysteine and cystine in the early history of the Earth.

This research was supported by the National Aeronautics and Space Administration, the Smithsonian Institution and the US Public Health Service. We thank Nurit Bar-Nun, Robert Goldberg, Jeremy Hribar, Fred McLafferty, James Bartholomew and Arabinda Guha for assistance and advice.

Bishun N. Khare
Carl Sagan

Laboratory for Planetary Studies,
Cornell University,
Ithaca,
New York 14850

Received January 28; revised April 15, 1971.

[1] Sagan, C., and Khare, B. N., *Science*, **173**, 417 (1971).
[2] Choughuley, A. S. V., and Lemmon, R. M., *Nature*, **210**, 628 (1966).
[3] Steinman, G., Smith, A., and Silver, J., *Science*, **159**, 1108 (1968).
[4] Fruton, J. S., and Simmonds, S., *Biochemistry* (2nd ed.), 58 (Wiley, New York, 1958).
[5] Horowitz, N. H., *Proc. US Nat. Acad. Sci.*, **31**, 153 (1945).
[6] Forbes, W. F., and Savige, W. E., *Photochem. Photobiol.*, **1**, 1 (1962).
[7] Forbes, W. F., and Savige, W. E., *Photochem. Photobiol.*, **1**, 77 (1962).

Cannabivarin and Tetrahydrocannabivarin, Two New Constituents of Hashish

We have identified two more constituents of hashish to add to the constituents of hashish already known[1]. In 1969 Vollner *et al.*[2] identified cannabidivarin, a cannabidiol homologue in which the side chain is C_3H_7 instead of C_5H_{11}. All cannabinoids found until then had contained an olivetyl group (n-amylresorcinol). This was the first cannabinoid found which contained a divarinyl group (*n*-propylresorcinol). Because many constituents are derived from cannabidiol and cannabidiolic acid, Vollner *et al.* predicted that hashish would yield more constituents derived from cannabidivarin, and indeed Gill *et al.*[3] found in 'Tincture of Cannabis' (BPC) a Δ^1-tetrahydrocannabinol homologue with C_3H_7, instead of C_5H_{11} as the side chain.

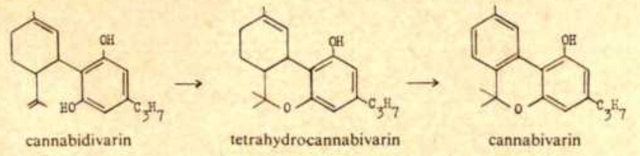


Fig. 1

Using a modification[4-11] of Korte and Sieper's[12] thin-layer chromatographic method we noticed an intense violet spot from a sample of Nepal hashish: the colour was similar to that of the cannabinol spot. We therefore isolated this constituent by preparative thin-layer chromatography and subjected it to mass spectrometry.

The mass spectrum had a molecular ion peak at *m/e* 282 and other peaks at *m/e* 267 (M^+-CH_3, base peak) 239 (M^+-C_2H_4), 238 (M^+-C_2H_5), 223, 209, 195, 165 and 141. From this fragmentation pattern, which is very similar to that of cannabinol[13], it can be concluded that the unknown constituent is a cannabinol homologue, in which the side chain is C_3H_7 instead of C_5H_{11}. We proposed for this constituent the name cannabivarin[14]. This compound can be derived in biogenesis from the tetrahydrocannabinol homologue, and so we searched for this homologue in the same samples of Nepal hashish.

We also found a red spot of unknown origin between the cannabinol spot and that of tetrahydrocannabinol. The red colour was very similar to that of the tetrahydrocannabinol spot. We succeeded in isolating this component by preparative thin-layer chromatography and we elucidated the chemical structure by mass spectrometry. The mass spectrum had a molecular ion peak at *m/e* 286 and peaks at 271 (M^+-CH_3), 243 (M^+-C_3H_7), 203 (base peak), 165. This mass spectrum has the characteristic fragmentation pattern of Δ^1-tetrahydrocannabinol[13], with the difference that all masses are twenty-eight units smaller (the difference between the mass of C_5H_{11} and C_3H_7).

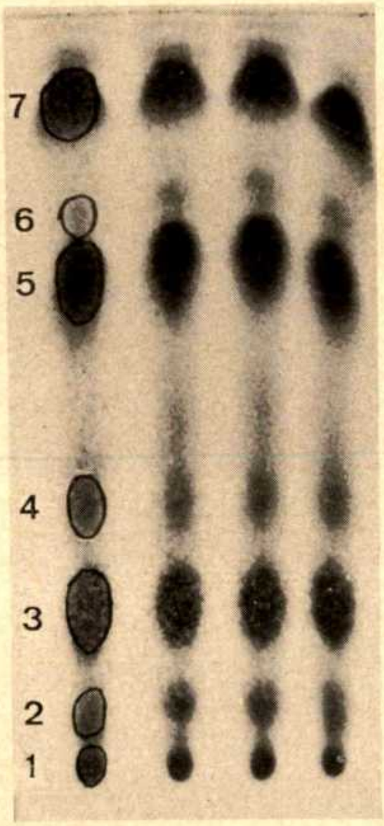


Fig. 2 Thin-layer chromatogram of a sample of Nepal hashish. The sample was extracted with petroleum ether and after evaporation of the petroleum ether the residue was dissolved in chloroform. The separation was carried out on a precoated silica gel plate, impregnated with dimethylformamide and carbon tetrachloride (6+4) and dried in air at room temperature for 30 min. The plate was developed three times with cyclohexane and finally sprayed with 0.5% fast blue salt B in water. The sequence of the separation and the colours of the spots are: (1) cannabidiolic acid (orange); (2) cannabidivarin (orange); (3) cannabidiol (orange); (4) cannabivarin (violet); (5) cannabinol (violet); (6) tetrahydrocannabivarin (red); (7) tetrahydrocannabinol (red).

Thus, the unknown component is a tetrahydrocannabinol homologue, in which the side chain is C_3H_7 instead of C_5H_{11}. We propose for this component the name tetrahydrocannabivarin, according to the names cannabidivarin, proposed by Vollner and coworkers[2], and cannabivarin, proposed by us[14]. Subsequently, we established the identity of a hitherto unidentified orange spot between the starting point and the cannabidiol spot as cannabidivarin.

In all the Nepal samples we studied cannabidiol, tetrahydrocannabinol and cannabinol were also present, and so it seems that two parallel biosynthetic processes are possible in the same cannabis product: cannabidiol (CBD)→tetrahydrocannabinol (THC)→cannabinol (CBN) and cannabidivarin (CBDV)→tetrahydrocannabivarin (THCBV)→cannabivarin (CBV) (Fig. 1).

The sequence of the R_F values of the different constituents can be explained as follows (Fig. 2). The hydrogen bond between the stationary phase dimethylformamide and the phenolic group of the three cannabinoids, containing the propyl group, is stronger than the bond between dimethylformamide and the cannabinoids containing the amyl group. Supposing an equal solubility in the solvent cyclohexane, the R_F values of the three cannabinoids with the propyl group are lower than the R_F values of the corresponding cannabinoids with the amyl group. Indeed the following sequence of R_F values is obtained: cannabidivarin < cannabidiol <, cannabivarin < cannabinol and tetrahydrocannabivarin < tetrahydrocannabinol. The quantitative determination was carried out by gas liquid chromatography on a 'SE 52' column (Fig. 3).

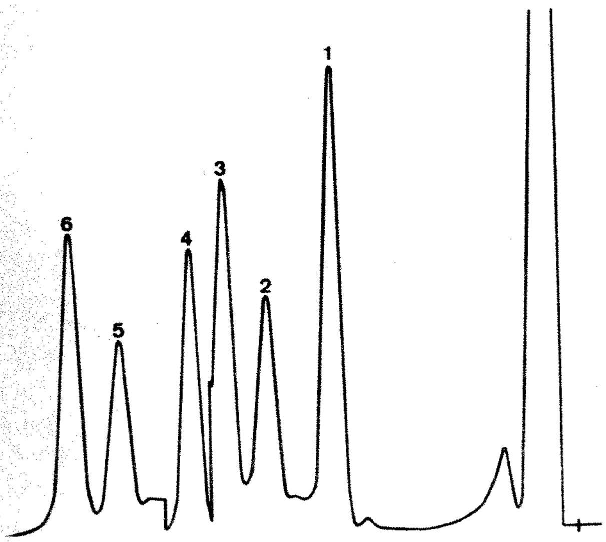


Fig. 3 Gas liquid chromatography of a sample of Nepal hashish. The sample was extracted with petroleum ether and after evaporation of the petroleum ether the residue was dissolved in chloroform. The separation was carried out on a column of 'SE 52' (5% on 'Gas Chrom Q'), column temperature 225°–280° C, 6°/min, N_2 flow 40 ml./min, flame ionization detector. Peak numbering: cannabidivarin (1), tetrahydrocannabivarin (2), cannabivarin (3), cannabidiol (4), tetrahydrocannabinol (5), cannabinol (6).

The retention times of the chief constituents of the samples of Nepal hashish were on the 'SE 52' column (column temperature 225°–280° C, 6°/min, N_2 flow 40 ml./min): cannabidivarin 4 min 19 s, tetrahydrocannabivarin 5 min 29 s, cannabivarin 6 min 12 s, cannabidiol 6 min 48 s, tetrahydrocannabinol 8 min 00 s, and cannabinol 8 min 53 s. The peak areas were measured by height × width at half height. Table 1 summarizes the quantitative determination of three samples of Nepal hashish. The three cannabinoids containing the divarinyl group seem to be present in large quantities in proportion to the other well known cannabinoids. The maximum percentages of the total amount of the six chief constituents we found were 15.8% for cannabivarin, 5.7% for tetrahydrocannabivarin and 12.3% for cannabidivarin. These findings are significant not only for chemistry but also in assessing the biological activity of samples of hashish containing these constituents.

Table 1 Amounts of Single Components in Nepal Hashish as Percentage of Total Cannabinoids: Cannabinol, Tetrahydrocannabinol, Cannabidiol, Cannabivarin, Tetrahydrocannabivarin and Cannabidivarin

	Sample A	Sample B	Sample C
Cannabinol	22.1	24.8	30.3
Tetrahydrocannabinol	11.5	3.4	5.5
Cannabidiol	35.9	41.1	41.2
Cannabivarin	12.5	15.8	11.6
Tetrahydrocannabivarin	5.7	3.0	2.3
Cannabidivarin	12.3	11.9	9.1

The quantitative determination was carried out by measuring the peak areas on a gas chromatogram, made on an 'SE 52' column.

Tetrahydrocannabivarin will undoubtedly have psychotomimetic properties and is a more polar compound than tetrahydrocannabinol. Its biological activity will be smaller, since from earlier studies[15] with synhexyl (a THC-like compound), in which the side chain was varied, it seemed that the activity in the n-alkyl series rises to a maximum at n-hexyl and then falls off again. But we think the more polar character of tetrahydrocannabivarin may influence the resorption, the onset of action, the formation of polar metabolites and the excretion in man.

In relation to this, two recent studies are of interest. Gill *et al.*[3] found that tetrahydrocannabivarin is 4.8 times less active than tetrahydrocannabinol in producing a cataleptic effect in mice. Tetrahydrocannabivarin, however, contributes significantly to the early part of the response of mice to crude cannabis, while tetrahydrocannabinol itself acts very slowly. Second, from the investigations of Lemberger, Silberstein, Axelrod and Kopin[16] it can be concluded that tetrahydrocannabinol persists for many days in normal volunteers and that it is metabolized in man and excreted as polar metabolites in urine and faeces. These observations enable us to conclude that the presence of tetrahydrocannabivarin in a sample of hashish will contribute significantly to the psychotomimetic activity of that sample.

It is also likely that tetrahydrocannabivarin will have an additive or a synergistic effect to the tetrahydrocannabinol effect. We have found that in many samples of hashish a considerable quantity of cannabidivarin, tetrahydrocannabivarin and cannabivarin is present. The identification and the quantitative determination contribute to a more exact chemical knowledge of the constituents and their biosynthesis and allow a better understanding of the different biological activities of hashish samples.

FRANS W. H. M. MERKUS

RC Hospital,
Sittard,
The Netherlands

Received April 18, 1971.

[1] Mechoulam, R., *Science*, **168**, 1159 (1970).
[2] Vollner, L., Bieniek, D., and Korte, F., *Tetrahedron Lett.*, 145 (1969).
[3] Gill, E. W., Paton, W. D. M., and Pertwee, R. G., *Nature*, **228**, 134 (1970).
[4] Merkus, F. W. H. M., *Pharm. Weekblad*, **106**, 49 (1971).
[5] Merkus, F. W. H. M., *Symposium 6, Chromatography and Electrophoresis*, Brussels (in the press).
[6] Aramaki, H., Tomiyasu, N., Yoshimura, H., and Tsukamoto, H., *Chem. Pharm. Bull.*, **16**, 822 (1968).
[7] Karlsen, J., Exler, Th. J. H., and Baerheim Svendsen, A., *Pharm. Weekblad*, **104**, 583 (1969).
[8] Machata, G., *Arch. Toxikol.*, **25**, 19 (1969).
[9] de Faubert Maunder, M. J., *J. Pharm. Pharmacol.*, **21**, 334 (1969).
[10] Parker, K. D., Wright, J. A., Halpern, A. F., and Hine, C. H., *Bull. Narcotics*, **20** (No. 4), 9 (1968).
[11] Mechoulam, R., and Gaoni, Y., *Tetrahedron*, **21**, 1223 (1965).
[12] Korte, F., and Sieper, H., *J. Chromatog.*, **13**, 90 (1964).
[13] Budzikiewicz, H., Alpin, R. T., Lightner, D. A., Djerassi, C., Mechoulam, R., and Gaoni, Y., *Tetrahedron*, **21**, 1881 (1965).
[14] Merkus, F. W. H. M., *Pharm. Weekblad.*, **106**, 69 (1971).
[15] Todd, A. R., *Experientia*, **2**, 55 (1946).
[16] Lemberger, L., Silberstein, S. D., Axelrod, J., and Kopin, I. J., *Science*, **170**, 1320 (1970).

Acid Monoamine Metabolites in Cerebrospinal Fluid during Methadone Maintenance

SOME studies of the interaction of narcotic analgesics and brain monoamine systems have implicated brain monoamines in acute and long term effects of the principal narcotics[1], but interspecies variation makes it difficult to apply these results to man. Techniques are now available which use monoamine metabolites in cerebrospinal fluid (CSF) as indicators of central monoamine metabolism[2–4]. Using measures which reflect central 5-hydroxytryptamine (serotonin) and dopamine synthesis, we studied patients on methadone maintenance therapy for heroin addiction.

Twenty-five determinations were made in sixteen male (ages 25–46) and five female (ages 25–32) heroin addicts who were taking single daily doses of methadone (25–135 mg/day). For ten determinations, 4 ml. CSF was obtained by lumbar tap while a group of subjects was taking methadone only. Before fifteen further determinations, another group of subjects were given probenecid, 3.0 g/day orally for 2.5 days before CSF sampling as described before[5,6]. Probenecid blocks the egress of acid monoamine metabolites from CSF and brain and as a result metabolite concentrations increase[7–9]. Acid monoamine metabolite values in CSF obtained after the use of probenecid may therefore reflect more closely than baseline values the formation rates of the metabolites within the central nervous system. Samples were assayed fluorimetrically for 5-hydroxyindoleacetic acid (5HIAA, acidic metabolite of serotonin) and homovanillic acid (HVA, acidic metabolite of dopamine)[2–4]. Similar non-paired samples with and without probenecid were obtained from a group of psychiatric inpatients (within 10 days of hospital admission, no drugs for at least a week) and from a group of paid inmate volunteers. All subjects gave informed consent. Methadone added to CSF caused no quenching or other interference with acid monoamine metabolite readings except at very high concentrations (1 mg methadone/ml. CSF). In studies involving mice, methadone in substantially larger doses did not interfere with the ability of probenecid to block the egress of acid monoamine metabolites from brain[10].

Unpaired comparisons for 5HIAA and HVA were made between subjects in each category (addicts, psychiatric patients, and inmates) who took probenecid and those who did not. Intergroup comparisons were made for the probenecid and non-probenecid conditions. Addicts in the probenecid condition taking less than 90 mg methadone/day were compared with those taking 90 mg/day or more. Mean values for 5HIAA and HVA were compared by a t test (two tailed).

Without probenecid, compared with inmates, 5HIAA values were significantly lower in the psychiatric and addict groups ($P<0.001$, Table 1). Within the groups, subjects taking probenecid, compared with those not taking probenecid, had higher values for CSF 5HIAA in the case of inmates ($P<0.005$) and psychiatric patients ($P<0.001$), but not in the addict group. On probenecid, the addicts (mean methadone dose 87.4 mg/day) showed a significantly lower value for CSF 5HIAA than inmates ($P<0.001$) and psychiatric patients ($P<0.005$). Without probenecid, the three groups' means did not differ in HVA. Within each group, subjects on probenecid had a higher CSF HVA value than those without probenecid. But the addict group on probenecid showed the lowest HVA value, significantly lower than the psychiatric group ($P<0.01$) but not the inmate group. Addicts taking less than 90 mg of methadone/day as a group had a significantly higher mean value for CSF 5HIAA than those taking 90 mg or more/day. The means (± s.e.) were 40.8 ± 7.3 ng/ml. and 23.8 ± 3.2 ng/ml. respectively ($P<0.05$). Although not significant, the same trend was found for CSF HVA values where the corresponding means were 77.9 ± 10.4 ng/ml. (actual mean methadone dose 66 mg/day) and 52.4 ± 16.2 ng/ml. (actual mean methadone dose 107 mg/day). A daily 90 mg dose is approximately the average maintenance dose in our subjects. Taken altogether, these data indicate that methadone suppressed the formation of 5HIAA and possibly HVA in the central nervous system of the addicts. These results do not differentiate definitely the possible effects on CSF 5HIAA and HVA of addiction to heroin compared with methadone maintenance. But, whatever the effect of heroin addiction, we can say that full maintenance doses of methadone resulted in a greater decrease in CSF 5HIAA and HVA than lower doses. The mechanism underlying this effect cannot be specified at this point, for methadone may be affecting processes of synthesis (including availability of amine precursors) or storage and release in our subjects. Although not the focus of this report, the finding of a decrease in CSF 5HIAA in a psychiatric population is consistent with previous studies[11–13]. The mechanism of this finding is under investigation.

Table 1 Concentrations of 5HIAA and HVA in the Lumbar CSF of Human Subjects with and without Probenecid Pretreatment

Group	5HIAA Without probenecid	5HIAA With probenecid	HVA Without probenecid	HVA With probenecid
I	23.2 ± 2.9 (10)	32.0 ± 4.2 (15)	25.3 ± 7.5 (10)	64.3 ± 10.1 (15)
II	29.6 ± 2.5 (36)	49.9 ± 4.1 (15)	26.0 ± 4.2 (34)	111.5 ± 13.3 (15)
III	43.5 ± 1.7 (18)	59.4 ± 4.6 (20)	23.2 ± 5.9 (17)	86.1 ± 8.2 (16)

Group I are addicts on methadone, group II are psychiatric patients, group III are inmates. Values are ng of the free acid/ml. CSF ± s.e. with numbers of determinations in parentheses. Mean dose of methadone for addicts with and without probenecid was 87.4 and 65.5 mg per day respectively. Mean doses are not significantly different ($P=0.2$).

A number of studies in animals suggest that functional monoamine systems are necessary for both the acute and long term effects of the narcotic analgesics. In the rat, lesions of the midbrain raphe (which destroy the main serotonin neurone group) increased sensitivity to shock and decreased the analgesic effect of morphine[14,15]. Further, reserpine and tetrabenazine antagonized analgesia and tolerance in mice[16,17]. These effects were reversed by 5-hydroxytryptophan and L-dihydroxyphenylalanine, precursors of serotonin and dopamine respectively[14–19]. Monoamine oxidase inhibitors increase the toxicity of narcotic analgesics, probably by increasing levels of serotonin[19]. Further, inhibitors of serotonin and catecholamine synthesis have been reported to increase sensitivity to pain and to antagonize acute and long term narcotic effect[20–22]. These studies, which employ techniques that disrupt monoamine systems, do not explain the effect of narcotics on monoamine turnover, but they suggest that monoamines are utilized in the production of acute and long term narcotic effects. Earlier studies of the effects of narcotics on brain levels of serotonin and noradrenaline showed minimal changes[1]. Some investigators have utilized measurements of monoamine synthesis and turnover. Way and Haubrich found evidence of increased synthesis of serotonin after morphine had been given to mice and rats[23–25]. Others have been unable to reproduce these results[26,27]. We have shown that acute and chronic doses of methadone increased brain 5HIAA in mice[10]. In studies utilizing radioactive tyrosine, Clouet and Ratner reported increased incorporation of radioactivity into both dopamine and noradrenaline after acute and long term treatment of rats with morphine[28]. The results reported here are in contrast with most of the animal data[23–27]. As measured by the formation of acidic monoamine metabolites in CSF, serotonin and probably dopamine synthesis were decreased, at maintenance levels of methadone in our patients. Whether these results reflect a different effect of methadone or narcotics in general on monoamine systems in man compared with other species is still unclear.

While this paper was being reviewed, Tamarkin, Goodwin and Axelrod reported similar findings in three addicts on methadone[29].

This work was supported by a US Public Health Service grant (M. B. B., jun.). Amanda Smith and Angelica Rozitis gave technical assistance.

MALCOLM B. BOWERS, JUN.
HERBERT D. KLEBER
LINDA DAVIS

Yale University Department of Psychiatry,
and Drug Dependence Unit,
Connecticut Mental Health Center,
New Haven,
Connecticut

Received January 11; revised April 23, 1971.

[1] Harris, L. S., *Fed. Proc.*, **29**, 28 (1970).
[2] Anden, N. E., Roos, B. E., and Werdinius, B., *Life Sci.*, **2**, 448 (1963).
[3] Ashcroft, G. W., and Sharman, D. F., *Brit. J. Pharmacol. Chemother.*, **19**, 153 (1962).
[4] Gerbode, F., and Bowers, jun., M. B., *J. Neurochem.*, **15**, 1053 (1968).
[5] Olsson, R., and Roos, B. E., *Nature*, **219**, 502 (1968).
[6] Bowers, jun., M. B., *Brain Res.*, **15**, 522 (1969).
[7] Guldberg, H. C., Ashcroft, G. W., and Crawford, T. B. B., *Life Sci.*, **5**, 1571 (1966).
[8] Bowers, jun., M. B., and Gerbode, F., *Life Sci.*, **7**, 773 (1968).
[9] Bowers, jun., M. B., *Life Sci.*, **9**, 691 (1970).
[10] Bowers, jun., M. B., and Kleber, H. D., *Nature*, **229**, 134 (1971).
[11] Ashcroft, G. W., Crawford, T. B. B., Eccleston, D., Sharman, D. F., MacDougall, E. J., Stanton, J. B., and Binns, J. K., *Lancet*, ii, 1049 (1966).
[12] Dencker, S. J., Malm, U., Roos, B. E., and Werdinius, B., *J. Neurochem.*, **13**, 1545 (1966).
[13] Bowers, jun., M. B., Heninger, G. R., and Gerbode, F., *Intern. J. Neuropharmacol.*, **8**, 255 (1969).
[14] Lints, C. E., and Harvey, J. A., *J. Comp. Physiol. Psychol.*, **67**, 23 (1969).
[15] Saminin, R., Gumulka, W., and Valzelli, L., *Europ. J. Pharmacol.*, **10**, 339 (1970).
[16] Rudzik, A. D., and Mennear, J. H., *J. Pharm. Pharmacol.*, **17**, 326 (1965).
[17] Takagi, H., and Kuriki, H., *Intern. J. Neuropharmacol.*, **8**, 195 (1969).
[18] Lints, C. E., and Harvey, J. A., *Physiol. Beh.*, **4**, 29 (1969).
[19] Rogers, K. J., and Thornton, J. A., *Brit. J. Pharmacol.*, **36**, 460 (1969).
[20] Tenen, S. S., *Psychopharmacologia*, **10**, 204 (1967).
[21] Tenen, S. S., *Psychopharmacologia*, **12**, 278 (1968).
[22] Eidelberg, E., and Schwartz, A. S., *Nature*, **225**, 1152 (1970).
[23] Way, E. L., Loh, H. H., and Shen, F., *Science*, **162**, 1290 (1968).
[24] Loh, H. H., Shen, F., and Way, E. L., *Biochem. Pharmacol.*, **18**, 2711 (1969).
[25] Haubrich, D. R., and Blake, D. E., *Fed. Proc.*, **793** (1969).
[26] Marshall, I., and Grahame-Smith, D. G., *Nature*, **228**, 1206 (1970).
[27] Cheney, D. L., Goldstein, A., Algeri, S., and Costa, E., *Science*, **171**, 1169 (1971).
[28] Clouet, D. H., and Ratner, M., *Science*, **168**, 854 (1970).
[29] Tamarkin, N. R., Goodwin, F. K., and Axelrod, J., *Life Sci.*, **9**, 1397 (1970).

Life Cycle of *Tripedalia cystophora* Conant (Cubomedusae)

THE Cubomedusae, commonly called "box jellies" or "sea wasps" because of their shape and, for some, their severe sting, are inhabitants of neritic tropical regions of the oceans. Though they have been known for more than 200 yr and the morphology of several species has been investigated[1], little is known of their life histories. Conant[1], in Jamaica, was able to rear the planula of *Tripedalia cystophora* to a four-tentacled polyp. Okada[2], in Japan, reared *Carybdea rastoni* to approximately the same stage. In neither case did the polyps live to an age which revealed their unusual nature. The opinion, held by many zoologists, that the Cubomedusae are an aberrant order of the Class Scyphozoa has therefore derived from a knowledge of the medusa generation alone. This belief, and the view of some authors[3-6] that the polyp is the stem form of all recent Cnidaria, have led to efforts to elucidate the life cycle of a member of this order.

Unlike other Cubomedusae, the fertilized eggs of *Tripedalia* develop into planulae in the gastral pockets of the female medusa. When the planulae are mature, they are released within 2 days. They are then slightly pyriform, have a diameter of 0.13 mm, and contain a yellow-brown pigment. The planulae settle within 2 days. They have a preference for bivalve shells and darkness but will settle on glass or plastic. Two to three days after settlement a polyp with a mouth and four tentacles is formed. Although naked at first, the polyp's base is enveloped by a thin transparent periderm when the four-tentacle stage is achieved. When the polyps begin to feed they grow rapidly.

When 0.5 mm long, with five or six tentacles, the polyps begin to produce asexual buds from the basal body wall. The buds resemble those of *Hydra* and acquire a functional mouth and tentacles before they are released. On detachment, the two to four-tentacled bud or secondary polyp elongates and one of its tentacles becomes much longer than the others. It is capable of gliding, crown first, over the substrate by use of this elongated tentacle. This secondary polyp soon attaches, becomes pyriform, develops a peridermal sheath about the base, and buds off more polyps in the same manner as the primary polyp. At an age of 8–10 weeks the polyps, either primary or secondary, have a single whorl of seven to nine tentacles, a body length of 0.8–1.0 mm, and appear to be fully grown (Fig. 1).

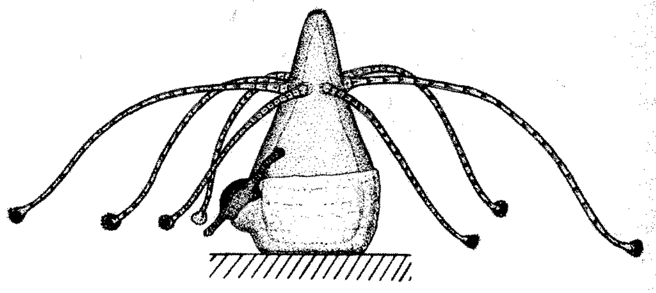


Fig. 1 *Tripedalia cystophora*. Adult polyp; basally, on the left, is a young secondary polyp just before detaching. Length = height of the body is 1.0 mm (drawn by F. Heckmann).

The chief features of the solitary polyp are its long, contractile, cone-shaped peristome and solid, completely retractile, capitate tentacles which bear nematocysts only at their tips. Scyphopolyp features such as a cruciform-shaped mouth, peristomial pits, gastric septa and pockets, septal funnels and muscles, and thick mesogloea are lacking. Only at the time of transformation to a medusa does a tetraradiate symmetry appear. The *Tripedalia* polyp thus more closely resembles a hydropolyp than a scyphopolyp.

The polyp of *Tripedalia* is also unique in that it undergoes metamorphosis into a single medusa. The first evidence of medusa formation is the transformation of the polyp crown to a quadrangular shape. The polyp tentacles then, in no particular order, congregate at the four corners (Fig. 2*A*). The bases of the tentacles, or coalesced bases of several tentacles, thicken and develop small pigment spots (Fig. 2*B*). At the same time, the distal parts of the tentacles are resorbed. Ultimately polyp tentacles, either singly or as a fused group, become the four rhopaloids. Before the polyp tentacles are resorbed, the four primary medusa tentacles appear, *de novo*, between the four rhopaloids. These are hollow, very long, pointed, and are fringed with annular nematocyst batteries. The histology of the developing medusa will be described in a later report, but it can be stated here that the substance of the polyp is completely converted into the tissues of the medusa. At water temperatures of 28°–29° C, metamorphosis to a medusa is completed in 4–5 days. Growth time from planula or newly liberated bud to onset of metamorphosis is 10–12 weeks.

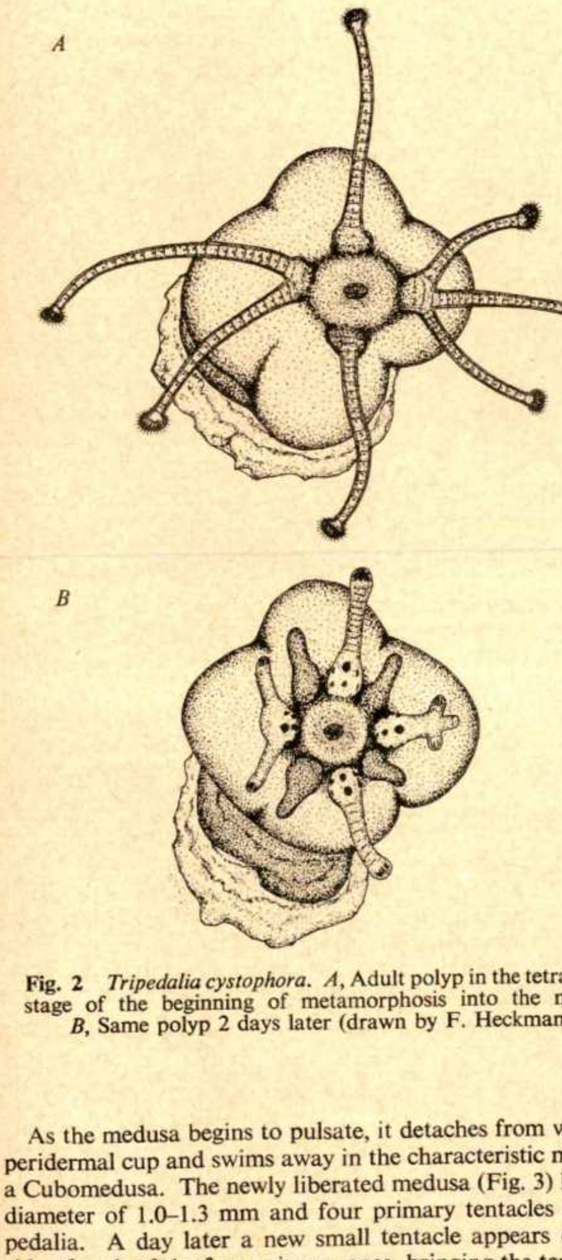


Fig. 2 *Tripedalia cystophora*. *A*, Adult polyp in the tetraradiate stage of the beginning of metamorphosis into the medusa; *B*, Same polyp 2 days later (drawn by F. Heckmann).

As the medusa begins to pulsate, it detaches from within the peridermal cup and swims away in the characteristic manner of a Cubomedusa. The newly liberated medusa (Fig. 3) has a bell diameter of 1.0–1.3 mm and four primary tentacles that lack pedalia. A day later a new small tentacle appears on either side of each of the four primary ones, bringing the tentacles to full complement. Later, the proximal part of each tentacle flattens to become the pedalium. In 10–12 weeks the medusa attains full size and sexual maturity.

Several features of the cubopolyp distinguish it from both a hydropolyp and a scyphopolyp. The more salient of these are the total conversion of the polyp into a medusa, a radial symmetry, and the absence of most of the typical scyphopolyp features. These, together with the morphological peculiarities of the medusa generation, suggest that the Cubomedusae may not be Scyphozoa at all. They possibly may represent an evolutionary link between the Scyphozoa and Hydrozoa and conceivably could be considered to comprise a taxon of comparable rank.

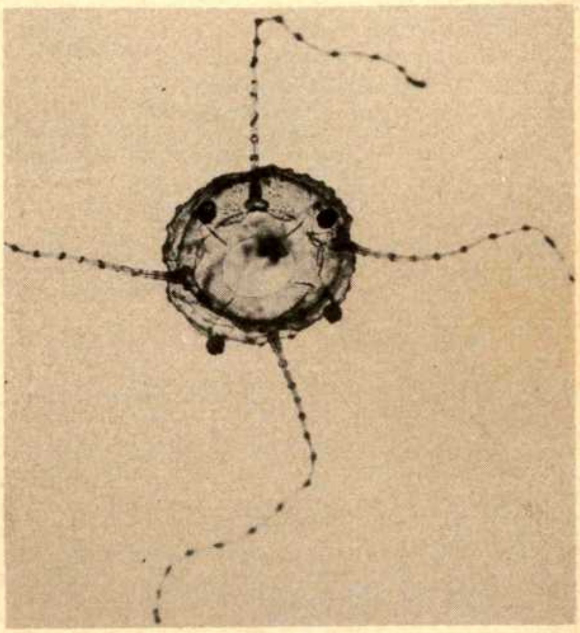


Fig. 3 *Tripedalia cystophora*. Young medusa just after metamorphosis from polyp, reared in laboratory; bell margin above; diameter of the bell is 1.3 mm (photograph by B. Werner).

This study was conducted at the Biologische Anstalt Helgoland and the Department of Marine Sciences, University of Puerto Rico, Mayagüez. It was supported by a grant from the US Department of the Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, with matching funds from the University of Puerto Rico and was coordinated by the Fisheries Development Program, Department of Agriculture, Commonwealth of Puerto Rico. We thank Sir Frederick S. Russell for reading the manuscript.

BERNHARD WERNER

Biologische Anstalt Helgoland,
2 Hamburg 50,
Palmaille 9

CHARLES E. CUTRESS
JOHN P. STUDEBAKER

Department of Marine Sciences,
University of Puerto Rico,
Mayagüez

Received April 27, 1971.

[1] Conant, F. S., *Mem. Biol. Lab. Johns Hopkins Univ.*, **4**, 1 (1898).
[2] Okada, Y. K., *Bull. Biol. France Belg.*, **61**, 241 (1927).
[3] Chapman, D. M., in *Symp. Zool. Soc. London* (edit. by Rees, W. R.), **16** (Academic Press, London, 1966).
[4] Thiel, Hj., in *Symp. Zool. Soc. London* (edit. by Rees, W. R.), **16** (Academic Press, London, 1966).
[5] Uchida, T., *Bull. Mar. Biol. Stat. Asamushi, Tôhoku Univ.*, **13**, 247 (1969).
[6] Werner, B., *Zool. Anz. Suppl.*, **33**, 159 (1970).

BOOK REVIEWS

Science and Society

Science, Man and Society. By Robert B. Fischer. Pp. viii+124. (Saunders: Philadelphia, London and Toronto, March 1971.) £1.25.

The Scientist's Role in Society : a Comparative Study. By Joseph Ben-David. Pp. xi+207. (Prentice-Hall: Englewood Cliffs, New Jersey, June 1971.) $6.95 cloth ; $2.95 paper.

DR FISCHER observes that traditional science courses are organized around particular parts of the subject matter of science. They do not give insight into the relevancy of science to man and to society, when people have to face serious social and political issues in which science is a major factor.

Various approaches are being tried to remedy this situation. One is for the science faculties to turn the problem over to the faculties of philosophy and history. Dr Fischer dismisses this as "passing the buck", as it usually turns out to be no solution. Courses in science may be replaced with courses about science. He thinks this is liable to leave the student with no clear grasp of any important principle. Nor is he in favour of organizing science teaching around topical themes such as pollution: these are subject to fashion. His book contains the selection which he considers suitable.

He stresses that science is a dynamic, human activity, limited to things accessible to the senses. He warns against non-dynamic words such as "systematic" and "organized". The understanding of concepts is gained from experience, and, as Einstein says, "the whole of science is nothing more than a refinement of everyday thinking".

Dr Fischer quotes briefly sketches of Priestley, Cavendish and Faraday as contrasting scientific types. Then he compares the scientists of today with those of two centuries ago. They have more to learn, and even then are deeply acquainted with only a limited field. The long training uniformalizes their knowledge and habits, to fit them for niches in the vast modern scientific activity.

He relates the present crisis in universities to the growth of science. The universities have become a complex of competing subjects and factions, described by Clark Kerr as the "multiversity", in contrast to the ancient "university". Some of the courses, intended to deepen students' general understanding of science, have increased their alienation. The more they know about science, the less they like it. Man has got to the Moon, but not solved the problems of poverty and crime.

It is essential to emphasize that science is subject to man, who is a thinking, feeling, doing being. It is up to man to decide whether science shall be allowed to have dehumanizing effects.

Dr Fischer has written a wise and useful little book which should be helpful to many students, and their teachers.

What help is provided by Dr Ben-David's sociological monograph? He discusses the development of science especially in Britain, France, Germany and the United States. He says that "social conditions do not generate intellectual ability and moral responsibility, they only provide the conditions for their exercise". Accordingly, he interprets the foundation of modern science in seventeenth century Britain as due to people who found empirical natural science "a cognitive structure consistent with their interests in a changing, pluralistic and future-oriented society". The conceptual development of empirical natural science was, he says, quite independent of social circumstances.

In the society of twentieth century United States, the "enterprising system of universities working within a pluralistic, educational, and economic system has created an unprecedentedly widespread demand for knowledge and research and has turned science into an important economic resource". This system has encouraged scientific creativity for its own sake by creating a very wide foundation for pure research. However, the system is in a state of delicate balance between scientific and scholarly creativity and the demands of the economic and political powers. This balance is more delicate than elsewhere because "the pluralistic entrepreneurial structure and expansive system of science and higher education require a much greater involvement of the universities in the affairs of society. This is the price paid for the greater support of science and scholarship". A large section of American youth now receives university education in science. Consequently, "in spite of the greater decentralization of political and economic life", US universities have become "one of the most suitable centres of political activism".

Dr Ben-David believes that there was a comparative decline in British science in the second half of the eighteenth century. It is true that Newtonian science declined, but he does not see that that was already no longer in the first line. The industrial revolution posed new problems, symbolized by the steam engine and the concept of energy, which led to modern physics. Science in Britain changed its direction, founding the line of the future. In France Lagrange and Laplace carried Newtonian science to its apogee, but they were completing old science rather than founding new. If the theory of gravitation is the scientific apotheosis of the British mercantile civilization, the theory of energy is that of the industrial revolution. Ben-David does not perceive in these major social movements examples of the prime forces which have created science.

He comments on the intellectual freedom in German universities in the nineteenth century, while they were the media for nationalistic and anti-semitic propaganda. He says: "it is difficult to determine with any certainty the reason that so many of the 'liberal' elements accepted this situation". Then, he remarks that in the early 1930s "the visiting scientists saw the German university in the light of its ideal self: as a seat of the purest learning for its own sake and an unequalled centre of overall excellence".

In fact, as a visitor to Germany at that period, I was painfully struck by the gap between the high Berlin scientific life and the brutalities done to and by the rank-and-file population. The reason why German scientists put up such a poor show against both German imperialism in the nineteenth century and Nazism in the twentieth was that they had a false philosophy. They believed that "science" was a thing in itself, an affair of the "scientific community", an exalted intellectual endeavour, above the vulgarity of common affairs. Dr Ben-David's book illustrates why "passing the buck" to the sociologists also provides no solution of the problem of science, man and society.

J. G. CROWTHER

Objective Sense

Rationality. Edited by Bryan R. Wilson. (Key Concepts in the Social Sciences.) Pp. xviii+275. (Blackwell: Oxford, November 1970.) £2.75.

ALTHOUGH this collection will particularly interest investigators who regard human actions or utterances as a necessary part of their data, those with a general interest in the nature and scope of scientific thought should also find it rewarding. The contributions represent a coherent tradition of inquiry in areas too abstract and complex for easy intelligibility, but lucid writing, with a minimum of esoteric terminology, comes close to eliminating the difficulties involved.

The central issue is how social scientists should formulate a general strategy for understanding and explaining human beliefs and actions. Such a strategy, it is agreed, must take account of the subjective, meaning which distinguishes "action" from "behaviour". Compare a human being seeking money with a rat seeking food. The desire for money becomes intelligible only through its meaning—its subjective significance in a wide range of social relationships. So for beliefs and actions in general; they can be understood only contextually, as elements in meaningful systems; particular beliefs are intelligible only in so far as the total context of belief is intelligible.

This raises important problems concerning the degree to which mutual understanding is possible between any set of actors. It is in the work of social anthropologists, however, that these problems emerge with particular clarity, and the understanding of alien cultures consequently provides the principal subject of discussion here. How can we know when the meaning of an alien utterance has been captured in our own language? To what extent can the properties of alien belief systems be assumed to resemble our own? And what is to be made of beliefs which seem to us self-evidently false, contradictory or otherwise irrational?

With respect to this last question, two basic positions can be identified. One accepts the existence of "irrationality" and seeks to account for it as such: it is the product of bias, or of a mistaken world view, or illogicality: it persists in a culture because social norms discourage rational criticism, or because scientific method is not available to refute it. The other holds that seeming irrationality can become intelligible if its relationship to its context is more closely scrutinized: if beliefs are given significance only in relation to those actions with which they are concretely associated, their "irrationality" may disappear or become irrelevant: if contextual evidence can make a metaphorical or symbolic interpretation of beliefs plausible then, again, all is well.

The intensity of this conflict unfortunately obscures the fact that the two positions are not mutually exclusive. Irrational or false beliefs, as here defined, are endemic in all societies, and it is hard to apply symbolic explanations everywhere (consider, for example, the "irrationality" which these authors painstakingly demonstrate in each other's work). On the other hand, to interpret the Nuer belief that human twins are birds as symbolism is at least more promising than to treat it as a curious empirical error. Moreover, it is not difficult to combine the approaches: given that there are many ways of being "irrational", symbolism can help to explain why particular examples occur in particular contexts.

Both approaches assume that "irrational" beliefs need different explanatory treatment to "rational" ones, and hence that it is, in principle, possible to distinguish the two by external objective standards. But, in spite of its title, the book does not give this topic detailed consideration; "irrationality" is usually the starting point for analysis, not its product. Crude models of rationality, usually drawn from the philosophy of science, serve as a sketchy backdrop to the problems already discussed. Empiricism lies behind puzzlement at the incidence of false beliefs. Popper's philosophy is linked with interest in why alien actors hold beliefs uncritically. (Unfortunately, P. Winch relates his "all is rational" view of alien belief systems not to T. S. Kuhn but to their common precursor Wittgenstein, thus spoiling the pattern!)

What does emerge is that if objective criteria of rationality exist there is at present no hope of a consensus on what they are. It could in any case be argued, against the dominant approach in this volume, that such criteria are of little interest to the practical investigator. If he must account for the existence of "false" beliefs, he must do no less for "true" ones; if dogmatic adherence to beliefs is problematic, so too is open-mindedness and provisional commitment. Much of the discussion in this volume could be reformulated to apply to the aetiology and ecology of all beliefs, and merely loses perspective by being confined to the analysis of "irrational" residues. Robin Horton's admirable comparison of African traditional thought with that of Western natural science demonstrates the value of widening the scope of the debate, treating our own beliefs with genuine curiosity.

I would agree that any paradigm of rationality must be broadly compatible with the procedures of the natural sciences. Given the rich variety of scientific thought, however, and the way it consistently eludes any single formal description, I am led to adopt what Gellner would call a "charitable" view of alien belief systems, based on the weakest possible rationality criteria. Those who would use stronger or more objective ones should consider, among other things, how much of modern science they would thereby define as irrational. This position comes closest to that of Winch, who nevertheless misuses his own arguments. By treating all belief systems as rational, he does not show that beliefs are immune to causal explanation, but that the same forms of causal analysis can be universally applied to all beliefs—something not yet fully recognized, at least within the social sciences.

S. B. BARNES

Natural Selection

Coefficients of Natural Selection. By L. M. Cook. (Biological Sciences.) Pp. 207. (Hutchinson University Library: London, February 1971.) £2.50 boards; £1.25 paper.

THE problem of explaining mathematical arguments to biologists has driven many a promising teacher to despair. The biological undergraduate, and occasionally even the graduate, is prone to go into shock when faced with an algebraic equation as complex as $a = \frac{bc}{d}$. Nowhere is the problem more acute than in population genetics, where the underlying arguments are largely mathematical but the observations and experiments are firmly based in natural history. Dr Cook has attempted the dreadful task of bridging the chasm between them, and he has largely succeeded.

His book is neither a text for undergraduates, although there are many who would benefit from it, nor an account of methods in research. It is a stimulating, original and idiosyncratic assay about the genetic analysis of natural and experimental populations. He leads his readers gently through the complexities of selection differentials, the calculation of equilibria, and the analysis of selective forces depending on gene-frequency and population density. At times, understandably, he falls into the trap of rendering an argument more obscure by explaining it too much. Less often, but more seriously, the reader may be led astray by misprints (as on pages 29, 43 and 44) or other errors (as on pages 59, 142 and 160). There are some surprising gaps. For example, he chooses to discuss the phenomena of non-random mating without mentioning the work of Ehrman or Spiess.

These are matters, however, that can be remedied if, as devoutly we may wish, the book reaches a second edition. Its virtues far outweigh its vices. An example of these virtues, to a statistically naive reviewer, is a uniquely lucid exposition of the method of maximum likelihood.

I believe that the value of the book will prove to be inversely proportional to the mathematical ability of the reader. It should be welcomed by biologists and neglected by mathematicians.

BRYAN CLARKE

Food and Safety

Metabolic Aspects of Food Safety. Edited by Francis Roe. Pp. xxiii+612. (Blackwell: Oxford and Edinburgh, 1970.) £7.

ABOUT ten years ago the Nuffield Foundation became concerned with the toxicological risks associated with the increase in chemical and physical treatment of food. To evaluate the hazards involved it is necessary to work with small laboratory animals, but lack of knowledge of their diseases made the investigations difficult. In 1966 the first food safety conference lead to the publication of a useful book, The *Pathology of Small Laboratory Animals.* In 1969 the second food safety conference considered the tests used to assess the safey of food additives and contaminants, and the problems that arise in interpretation of experimental results. It is not as easy as might be supposed to decide whether a compound is toxic to an animal even in the conditions of the experiment, without the additional problem of extrapolation to a human population and the conditions in which the compound is or could be consumed. As toxicology is studied by physiologists, biochemists and electron microscopists, there was a need for different people concerned to meet to discuss the problems. The book of the conference is disappointing, although it should be of some use to those engaged in this work.

"Metabolic Aspects" is a very misleading title, as the book is not concerned chiefly with the metabolism of food constituents, additives or contaminants, or with their effects on metabolism of the animal. "Biological Aspects" would have been more appropriate. A very heterogenous collection of twenty articles discusses basic physiology of the small intestine and of gastrointestinal absorption, renal function tests in animals and in man, and the significance of such factors as gut flora, age, sex, hormones and nutritional status of the experimental animal. The fact that protein deficiency makes animals less sensitive to compounds such as heptachlor which exert their toxic effects after metabolism, and more sensitive to compounds such as aflatoxin, the toxicity of which probably does not depend on their metabolic conversion, underlines the difficulty of applying results both to the affluent society and to the undernourished countries.

One basic problem is whether any effect produced by a chemical should be regarded as evidence of toxicity, even if it does not apparently inconvenience the animal. Thus many food additives cause an increase in liver weight, and the problem of whether this is harmful or possibly even beneficial is discussed. Obviously to assess the significance of the increase in liver weight caused by chemicals it should be compared with changes occurring naturally. However, in the present context, the long detailed discussion of the increase in liver weight occurring during pregnancy and lactation seems out of place, in the absence of sufficient comparison with chemically induced enlargement. Only a shrewd question in the discussion brought out a fundamental difference: chemical-induced liver enlargement is frequently accompanied by hypertrophy of the smooth endoplasmic reticulum, that occurring during reproduction is not. The important question of the reversibility of natural and chemically induced changes is barely mentioned. The problem of whether an increase in weight is harmful leads to the question of what controls liver weight, and of the mechanism by which the changes are brought about. Thus it is essential "to find out not only what happened but how it happened" and to study the basic biochemical processes before the results of toxicity tests can be evaluated. Two good chapters get to grips with the problem in this way. One discusses metabolic aspects of liver tumour induction in animals by compounds known or suspected to be food contaminants, and the other discusses the ultrastructural changes.

Although apparently the aim of the book is to have a wide scope rather than to study any particular problem in depth, there are some important omissions. There is no mention of possible hazards to the brain, although the vulnerability of the central nervous system had been demonstrated spectacularly and tragically ten years before, when methyl-mercury contamination of fish was shown to be responsible for Minamata disease in Japan. More surprisingly teratogenesis is not discussed, although dyes, fungal metabolites and other possible food contaminants are known teratogens. The basic physiology, biochemistry and tests of kidney and liver function in man, are dealt with in standard textbooks. If these subjects could have been replaced by discussion more directly relevant in the present context, the book could have gone much further into the problems and would have been a more valuable contribution to the subject.

V. M. CRADDOCK

Tsetse and Ecology

The Role of the Trypanosomiases in African Ecology: a Study of the Tsetse Fly Problem. By John Ford. Pp. xiv+568. (Clarendon: Oxford; Oxford University: London, March 1971.) £6.50.

THE change of title of the East African Tsetse and Trypanosomiasis Research and Reclamation Organization (EATTRRO) to East African Trypanosomiasis Research Organization (EATRO) in 1956 signified a change of approach—from one in which the prime emphasis was on the insect vectors of trypanosomiasis—*Glossina* (tsetse flies)—to a more balanced one in which medical, veterinary, entomological and ecological aspects were to be studied as a whole. Also, the first organization was concerned with both research and reclamation, the second solely with research. John Ford served for many years with the earlier organization, only briefly with EATRO, as director, until he went to Rhodesia as director of tsetse control in 1957. He is primarily an entomologist, but a very thoughtful one, and his book is a fine attempt to put *Glossina* in proportion among the many other ecological factors which affect the African scene. This is a very different approach from that of the generality of British glossinologists, which was that the elimination of *Glossina* was a certain way of controlling trypanosomiasis; that this should be stressed to the near exclusion of all other approaches; and that economical control consistent with the poverty of the African territories affected should be able to be attained by minor interference with the *Glossina* vegetational environments—if only the ecology of these flies were sufficiently understood.

After extended experience of this approach in East Africa, Ford worked in Rhodesia where game elimination as a *Glossina* control measure had been particularly practised. He subsequently visited and studied in West Africa. He has, therefore, a unique first hand acquaintanceship with three different African regions, all sharply affected by trypanosomiasis, but in different ways. The description of trypanosomiasis situations in these three regions form the core of the book. After five introductory chapters giving essential basic information about the organisms concerned—trypanosomes, *Glossina,* animals and man—Ford devotes nineteen chapters to describing five sample areas, three in East Africa and one each in Rhodesia and in Nigeria. He ends with two summarizing chapters, one considering trypanosomiasis in its continental ecological aspects, the other proposing a new approach.

Basically, Ford's thesis is that, before the advent of the Europeans in Africa, the indigenous peoples had made very considerable advances towards overcoming the obstacle of trypanosomiasis by physiological and cultural adjustments in themselves and in their domestic stock and that this fact is usually overlooked. He considers the epidemics of trypanosomiasis in man and animals since the turn of the century to be direct results of the political and ecological aspects consequent on

European colonization, which acted particularly by disturbing the uninhabited no man's land (Grenzwildnisse) which separated tribal areas and kingdoms and which provided mechanisms for adjustment between different tribal groups and with their environment.

Ford marshals an impressive amount of information to support his thesis, delineating the development of affairs in all his five areas. The reader new to the field may find the diversity of the material bewildering, including, as it does, geology, palaeontology, historical fragments, excerpts from the diaries of early travellers, oral tradition, geographical description, entomology, and human and animal demography, all developed together with great profusion of detail so that any individual body of data is difficult to isolate, examine and assess. Nevertheless, the result is of great interest and, whether one agrees or not with Ford's thesis, the book will always be valuable, if only for the amount of data, otherwise difficult to obtain, amassed within it.

In his final chapter, Ford is apprehensive of epidemiological upsets at the termination of the colonial empires as profound as those which attended their establishment and cites present indications from the Congo and the Sudan. He emphasizes, again, the need to relate disease control to expected economic growth rate. He urges the development of the mathematical epidemiology of trypanosomiasis. He laments the neglect of entomologists to study, at the same time as the habits of their insects, also the habits of the people and the animals bitten. He hopes for a better balance under political freedom than has happened under colonial rule. This is questionable and indeed, under the more general approach to trypanosomiasis study in recent years, this lack has been realized and much done to repair it. To my mind, however, much of the uncertainty and misapprehension in the field of trypanosomiasis does indeed derive from the paucity of integrated studies. Trypanosomiasis contrasts sharply in this respect with some other tropical diseases, such as yellow fever, in which integrated teams fairly rapidly worked out the main essentials of the epidemiology, in both South America and Africa.

Considerable progress in integrated studies was indeed made by the East African Trypanosomiasis Research Organization and is indeed still continuing in spite of the dislocation caused by the loss of long-experienced personnel consequent on the granting of independence to East African territories in 1962–63.

The book is well illustrated with line diagrams and is fully indexed. There is a bibliography of some 600 sources.

W. H. R. LUMSDEN

Molecular Pharmacology

Essentials of Molecular Pharmacology: Background for Drug Design. By Andrejus Korolkovas. Pp. xv+339. (Wiley Interscience: New York and London, November 1970.) 160*s.*

THE term molecular pharmacology is of recent currency but may be taken to indicate a new determination among pharmacologists to account for the nature and specificity of drug action in terms of a detailed description of events at the atomic level. Speculation at this level has of course been rife for years but hard facts have been few, and it is really the arrival of new techniques, notably X-ray crystallography of proteins, that have given a great new impetus to this challenging subject. This book deals with the major areas of activity so far including adequate accounts of intermolecular forces and the analysis of chirality. The account of drug–receptor interactions is imaginative and stimulating, but it is here that the weaknesses in contemporary pharmacological arguments become so clear—the argument is based on analogy with enzyme–substrate interactions (themselves not understood unequivocally), and extrapolations from structure activity studies.

The position is rapidly developing where drug binding directly to receptors is possible so that the analogies may be put aside, but as the X-ray studies of binding of enantiomeric glycosides to lysozyme, and isomeric nucleotides to ribonuclease have shown, changes in structure of the ligand may entail a major change in the geometry of the complex that cannot be predicted from structure activity studies. The implication is that structural techniques, particularly X-ray crystallography and nuclear magnetic resonance, must be decisive tools for the molecular pharmacologist.

The theories of drug action discussed in this book are bound to become rapidly clarified as the study of isolated receptors proceeds. It is interesting to see how history repeats itself. The isolation and crystallization of enzymes was inhibited by vitalistic concepts, the isolation of receptors was similarly inhibited by beliefs that receptors could not survive isolation from their environment in membranes; lo and behold, when isolated they are as tough as old boots. Nevertheless, we must not be carried away by excessive optimism, isolation of receptors will not answer all the questions of drug action any more than isolation of enzymes solves the problem of how enzymes work, but it does have the enormous advantage of replacing mystery by tangibility.

For the rapid development of molecular pharmacology that is now just beginning, this book provides a useful and interesting primer that can be strongly recommended.

A. S. V. BURGEN

Complex Chemistry

Organometallic Chemistry. Edited by F. G. A. Stone and M. I. Bruce. (Plenary Lectures at the Fourth International Conference on Organometallic Chemistry, held at Bristol, UK, July 28–August 1, 1969.) (International Union of Pure and Applied Chemistry in conjunction with the Chemical Society, London. *Pure and Applied Chemistry,* Vol. 23, No. 4, 1970.) Pp. 375–503. (Butterworth: London, 1970.) £3.50.

PUBLICATION, in a hard-cover version, of the proceedings of symposia of the Internation Union of Pure and Applied Chemistry has complemented, for the past few years, their normal appearance in the journal *Pure and Applied Chemistry.* One would not have guessed that such a double-barrelled approach would have been profitable for the publishers—it must be a tribute to the balance and high quality of reviews which appear in this volume and in its predecessors.

So far, the only characterized organometallic complex occurring in nature is vitamin B_{12}, and its chemistry is better understood now through the systematic studies of metal complexes of porphins, corrins and corroles. These are reviewed by A. W. Johnson. K. Schlögl concentrates his attention on chirality in the metallocenes and the summary serves as a general reminder that the optical activities of organometallic catalysts and stereospecificity of polymerization reactions could be a renewed focus of interest in the near future. Gubin surveys the information which electrochemical methods have given on energy levels and bonding in the metallocenes and cyclopentadienyl metal carbonyls and Chini completes our exposure to transition metal chemistry by a succinct summary of the chemistry of a number of carbonyl clusters of the iron and cobalt triads.

It is good to see main group organometallic chemistry so well represented. Brown gives a particularly nice account of nuclear magnetic resonance studies of structure and exchange reactions of organolithium compounds; Seyferth summarizes insertion reactions of methylene bridges into group IV hydrides and halides while Neumann emphasizes the free radical chemistry of organotin compounds.

Obviously, individual volumes can have no claim to comprehensiveness but together they represent a most valuable addition to the secondary literature.

R. MASON

CORRESPONDENCE

Metric Chains

SIR,—You chose an unfortunate subject for your aside upon shortening cricket pitches “. . .(. . . a non-metric measure—who now knows what a chain is?)”[1].

It is unfortunate that, with the impending change to the metric system, we do not much use chains and furlongs and even the infamous rod, pole or perch, for they correspond respectively to 20 m, 200 m and 5 m with an accuracy of 0.6%.

Yours faithfully,

S. D. STEEL

Department of Inorganic,
Physical and Industrial Chemistry,
Donnan Laboratories,
Grove Street,
PO Box 147,
*Liverpool L*69 3*BX*

[1] *Nature*, **232**, 433 (1971).

Telescope Administration

SIR,—Twice each year your advertisement pages invite applications for observing time on the Isaac Newton Telescope. Lest any potential applicant be deterred by the “widespread feeling” noted in your editorial (*Nature*, **232**, 289; 1971) that the Royal Greenwich Observatory exerts a detrimental influence on the running of this telescope, perhaps you would allow an informed comment.

If “running” refers to the day-to-day management and maintenance of the telescope, then indeed any neglect here would, as stated, be inimical to the progress of British astronomy. But if this is what is alleged, the proper place to complain is on the spot at the time. No such complaints are in fact made by visiting observers, and those responsible for the care of the telescope must be forgiven if they decline to notice anonymous allegations by others, especially when they are wholly unsupported by facts.

If, however, the criticism is now, as it has been in the past, of the observatory's alleged monopoly in the design and construction of ancillary equipment, it is time to reiterate that no such monopoly exists. Any successful applicant for observing time may bring his own equipment—many of them do—and the only requirement is that it shall be compatible with the telescope itself. As to the larger spectrographs, it is true that these are of RGO design. It is equally true that had the Astronomer Royal not taken the initiative to get them built, no spectrographs for common use would now exist. It is this situation that would indeed have been inimical to the progress of British astronomy.

But maybe the rub lies in the allocation of observing time. This is done twice a year by a Science Research Council panel of six working astronomers, not all of them in the optical field and only one from the Royal Greenwich Observatory. Selection among the applications received is strictly according to the scientific merit of the programmes proposed, and I am sure the members of the panel would repudiate any suggestion that they unduly favour RGO applicants. It is true that RGO applications are in fact awarded more time than any others; but they are in a substantial majority and the panel plainly adjudges them more worthy of support.

If there are those who find the basis of allocation unfair, let them suggest a more equitable one. If they allege sinister influences by the major applicants on the process of allocation, let them produce something more substantial than hints in the scientific press.

Yours faithfully,

A. HUNTER

Secretary, Large Telescope Users' Panel,
Royal Greenwich Observatory,
Herstmonceux Castle,
Hailsham, Sussex

SIR,—Your article on “New Astronomy” (*Nature*, **232**, 289; 1971) was informative and interesting. Before commenting on points of substance, however, may I correct one misunderstanding which the article revealed ?

I refer to the “widely held” criticism, attributed to “many astronomers” who see the RGO as a “white elephant”—on the grounds, according to you, that the £1.2 million which the SRC spends on the RGO, the Royal Observatory Edinburgh and on the observatories in South Africa, gives poor returns compared with the running costs of the Hale Observatories of $1.7 million a year.

The Hale Observatories are not government observatories and have no national responsibilities. They spend nothing on the equivalent of the Nautical Almanac Office, the Time Service and the Meridian and Astrometry Departments of RGO which take up some 57% of the total RGO budget. If you then compare the remaining 43% of the RGO budget, plus the full cost of the South African Observatories and the Royal Observatory Edinburgh, the total comes out for all practical purposes the same as the cost of the Hale Observatories—in round figures, £700,000.

You were, of course, right to draw attention to the disquiet of the scientific staff of the RGO about the future of their scientific work. In part, the bad staff relations in the Science Research Council are a cause of this. The fact that they were given a categorical assurance that they would be consulted about any proposal that the Astronomer Royal post would be divorced from the directorship of the RGO, but nevertheless were not consulted about it at all, is only an unusually dramatic illustration of why the management is losing the confidence of its staff. Nor does the explanation which has now been given make things any better.

But it is the affair of the Northern Hemisphere telescope which has caused the greatest anxiety. As you point out, there have been powerful pressures in favour of creating a third administrative centre in the UK for the Northern Hemisphere telescope. This should be a scientific and administrative responsibility of the SRC as such, one of whose responsibilities it is to provide facilities for common use by a variety of university scientists. The logical site for the centre is at the RGO—or, alternatively, at the Royal Observatory Edinburgh—but it appears as if there has been tremendous opposition to this. To establish one university rather than another as a third centre for the handling of common facilities would surely be contrary to the purpose of the SRC, as well as being unnecessarily costly; and there is no point in having a third centre all by itself.

It is also the function of the SRC to do its own research at its own establishments, and a considerable number of first-rate scientists are employed on its staff for this very purpose. Collaboration with universities in the use of scarce facilities and equipment held in common by the SRC is fully accepted by the staff and by the institution. It is one of the purposes of SRC to bring about this collaboration, and such differences as there may be from time to time between the SRC's own scientists and those from universities should be concerned with nothing more than matters of balance. The affair of the Astronomer Royal has raised the question, however, of whether it is a question of balance. The policy of the Science Research Council is in the hands of the majority of university representatives on the council, and in a period in which there is keen competition for the relatively limited amount of money available, the SRC's own scientists are looking for reassurance about the long-term standing of their own research work.

Not only are they not getting it, but they can see no means by which, within the SRC as it stands, they can right the balance if it tips too much in the universities' direction.

Yours faithfully,

JOHN LYONS
Deputy General Secretary.

The Institution of Professional Civil Servants,
Northumberland Street,
*London WC*2

Orbital Planes of Planets

SIR,—Recently (April 26) we attended a lecture at the University of the South, Sewanee, Tennessee, by Dr Peter Van de Kamp of Sproul Observatory, Swathmore, Pennsylvania. He spoke on "The Search for Extra-Solar Planets", his work for 35 years, which indicates that Barnard's star has two planetary companions bearing a similarity to Jupiter and Saturn.

This lecture restimulated an idea held by one of us (Wells) for several years. We know the ecliptic plane is practically at right angles to the plane of the galaxy, but we don't know if this is only by chance or of deep significance. After the lecture Dr Van de Kamp was asked if enough was known about the orbits of stars with companions to determine orbital planet orientation. He said he had not considered it but some work might well be devoted to the question. He commented on the planes of binaries as being apparently random.

During the 70 mile drive home, we discussed this further. It was decided that, since Dr Van de Kamp had not investigated this relationship, it may not ever have been properly considered.

It seems that one of the most fundamental bits of information that could come of extra-solar planet searches would be a determination of the distribution of orbital planes. If other solar systems besides ours should be found with the orbital planes also at right angles it would be suggestive, with probable implications for galactic evolution, as indeed would any significant deviation from random orientation of orbital planes.

Should a preferred orientation be established, it could have a great bearing on theories of galactic dynamics. It would seem that our solar system may have been formed approximately 90° from the present galactic location initially, since difficulties are foreseen in trying to reconcile the ecliptic angle with fluid mechanics or condensation theories.

If we should be able to determine the plane of other ecliptics around other stars, it would greatly simplify the search for extra-solar planets by determining the directions from the stars to expect planets; a great advantage in radio astronomy or other scanning methods. For instance, Project Ozma was apparently limited in scan directions.

It was felt that this letter might bring comments from others on this question and help keep it before the attention of astronomers who might be able further to evaluate and pursue the phenomena.

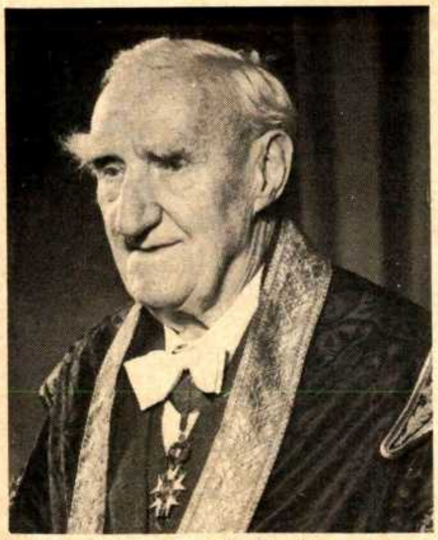
Yours faithfully,

ERNEST H. WELLS
DANIEL PAYNE HALE

National Aeronautics and Space Administration,
George C. Marshall Space Flight Center,
Huntsville, Alabama 35812

Obituary

Lord Boyd Orr



LORD BOYD ORR of Brechin Mearns, Nobel Peace Laureate, Chancellor of the University of Glasgow, first director-general of the United Nations Food and Agricultural Organization, first director of the Rowett Research Institute and founder of the World Food Council, died on June 25 at the age of ninety.

John Boyd Orr was a graduate in arts, science and medicine of the University of Glasgow, where he also obtained his MD and DSc. His first external appointment was to a new research post in Aberdeen linked to the College of Agriculture and the university. This subsequently grew into the Rowett Research Institute. However, before he could get established the First World War began. Until 1917, he served with the RAMC, winning the DSO and MC with bar. Later he undertook an investigation of the physical requirements of servicemen for the navy.

Some of his first experience in nutritional investigation was gained in collaboration with the late Professor E. P. Cathcart at Glasgow. Here he worked chiefly on water and protein metabolism and on energy expenditure during work. There he was influenced by research on the diet of the working classes. On moving to Aberdeen, he took the advice of Cathcart and studied the mineral metabolism and needs of farm livestock, developing both topics with characteristic energy and insight. Some of his most impressive work was on mineral deficiencies and soil exhaustion in relation to pasture production both at home and abroad. This led him to establish a clinical unit to investigate the effects of husbandry techniques on the human populations of Masai and Kikuyu. It also established his interest in nutrition and disease and their interrelations.

He was responsible for great improvements in the production of meat, wool and milk, and helped to bring prosperity to the livestock industry. He gave the farmer more precise knowledge in the selection of food mixtures as a substitute for growing pastures, particularly in the wintering of his stock. He also gave some definition to the biochemical and immunological changes which precede and accompany the general symptoms of disordered metabolism caused by nutritional deficiency or imbalance. After starting work at the institute in a cellar of Marischal College, Orr received the financial backing of such men as Dr John Quiller Rowett (after whom the institute was named) and was able to establish at the institute the Imperial Bureau of Animal Nutrition.

Orr's professional concern was with animals of agricultural importance, but he found that whereas he had no difficulty in persuading farmers of the value to their stock and pockets of the application of sound nutritional principles, he could not convince anyone that the same was true of children. At that period the nutrition of man himself was an art rather than science, empiricism rather than experimentation, general impressions rather than controlled observation. But now, the newer knowledge of nutrition was growing, requirements could approximately be defined, and dietary surveys could expose the gap between requirement and that consumed. A large scale demonstration of the nutritious value of milk for school children partly done to promote the increased consumption of milk for the benefit of the dairy industry, but primarily done to promote the health of the children whom he suspected of being undernourished, led to further work on human nutrition. With government financial help he undertook the survey which became a classic: *Food, Health and Income,* and was first published in 1936 and had world-wide repercussions.

Then there followed the more extensive dietary and clinical surveys of representative groups of families with children of varying income levels in many parts of Britain, which further demonstrated the relation of food, health and income, and the consciousness of this on a world scale grew into a natural and later international food policy based on human needs. This was supported by Carnegie UK Trust funds. Such was the value of this accumulated knowledge of human needs and intakes that Orr was able to so advise the Government on the scientific food policy during World War II that we were able to emerge in a better nutritional state than when we entered, for rationing was based on needs and not on purchasing power. Further, these observations and knowledge displayed by Orr so impressed the United Nations that Sir John Orr—as he then was, having been knighted in 1935—was appointed at the Quebec Conference the first director-general of the Food and Agricultural Organization, coinciding with his retirement from the Rowett Research Institute in the autumn of 1945, and this post he held until 1948. It was on the platform or at the conference table that his biggest personal impacts were made.

He was lean with craggy eyebrows, long jawed, and although he had no tricks of oratory, the single-mindedness of his argument—one thing at a time—and his prophetic sincerity generally won the day. Some thought him naïve, but it was an expressed simplicity with a purpose. His hunches were very sound: he was sometimes impatient of the slowness of the research worker to corroborate what he felt in his bones to be valid and vital.

Orr inherited much of his strength of character from his mother, who was a Boyd. In 1915 he married Miss Elizabeth Pearson Callum, of West Kilbride. They were seldom separated and travelled the world together. She was a wonderful partner and ambassadress. He is survived by his wife and two married daughters. Their only son was killed on a Coastal Command mission during the war.

Announcements

International Meetings

September 15–17, **British Society for Cell Biology Meeting,** Bristol (Dr J. M. N. Boss, Department of Physiology, Veterinary School, Park Row, Bristol BS1 5LS).

September 20–21, **Small Craft,** Southampton (Royal Institution of Naval Architects, 10 Upper Belgrave Street, London SW1X 8BQ).

September 21–23, **Infrared Techniques,** Reading (Conference Department, Institution of Electronic and Radio Engineers, 8–9 Bedford Square, London WC1B 3RG).

September 28–29, **Biochemistry, Genetics and Therapy of Schizophrenia,** London (Schizophrenia Association of Great Britain, Llanfair Hall, Caernarvon).

September 28–29, **Ultrasonics 71,** London (Mr A. H. Crawford, Ultrasonics 71, 32 High Street, Guildford, Surrey).

October 11–12, **Natural Slopes Stability and Conservation,** Naples and Cosenza (Dr G. Guzzetta, Istituto di Geologia, Naples, Italy).

October 18–22, **Eurocon 71,** Lausanne (Eurocon 71 Office, 24 Chemin de Bellerive, CH-1007 Lausanne, Switzerland).

November 10, **Medical Thermography,** London (Warren Cook & Partners, Woodfield House, Goudhurst, Kent).

November 11–12, **Coatings,** Great Malvern (M. G. Bader, Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey).

November 11–12, **Endocrine and Non-endocrine Hormone Producing Tumours,** Houston (Mrs Jane Brandenberger, Information Coordinator, University of Texas M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas 77025, USA).

Reports and Publications

not included in the Monthly Books Supplement

Other Countries

Australian Journal of Zoology. Supplementary Series. Supplement No. 1: The Tipuldae (Diptera) of Australia. 3: The Genus *Ptilogyna* Westwood. By N. V. Dobrotworsky. Pp. 41. (East Melbourne: CSIRO, 1971.) [195

Australian Academy of Science. Reports No. 13: Education and the Environmental Crisis. (Proceedings of a Conference held in Canberra, 24–26 April 1970 under the auspices of the Australian Academy of Science through its National Committee for the International Biological Programme.) Edited by Jeremy Evans and Stephen Boyden. Pp. 117. (Canberra: Australian Academy of Science, 1970.) [195

Evaluation of Food Additives: Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation—Some Extraction Solvents and Certain other Substances; and a Review of the Technological Efficacy of some Antimicrobial Agent. (Fourteenth Report of the joint FAO/WHO Expert Committee on Food Additives, Geneva, 24 June–2 July 1970. FAO Nutrition Meetings Report Series, No. 48. WHO Technical Report Series, No. 462.) Pp. 36. (Rome: FAO; London: HMSO, 1971.) 30p; $1. [205

National Academy of Engineering—Committee on Public Engineering Policy. Federal Support of Applied Research. Pp. 16. Priorities in Applied Research: an Initial Appraisal. Pp. 33. (Washington, DC: National Academy of Engineering, 1970.) [215

Mercury in the Environment—The Human Element. By Robin A. Wallace, William Fulkerson, Wilbur D. Shults and William S. Lyon. (ORNL-NSF Environmental Program. ORNL NSF-EP-1.) Pp. vii+61. (Oak Ridge, Tennessee: Oak Ridge National Laboratory, 1971.) [215

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature + Nature Physical Science	**£24**	**$83**	**$90**
Nature + Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, August 20 1971

NATURE Volume 232 AUGUST 27 1971

From Intelsat to Aerosat

REPRESENTATIVES of fifty-five countries turned up at the State Department in Washington last Friday to sign the inter-governmental agreement creating the new international communications satellite organization, Intelsat. The name of Intelsat, of course, has been wafting about for about seven years but it was referred to the temporary consortium, set up in 1964, to make a commercial global network out of communications satellites which at that time were merely in the experimental stages. The new Intelsat, unlike the old, will have juridical personality and an international executive. Yet the seven years of Intelsat's interim life were not happy ones. The practical success in putting geostationary satellites into service over the Atlantic, Pacific and Indian Oceans has been soured by the bitterness over the way that the organization representing the United Sates, Comsat, the communications satellite corporation, dominated the consortium. The European industrial nations who were among the participants of the original agreement never won anything like the share of the contracts that they had once hoped for. Of the dozens of costly Earth stations built around the world to work with the system, for which non-American suppliers were more readily available than for the satellites themselves, the great majority were built by American companies. So great was the resentment over this and over the American antagonism toward regional communications satellites outside the Intelsat system, that France, one of the original signers of the 1964 agreements, did not sign last week. Neither did Belgium, Mexico and West Germany, although all may at a later time.

In the new Intelsat the American influence is considerably reduced. Under the old arrangements, the United States held a majority of the voting shares and could not slip below 50.4 per cent. Britain was a poor second with less than 10 per cent (the percentages varied as new members—eventually 80—joined Intelsat). But the American dominance went beyond that. Comsat was the autonomous manager of the system and as such was not very forthcoming about the reasons and financial details about the contracts which it prepared on Intelsat's behalf. Both the American State Department and Comsat were dismayed to find, as the negotiations for a permanent agreement began, that Britain joined with the Europeans in fighting for a more internationally balanced organization.

That has been achieved but it is pyrrhic victory. In the first place, three expensive, tedious and acrimonious international conferences were needed to reach the two agreements (one for governments, one for telecommunications entities like Comsat and the British Post Office) which were opened for signature last week. In the second place, the organization that has resulted from all the wrangles and compromises is over-structured—over-defined to the point where it will be weak and inflexible in considering the new services which communications satellites may offer in the late 1970s and early 1980s.

The new Intelsat is to have four parts. The most powerful will be the Board of Governors on which the biggest investors will be represented, with their votes weighted according to the size of their investment. One restriction on Comsat has been built in here, for no country may have more than 40 per cent of the vote. The other significant thing about the board is that its representatives will be telecommunications men, which will severely limit Intelsat's capacity to develop satellites for air navigation, weather mapping or any kind of communications except public telecommunications services. Another part will be the Assembly, where each member country will have one vote. The Assembly will have no power to make decisions or to dictate to the Board of Governors. All it can do is to make recommendations about general policy—a victory for the United States and a defeat for the developing countries, which wanted a strong Assembly as they will have little or no voice in the Board of Governors. There will also be an international directorate to handle the day-to-day affairs of the organization. Nothing like this existed under the temporary arrangements. The directorate will be headed for the six years after the agreement comes into force by a secretary general, whose function will be to monitor Comsat in its performance as manager. Then after six years, he will be superseded by a director general.

The fourth part of Intelsat is the management, which handles the practical operations of the system. The contentious question—will Comsat continue or not?—has effectively been shelved for six years. Comsat will continue for that period but under a contract limiting its tenure and then by 1978 must apply for the job together with any other organization which may present itself. Comsat is by no means certain of holding on. A European aerospace consortium might by then be qualified to manage a satellite system. The Hughes Aircraft Company, the world's major satellite builder, would qualify now; so would the National Aeronautics and Space Administration. Thus the fights over management seem doomed to be prolonged and renewed during the very years when Intelsat, as it tries to pull itself together as a fully-fledged autonomous organization, should be coming to grips with more important questions, such as persuading the Russians to join, at least to agree to terms of practical cooperation between Intelsat and its own proposed Intersputnik international satellite system.

Just how limited Intelsat is in scope was shown by the agreement reached in Madrid earlier this month on an experimental aeronautical satellite system. The project was agreed on and will be carried out by the American Federal Aviation Administration and the European Space Research Organization and other participants. The plans are firm and clear: a pre-operational network of four geostationary satellites by 1974 with the aim of a fully operational network by 1980. It is hoped that a formal memorandum of understanding will be signed by November. Not only will the experiment have nothing to do with Intelsat, it will not even use Intelsat as a model. (Great hopes had been fixed upon Intelsat as a model for international commercial technological cooperation.) In fact, it will be at some pains to avoid Intelsat's mistakes.

A council called Aerosat is to be formed, composed of an equal number of representatives of the United States and Europe, and one representative from the government of each of the other interested countries (Australia, Japan and the Philippines). At the same time, there will be an executive office with its staff equally balanced internationally. The council will attempt to prepare a final document proposing the specifications of the desired satellite. There is no question but that the satellites will use the UHF band, something that both the FAA and ESRO have agreed on, to the chagrin of commercial international airlines who would prefer a less costly VHF system. The money—about $150 million—will be provided by the participating governments.

The advantages will be great. Various governments will not have to bear the expense of proceeding with aeronautical satellite experiments separately and perhaps incompatibly. Eventually separation between aircraft flying routes such as the heavily congested North Atlantic will be substantially reduced. The present methods, in which aircraft cannot be pinpointed more accurately than to within ten miles, requires a horizontal separation of 120 nautical miles and a vertical separation of 2,000 feet. The specifications laid down for the experimental project would permit aircraft to be placed within one half mile of their actual position and to fly as close as 30 nautical miles apart, separated vertically by 1,000 feet.

What Market for Computers?

THESE have been bad times for computer manufacturers in Britain, those owned by British interests and those which operate in Britain as subsidiaries of parent companies in the United States. For several weeks now, there has been a steady stream of news of how computer manufacturers have been shedding their labour forces. A week ago, the Department of Trade and Industry provided statistics to show how sales of computers have declined both in Britain and in the British export market. The decline of business has been dramatic. The total deliveries of computers from British manufacturers declined between the last quarter of 1970 and the first quarter of 1971 from £79 million to £64 million, a decrease of 18 per cent. This is more than a seasonal effect. Deliveries of computers have risen steadily, quarter by quarter, since 1966 but appear to have reached a peak of £85 million in the third quarter of 1970. The decline in the export business has been particularly sharp. Home deliveries fell by ten per cent between the lasts quarter in 1970 and the first quarter in 1971, but export deliveries fell by no less than 33 per cent. In practice, roughly half the lost market consists of machinery which is assembled in Britain from components imported from elsewhere. There was actually a small increase of six per cent in the value of British-made computers delivered to the British market. And if the manufacturers are cast down by their statistics for equipment delivered, they must be even more depressed by the order books, which have been falling since the beginning of 1970 and which, by the beginning of this year, had become so meagre that there is bound to be a further decline in sales as this year wears on.

What has gone wrong? Why has the great fashion for computers been undermined so quickly? In the past few weeks, it has been frequently suggested that the potential purchasers of computing machinery have been discouraged by their own experience of the failure of computers to function properly, or at least as well as the prospectuses for them claim. Is it the case that as with other technical innovations of the past few years—nuclear power, for example—the usefulness of computing machinery has been diminished by optimistic and unattainable promises of what these innovations might accomplish? By all accounts, there is a good deal in this view. After all, the British experience in the past few months is only a more dramatic representation of what is happening elsewhere, particularly in the United States. Small and smallish companies of all kinds are everywhere to be heard complaining that the machinery which they have installed in the past few years has failed to do the job required of it. Core storage turns out to be too small for what it has to remember. Processors turn out to be much slower in practice than they are in theory. The costs of producing software, in any case, substantially incalculable, turn out to be formidably high. And all too often the purchasers of computing machinery discover that their new computer does not help them to reduce the costs of operations or even to undertake fast clerking operations without anxiety.

Salesmen are not solely to blame, although many companies have done themselves a lot of harm by over-selling. There are, however, serious problems of a technical character. Computer people are the first to say how important is the systems approach to practical problems. For an innocent company about to buy a computer for itself, what this implies is that the potential customer has—or should have—a clear idea of what he would like the machine to do but no great concern about the precise manner in which it will function. To be sure, there is much in the view that one of the uncovenanted benefits of acquiring a computer is that it compels those who would use it to understand the business in which they are engaged. But there are limits to what the systems analysts are entitled to claim in this respect. After all, the installation of a new computer system is not an end in itself, or should not be, and it is beyond dispute that the upheaval entailed is in itself a real disincentive. The moral is that manufacturers should as a matter of course sell (or offer to sell) more than a machine—the software should go with it. But it is by now quite clear that the software as sold commercially is often outrageously expensive. If manufacturers want to keep their order books full, they will take steps to see that the machines they sell do actually perform as advertised. Such a goal could bring quite a different structure for the computer industry. As things are, manufacturers have assumed that their profitability is best ensured by apeing the motor car industry and setting up production lines for turning out replicas of more or less identical machines. Although there is undoubted scope for arrangements under which computer complexes can be assembled from smaller parts so as to provide each potential customer with a custom-assembled complex, this is only a beginning. Might it not be more profitable for the manufacturers to find some way of standardizing on software even if this meant that the machinery had to be more sensitively adapted to the customer's needs.

Compromise Without Solution

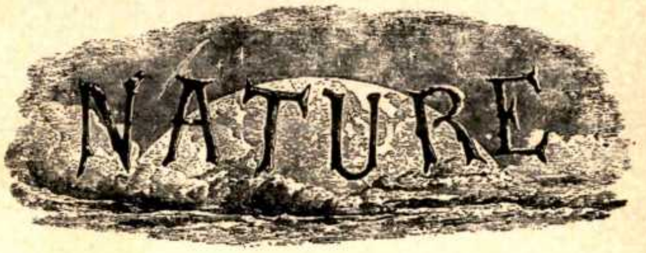
EVERYBODY knows by now that an arbitration tribunal inquiring into a pay dispute can usually be relied on to split the difference between the two opposing sides. This, predictably enough, is what the public inquiry into the salaries of scientists in the British civil service has done (see page 594). Those who had been offered nothing are now to get an extra five per cent. Those who had been offered something, for practical purposes about ten per cent, will have to be satisfied with that. With masterly empiricism, the tribunal says that the award "seeks to apply the principle of fair comparison and pay research taking into account the special features of a scientific career in the civil service and the fact that the pay of the scientific grades has been determined hitherto by horizontal relativity" without saying why it considers that the arguments put forward by the Civil Service Department in the past few months were invalid, as the Institution of Professional Civil Servants has been claiming. Indeed, the tribunal has the gall to say that "the award should in no way be taken to prejudice future decisions about the unified grading of the scientific and other classes". In other words, the tribunal has casually discarded one of the principles used in previous inquiries like this but has neglected to suggest a replacement. Nobody can be blamed for reacting vigorously to this development. The Civil Service Department will have only itself to blame if the Institution of Professional Civil Servants now becomes a constant thorn in its flesh. Unhappily, even the institution is a long way from recognizing that the time has come to take a much more radical view of the framework within which professional scientists should be employed by the British government.

The most obvious difficulty is that the scientific civil service has become a genteel blind alley for a great many of those who work in it. This is the point that the Fulton commission made. In practice, it has been exceedingly difficult for civil servants who begin as scientists to become, in the course of time, administrators of a more conventional kind. A part of the reason, no doubt, is that many working scientists in the civil service prefer to stick to science—not all of them complain. But it is beyond dispute that for a great many people who would normally expect in other walks of life, in industry for example, to move sideways from research into administration, the present rules are cramping. It is true that the Civil Service Department has been looking for recipes for transforming the civil service along lines that might have been suggested by the Fulton commission and that, ironically, discussions on these have been in part held up by the pay dispute. In the meantime, however, it is plain that the very large proportions of people who have accumulated in the higher grades of the scientific civil service are a measure of the extent to which there are few ways out at the top.

What is to be done? The first thing to recognize is that to the extent that many professional scientists in the civil service value their professional connexions as much as their status in the hierarchy, there is a strong case for hoping that it should be made comparatively easy for them to leave the civil service for other kinds of work. In other words, there should be much easier machinery by means of which scientists in the civil service could contract out of the arrangements which are conventional. In particular, it should be much easier for them to take their pensions with them if they are so inclined. The simplest way of arranging this would be to arrange that the Civil Service Department would convert into cash or its equivalent the accumulated pension rights of somebody wishing to leave. To be sure, this might often mean that the civil service would lose many men of promise and attainment, but then the question arises what in any case is the civil service for? Is it really meant to be an organization primarily concerned with its own perpetuation? Or should it be more eager than it is to help spread its skill about the country? These and related questions are the ones which the Institution of Professional Civil Servants should now be asking. It would be a great misfortune if they become entirely lost in detailed quibbles about the most equitable way of comparing dissimilar kinds of careers.

---

100 Years Ago



IN his last weekly return, the Registrar-General refers to the westward advance of Asiatic cholera as investing with more than ordinary interest Dr. Frankland's usual monthly report upon the quality of the metropolitan water supply. The water supplied by the New River and Kent Companies is again reported freest from organic impurity, that of the East London and Chelsea Companies the most impure. With reference to the advantage which would be derived from a general application of Dr. Clark's softening process to the London water supply, the Registrar-General adduces the following valuable facts, which have been communicated by Mr. Robert Rawlinson, C.B., C.E.:—"The average daily water supply to the metropolis was 111,292,104 gallons in June, and 112,107,697 gallons in July. Now in each million gallons of these waters there is about one ton of bi-carbonate of lime, or 111¼ tons in June and 112 in July. About two-thirds of this weight of lime or chalk would be removed by Dr. Clark's softening process—that is, in June 74 tons, and in July about 75 tons. In each year about 25,000 tons of useless lime would be removed from the metropolitan waters by the simple and easy process now in use at Canterbury." The Registrar adds: "This riddance of the foreign matter, which deprives water of some of its cleansing properties, is in itself an advantage; but, besides this, the fine precipitate of chalk carries down with it suspended impurities and probably frees it from choleraic and other contagions. It is a most effective filtration." It is a comfort to know that the working classes are beginning to feel their strength. When that is put forward it is certain to be in the direction of sanitary and educational measures. The Registrar-General appends to his report a concise sketch of the steps proper to be taken in view of the threatened epidemic.

From Nature, **4**, 349, *August* 31, 1871

Pure Che :stry Lib
SCIENCE COLLEG

OLD WORLD

SCIENTISTS' PAY

Splitting the Difference

The announcement last week of the award of the Civil Service Arbitration Tribunal to scientists employed in government laboratories brings to an end the first round of the dispute between the Institution of Professional Civil Servants and the Civil Service Department that has gone on since early in June. The IPCS, however, is far from satisfied with the result and is already preparing a campaign to change the whole process of pay determination for government scientists.

The IPCS, after asking for an overall increase of 14.1 per cent for members of the scientific classes, was offered 2.5 per cent by the Civil Service Department—with no increase for the higher grades. The arbitration tribunal awarded 5 per cent to the higher classes not offered any increase originally but was more open-handed with the Scientific Assistant classes. Otherwise the tribunal mostly confirmed the original Civil Service Department offer, details of which were given in *Nature*, **232**, 512; 1971. The Senior Scientific Assistant class was awarded a 5 per cent increase compared with the 10 to 15 per cent asked for and the 2.5 to 3 per cent offered. The Scientific Assistant class received the largest increase with the minimum of the scale being increased from £490 to £550 and the maximum from £1,253 to £1,395—very near to that demanded by the IPCS. The total award amounts to a 7.5 per cent overall increase and will cost the government nearly £2.5 million annually.

The Civil Service Department has cause to be pleased with the award as Mr Mustill, QC, the tribunal chairman, and his fellow members, state that "the award seeks to apply the principle of fair comparison and pay research that also takes into account the special features of a scientific career in the Civil Service".

The IPCS has argued vehemently against this principle (see *Nature*, **232**, 512; 1971) and believes that the pay of the scientist should be based solely on internal relativities. Mr William McCall, the general secretary of the IPCS, considers the award to be thoroughly unsatisfactory and he said last week "(the award) has attempted to produce a compromise between internal relativities and pay research. What it has achieved in fact is a complete mess. The award resolves none of the basic issues involved in the arguments to the tribunal. Indeed, the award marks the beginning of a dispute, not the end of it. It is plain from the statements of cases submitted to the arbitration tribunal that the salaries indicated by internal relativities cannot be reconciled with pay research information".

The IPCS will face an uphill task in its campaign to determine scientists' salaries in the future by some means other than pay research. Most other salaries within the Civil Service are directly or indirectly determined by pay research, and it will need a very strong case to place the scientific classes outside this scheme. The IPCS is confident, however, that this can be done, and at the moment is pressing for a joint review body of the Civil Service and IPCS to be set up to decide how scientific salaries are to be determined in future. As yet there is no reaction from the government to this suggestion, but it does seem likely that round two is about to start.

ELECTRONICS

Microcircuit Trouble

The British microcircuit industry is in decline as the world demand for integrated circuits continues to dwindle and overproduction increases. Companies are now faced with the extraordinary situation in which integrated circuits are being sold in the United States for less than the estimated cost of the raw materials and at only slightly higher prices in Britain. Manufacturers such as GEC-Marconi seem to be resigned to cutting production and closing factories.

What has caused such a state of affairs in which an American company, Texas Instruments, can now sell for £0.05 an integrated circuit that would have cost £3.00 in 1967? The first thing to say is that manufacturers of integrated circuits throughout the world are experiencing similar difficulties. Overproduction in the past few years has been the inevitable result of a rapid increase in production efficiency—better crystal growing techniques and a smaller rejection rate for completed circuits, for example—coupled with a reduction in the rate of increase of world demand. Companies that expanded their manufacturing facilities a few years ago, and then gritted their teeth while prices tumbled, are now obliged to prune manufacturing capacity thus losing skilled workers and running the risk of falling behind technologically. Those companies that can resist this inexorable process will presumably reap the benefits eventually.

During the past few months GEC-Marconi has announced the impending closure of its integrated circuit factories at Witham and Glenrothes after sustaining a loss of £1 million on these activities in 1970. Mullard lost £1.4 million on its microcircuit sales last year and Texas Instruments has made several hundred people redundant at its factory in Bedford. The state of the British market during the past few years is shown in Table 1; price reductions and the small growth in demand have both contributed to the relatively meagre increase during 1970.

Table I. Annual Sales of Microcircuits in Britain

1966	£2.6 million
1967	£4.8 million
1968	£7.8 million
1969	£15.4 million
1970	£17.0 million

About half of the British integrated circuit requirements in 1970 were imported and 43 per cent of these were supplied duty free from the Free Trade Area and from Singapore (where they are chiefly manufactured by American companies). Most of the other imports were of integrated circuits manufactured in the United States and packaged in cheap labour areas such as Taiwan, Korea and Portugal; these are still relatively inexpensive in Britain in spite of the 20 per cent tariff.

The total British market is much smaller than the domestic market in the United States (about £180 million in 1970) and fluctuations in the American market obviously have serious repercussions in Britain as American manufacturers try to keep production lines going by continuing to sell at a loss. Some Members of Parliament have indeed suggested that integrated circuits are being dumped in Britain and have called for additional tariffs or import quotas, but there is no doubt that dumping, in the strict sense of the word, cannot be taking place when the same integrated circuits are being sold more cheaply in the United States.

ENGINEERING

More Grades Invented

The Privy Council has now approved the request by the Council of Engineering Institution to set up an Engineers' Registration Board (ERB) providing a composite register of Chartered Engineers, Technician Engineers and Engineering Technicians. The ERB will comprise institutions and societies that are involved in education and training of their members in one of the above categories.

The aim of the ERB, according to the Council of Engineering Institutions, is to rationalize the qualifications of engineers and to give the engineer an identity. The three classes of membership suggested will carry the letters C.Eng., T.Eng.(CEI), and Tech.(CEI), and the qualifications for membership are a degree or equivalent in engineering for the C.Eng., a Higher National Certificate or City and Guilds Full Technological Certificate for a T.Eng. (CEI) and an Ordinary National Certificate or a City and Guilds Part II Final Technicians Certificate for a Tech.(CEI). Membership also requires appropriate training and experience in all categories.

The C.Eng. qualification is not new and was first instituted when the Council of Engineering Institutions was formed in 1965. The two new classes now instituted will, it is hoped, supersede 50 or 60 titles that have been in use for many years all with different qualification standards. Whereas the Council of Engineering Institutions does not expect all engineers to apply for membership immediately, a spokesman said this week that the eventual goal is to have the presently instituted qualifications accepted as the standard by all British engineers.

Engineers can be nominated to the register by one of the fifteen constituent members of the ERB. There will be no fee directly paid to the board by the engineer—his membership of the parent institution will be sufficient to qualify for membership. The member institutions are enthusiastic about the setting up of the Engineers' Registration Board, but as yet they have not tended to approach their corporate members to ask their views. There seems to be no reason why members should not share this view.

SCIENCE MUSEUMS

Summer Boom in London

IN the summer months, attendance at the London museums reaches its annual peak and the Science Museum and the Natural History Museum—the British Museum (Natural History) as it calls itself—is sharing in the popularity. The Science Museum has had more than 2,000,000 visitors a year since 1968 and the Natural History Museum has some 1,500,000 visitors a year—a figure that is increasing at 10 per cent a year.

But who comes to the museums, and what do they offer? There are no detailed statistics, although a survey that would establish this has been suggested at the Central Office of Information—but both museums know they have to cater for large numbers of children. The Natural History Museum has it down to a considerable art, with a special Children's Centre open on Saturdays during term and six days a week during school holidays. This helps children to find their way constructively around the museum by providing them with "Nature Trails" and encouraging those showing a genuine interest to join the Natural History Club which meets on Saturdays, when a certain amount of field work is done. There are close links with schools and teachers, and school parties are frequent. From a small random survey last winter, the Natural History Museum has some idea of the age distribution of its visitors—65 per cent of them are under thirty, over half of those are under twenty, and the survey excluded school parties, which provided just under 120,000 visitors in 1970.

The Science Museum has no such figures, but it thinks that one third of its visitors are children and that an appreciable proportion of them come time and again. Arrangements for seriously interested children are meagre, although the museum does run an increasingly comprehensive and varied set of lectures and tours. There is no equivalent of the "Nature Trails"—an experiment with a similar scheme is said to have turned out to be administratively and technically unmanageable. There is, however, some contact with schools and the special Children's Gallery, transferred to a larger location in the basement some two years ago, is as popular as ever. The transfer has allowed for several new exhibits to be included together with the old favourites.

Since 1963, the work of modernizing the exhibitions has progressed gallery by gallery, for the museum is aware that even if new exhibits are continually installed, the design of a gallery dates over the years. A spokesman said that plans are also afoot to redesign the mathematics gallery to include information on computing—a feature that has been sadly neglected to date—with the possibility of a working computer terminal. A section on production engineering is also planned, and both schemes should increase the topicality of the museum.

Although the museum itself makes no complaint, the sum available for new acquisitions is only £8,000 a year, a frighteningly small amount of money if the museum is expected to keep any sort of pace with scientific and technological developments. In spite of the attraction for children in the many working models, the museum does not consider itself primarily for children. They feel that their research facilities are an important part of their work, and that the main aim of the public galleries is to arouse people's curiosity, and possibly stimulate them to a deeper interest.

The Natural History Museum fills rather a different role from the Science Museum. Despite its large public halls, its purposes lie rather in the fields of active research, which the new wing, and the new building at Tring, at present under construction, will help to further. Established in 1881 on a taxonomical basis, the museum is now questioning the validity of this as current research shows more and more that animals and organisms should be considered in their environments, and a spokesman said there may be changes in the future to base the layouts on ecological principles. Before this can be fully accomplished more exhibition space will be needed.

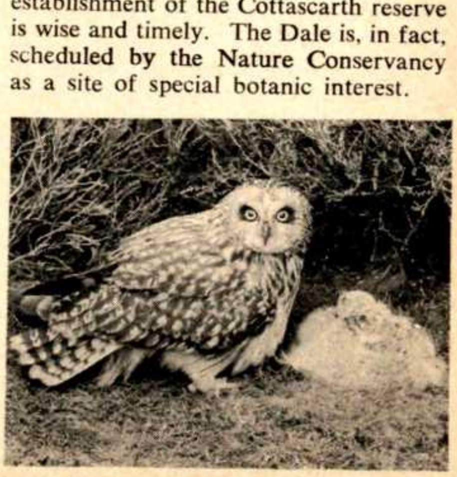
Nevertheless the main purpose of the museum is always likely to be one of research, partly because of the impossibility of displaying more than a small percentage of the natural world—for example the museum owns some 250,000 mineral specimens alone, of which only 4 per cent are on display—and partly because there is much still to be made known about the natural world. After all, although there are some 750,000 known species of insects, it is estimated that there are between two and four times as many yet to be identified.

CONSERVATION

Just for the Birds

THE Royal Society for the Protection of Birds has recently established a 335 acre reserve in the Dale of Cottascarth on the mainland of Orkney. The society, which manages more than forty other bird sanctuaries throughout the British Isles, chose this northern site because there are a number of typical habitats of the birds that breed in Orkney within its boundaries. These reserves are an attempt to maintain the natural integrity of wildlife communities and those that have been in existence for some time have been very successful in this purpose. Access is restricted to members of the society, although this year saw the well established reserves open their doors a fraction to let in small numbers of the interested public. The doors are unlikely to open much wider, and understandably so for the birds' sake.

With the ever-increasing popularity of Orkney with holiday-makers, the establishment of the Cottascarth reserve is wise and timely. The Dale is, in fact, scheduled by the Nature Conservancy as a site of special botanic interest.



Short-eared owl with young.

NEW WORLD

Drug Office Wins Friends, Disarms Enemies

by our Washington Correspondent

ONLY two short months have now gone by since President Nixon announced to Congress and the nation his war against drug abuse, and already two Congressional committees have concluded hearings on legislation designed to put into effect his package deal setting up a Special Action Office for Drug Abuse Control, with additional funding of $155 million for the control of drugs and the rehabilitation of addicts. Both committees—the House Subcommittee on Public Health and Environment and the Senate Subcommittee on Intergovernmental Relations—are expected to report to Congress soon after the recess, and it seems from the evidence submitted to the committees that the President's proposals should emerge substantially unchanged. The programme has met with approval even from the agencies whose work will be subordinated to the new special action office.

Chief among the tasks of the proposed action office is to coordinate the federal programmes for drug control and rehabilitation, at present spread over nine agencies of the Federal Government. To accomplish this task, the Special Action Office will have at its disposal the effective and overtly simple expedient of complete budgetary control over all federal drug abuse programmes. But creation of the special action office will also underline a marked shift in the government's attitude to drug control, for in the past it has relied heavily on the courts to deter potential addicts from taking drugs, often at the expense of seeming to neglect the other side of the coin—the rehabilitation of existing addicts. Only the full realization of the extent of heroin addiction among troops in Vietnam which came to light about a year ago, and the potential impact of thousands of addicts returning to the United States from Asia has led to the tardy adoption of a crash programme aimed at rehabilitation.

While the new emphasis away from severe court action brought against drug users and towards education and rehabilitation has been widely applauded, there are still many doubts surrounding the way in which the new special action office will function. One such doubt is whether the director of the office will be able to exercise complete control over the budgets of the agencies in his jurisdiction without at the same time running up against resentment within the agencies themselves at their loss of effective power. As James W. Symington, a member of the Subcommittee on Public Health and Environment, said to Dr Jerome H. Jaffe, Nixon's nominee to head the action office, "in every one of those agencies there is a fellow that has the responsibility right now to see how much they can afford, where it ought to go, and how he exercises that responsibility depends on the multiplicity of contacts that agency has in the states and localities. But you are going to understand all of that for every agency, put it together and then explain to them how to handle it. Congratulations and best wishes".

In many respects, the appointment of Dr Jaffe to the post of director has circumvented a few of those fears, for Jaffe is respected by all the agencies with which he will be dealing. In particular, he has served for several years on the board of the National Institute of Mental Health, whose Division of Narcotic Addiction and Drug Abuse will consume some 70 per cent of the Special Action Office's funds. During that time, Jaffe has built up a strong working relationship with the NIMH staff which will stand him in good stead for regulating their budgets. One official of the NIMH said last week that he regards the special action office as a powerful ally to fight for funds and that there should be no problem in the transfer of power from the NIMH to its drug overlord.

How Jaffe visualizes the work of the special action office became clear during his second appearance before the subcommittee earlier this month. He explained that the office would not seek to meddle closely in the work of the various agencies, and that it would take over the running of a particular programme only as a last resort. The office is therefore intended to be an overlord, working chiefly by informal contact and discussion with the members of the agencies under its control, acting as their mouthpiece in Congressional hearings, and using its powers to seek appropriations for drug abuse control and to distribute federal monies.

Applications for funding for new projects will be channelled through the office, and Jaffe will be concerned especially to see that there is no duplication of effort among federal agencies. The arrangement also has the potential advantage that it may help to speed up the adoption and funding for new projects, for they will no longer be buried within the bureaucratic layers of each individual federal agency.

If the house subcommittee on Public Health and Environment does alter the Administration's legislation, it seems likely that the powers of the proposed special action office will be widened rather than cut down, for there has been some disquiet that the Department of Defense is not included among the Federal agencies controlled by the office. Jaffe, however, has said that there would be no particular advantage in his having budgetary control over the Defense Department's drug abuse programmes, because any necessary coordination can be achieved by informal discussion.

One aspect of the drug problem in which the attitude of the Department of Defense is particularly important, however, is to establish the terms under which a member of the armed services is given a dishonourable discharge. In the past, services personnel have been given a dishonourable discharge for drug addiction, and this has prevented them from receiving treatment in the clinics run by the Veterans Administration.

There is also the problem of administering tests on servicemen for the presence of opiates in the urine, prior to discharge. It has recently been found from such tests that 5.4 per cent of American servicemen in Vietnam had used heroin in the two days before the test was given. The test, which was given on a random basis, almost certainly provided an underestimate of the number of servicemen taking hard drugs, and Jaffe himself believes that the true figure is probably nearer 10 per cent. It therefore seems likely that the subcommittee will add the Defense Department to the legislation in the hope that the Special Action Office will be able to coordinate not only the testing of American servicemen for the presence of hard drugs, but also the treatment of addicts in the armed services.

If the Special Action Office is set up, it will have an initial lifespan of three years, when its operations will be reviewed by the President. There is little in either the proposed legislation or in the steadily increasing figures for drug addiction—it is estimated that there are now about 250,000 heroin addicts in the United States, and probably 150,000 in New York alone—to suggest that the problem will be any less acute in 1974. But at least a change of direction in favour of rehabilitation is a comforting sign of the Administration's intentions.

Ailing Health Services

by our Washington Correspondent

A BATTLE is developing between Congress and the Administration over plans to transfer a narcotics rehabilitation and clinical research centre at Fort Worth in Texas from the Public Health Service to the Bureau of Prisons. The Administration stands accused of effectively shutting down much-needed facilities for the rehabilitation of addicts at a time when a drive against drug addiction has been labelled a national priority.

Congressional opponents of the plan led by Paul G. Rogers, chairman of the House Subcommittee on Public Health and Environment, seem assured at least of staying the execution for another year. A congressional victory may already be too late, however, to enable Fort Worth to carry on business as usual, for while the execution order has been hanging over the hospital, staff morale has slipped so badly that 135 of the hospital's usual complement of 400 have either left or retired during the past year, leaving the establishment severely undermanned.

Fort Worth is one of only two federally run narcotics rehabilitation and research centres left in the United States and, like its sister institution at Lexington in Kentucky, it is administered by the Public Health Service. Between them, the two hospitals cater for about 2,000 narcotics addicts each year committed by the District Attorneys' offices under the Narcotics Rehabilitation Act of 1966. Patients are treated at the hospital for a period of about six months and are then sent to an aftercare centre, where they are prepared for return to normal life. In Fort Worth alone, 941 narcotics patients were treated last year.

The thrust of the Administration's policy is to rely less on large in-patient hospitals than on community-based centres. The rationale underlying this approach is that there is more likelihood of success when a patient is treated within his local community, because sending him away to a relatively isolated in-patient hospital causes undue emotional upheaval.

To this end, the National Institute of Mental Health, the agency in charge of the programme, has been setting up community-based treatment centres to cater for patients who would otherwise have gone to Fort Worth, and the intention was to transfer the Fort Worth hospital to the Bureau of Prisons on October 4 for use as a medium security facility for treatment of geriatric, alcoholic and drug addicted prisoners. Opponents of this plan have, however, criticized the Administration for providing inadequate facilities in the community centres to take up the overspill from Fort Worth, and they therefore point out that the plan will simply result in closure of drug rehabilitation munity centres to take up the overspill facilities.

Mr Robert Carrick, deputy director of the NIMH Narcotic Addict Rehabilitation Branch, accepts that more community based facilities are needed, and he pointed out last week that "to say that all are geared up for use is untrue", but he nevertheless suggested that the fifteen centres already in operation have a potential capacity greater than needed to take up the patients from Fort Worth. (The centres are located in Florida, New Jersey, Rhode Island, Puerto Rico, West Tennessee, North Virginia, Arizona, California, Minnesota, two in Mississippi—one of which will serve Kansas—New Mexico, Oregon, Texas and Washington, and there are plans for centres in Louisianna and three more in Texas.)

The centres are operated under contract from NIMH, and the Federal Government reimburses local money spent on the treatment of narcotics addicts under the Narcotics Rehabilitation Act of 1966. Local finance is therefore not a great barrier to operation of a community centre.

Increased capacity notwithstanding, if the Fort Worth facility is closed down, there will be areas of the US that will suffer because they have no local drug treatment facilities of their own. For example, during Congressional hearings on the proposal to set up a Special Action Office for Drug Abuse Control (see page 596), Mr Rogers pointed out that St Louis houses some 6,000 narcotics addicts but that it has only eleven in-patient beds. Similarly, New Orleans has about 6,000 addicts and only ten beds.

For the Administration's plans to treat addicts within their local community to have a chance of working, it therefore follows that drug treatment facilities should be extended to every community in which they are needed, otherwise addicts will still have to be sent away for treatment. This argument is already embodied in legislation introduced into Congress by Mr Rogers and which would prevent payment of grants to community mental health centres after June 30, 1972, unless the grant application is accompanied by assurances that the centre will provide drug rehabilitation facilities.

The legislation is, however, being opposed by the Administration on the grounds that drug rehabilitation facilities may not be needed in every community centre, but even a cursory reading of the Rogers Bill should show that there is just such a get-out clause, for the Bill specifically states that the requirement shall not apply if the Secretary for Health, Education and Welfare "determines that there is not sufficient need for such a program in such hospital or other facility". The Administration therefore seems to be opposing legislation which in effect supports its own position.

The Administration's attempt to close Fort Worth is not an isolated action, for it forms part of a concerted attack on the Public Health Service mounted by almost every successive Administration since the war. So far, Congress has managed to frustrate the present Administration's hatchet wielding against the service, and it looks like being successful in its attempt to keep Fort Worth functioning in its present form for at least another year. The House passed a resolution to that effect earlier this month, and there is no reason to expect that the resolution will be turned down by the Senate.

The House resolution was identical to a resolution passed a few days earlier by the Senate, in that it called for adequate funding for all the Public Health Service Hospitals to allow them to maintain their present functions throughout the present financial year, but the House version also referred specifically to the clinical research centres at Lexington and Fort Worth. The objective of the resolution is to grant a stay of execution to all the eight remaining public health service hospitals so that Congress and the Administration may jointly define a role for them.

Set up in 1798 by the Marine Hospital Act, the Public Health Service hospitals, as they later came to be known, were originally intended for the treatment of American seamen and coastguards. By the end of the nineteenth century, they also discharged some responsibilities in the public health and sanitation fields. The Public Health Service grew slowly in the period up to the Second World War, when it was operating twenty-four hospitals with more than 3,000 beds. Only in the postwar period, when demand for the facilities by American seamen injured in the war slackened off, did the Public Health Service begin to fall foul of the Administration.

All but twelve of the hospitals were closed or converted to outpatient clinics by 1955, and the Budget Bureau was already thirsting after the blood of the survivors, recommending, as it has done ever since, that they be closed. In 1965, the Administration recommended conversion of seven of the smaller hospitals to outpatient clinics, while the Office of Science and Technology recommended that the Public Health Service should continue to operate general hospitals, which should be modernized and given adequate funding.

In the event, only two were closed,

and Congress authorized funds for studies leading to modernization of the remaining ten hospitals. No funds for construction have since been requested, however, and two more hospitals were closed in 1969. The latest attempt to get rid of the Public Health Service came in this year's budget, which simply contained no request for funds to operate the hospitals. The idea was to close the eight Public Health Service hospitals and thirty outpatient clinics, and to turn the Public Health Service Beneficiaries over to the Veterans Administration, severe pressure on the VA's already overburdened facilities notwithstanding.

Although Congress has restored the funds to the Public Health Service, to bring the appropriations up to last year's level, the upshot of the Administration's repeated attacks on the system is that the facilities are rapidly becoming outdated and that the whole system has deteriorated through lack of finance. Repeated recommendations that the hospitals be modernized have been largely ignored, staff levels at the establishments have been allowed to fall and repeated threats of closure have done nothing to stimulate the morale of the Public Health Service's 5,000 doctors. But this deterioration of physical facilities has become one of the Administration's strongest weapons. The pattern is clear: by maintaining the funding for the Public Health Service at minimum levels, the facilities are allowed to deteriorate and the deterioration is later used as an argument for closing the hospitals.

As far as Fort Worth is concerned, there is no doubt that the threat of transfer to the Bureau of Prisons has had a deleterious effect on the staff. As one member said last week, of the 135 who have left during the past year, 85 have retired but expressed their willingness to return if the hospital carries on discharging its present functions.

DDT on Mock Trial

by our Washington Correspondent

A FULL-SCALE public inquiry, billed as the final trial to decide whether or not DDT should be banned from all use in the United States, opened last week in Washington. Chemical manufacturers, in alliance with the US Department of Agriculture are appealing against a cancellation order which the Environmental Protection Agency was forced by federal courts to impose on the pesticide in January this year. Ranged against the Department of Agriculture and the representatives of the chemical manufacturers is the Environmental Protection Agency itself and a variety of environmentalist groups headed by the Environmental Defense Fund.

On the face of things, the hearing could condemn DDT as a hazardous chemical only to be used outside the United States in countries where it is still regarded as an essential weapon in the armoury against malaria. On the other hand, the pesticide could be acquitted and freed for use on certain crops in the United States itself. But the issue is, unfortunately, not that simple. A decision against DDT will in effect only prevent the agent from being sold across state boundaries, and the whole history of the in-fighting and delaying tactics that have accompanied moves to ban DDT in the US shows up the federal pesticide regulatory mechanism for the cumbersome and unsatisfactory procedure that it has become.

Opening shots against DDT were fired in November 1969, ironically by the Department of Agriculture, one of the principal protagonists in the fight to defeat the present cancellation order against the chemical. Amid a blast of publicity, President Nixon then announced that the agriculture department had cancelled four of the existing uses of DDT: spraying tobacco crops and shade trees, use around the home and over water. Thirty days later, however, several producers of the pesticide exercised their rights under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) to request that a scientific advisory committee look into the cancellation order, and thereby set in motion the mills of the pesticides regulatory machinery.

Under the provisions of FIFRA, once an appeal has been filed against a cancellation order, companies are allowed to carry on manufacture and sale of the material under examination. After 1969, pesticide manufacturers were allowed to do this for the seven months that it took for the department of agriculture to set up the appeals committee. Four of the appealing companies withdrew their request for an advisory committee, however, and in July 1970, the Lebanon Company requested a public hearing, but this request was also later withdrawn. What usually happens is that an advisory committee has sixty days in which to file its report, an order either confirming or setting aside the cancellation order is made within ninety days of receipt of the report and sixty days are then available for objections to the second order. An objector has the right to insist on a public hearing on the second order, and a final order is then issued within ninety days of the completion of the public hearing. During the whole proceeding the product can still be legally marketed.

The next step in the campaign against DDT was taken by the Environmental Defense Fund, an environmentalist group, which on October 1, 1969, filed a petition with the Secretary for Agriculture, requesting cancellation and suspension of all uses of DDT. A suspension order would prevent the product from being sold until the whole regulatory process had been completed. This request was, however, ignored for six months, until the Environmental Defense Fund got a court ruling ordering the Secretary for Agriculture to reply, which he duly did, saying that he refused to cancel all uses of DDT. This decision was challenged by the Environmental Defense Fund in the courts.

In the meantime, the pesticides regulatory machinery was taken out of the hands of the Department of Agriculture and placed under the control of the newly-created Environmental Protection Agency, headed by William D. Ruckelshaus. On January 7 this year, a court in Washington DC ordered the EPA to cancel all uses of DDT, and to re-examine the decision not to suspend its use pending a final decision. Ruckelshaus duly cancelled all remaining uses of the chemical on January 15, but after seeking outside advice he decided not to suspend its use. It is that cancellation order which is now being appealed against in the public hearing which opened last week, and which will probably continue into 1972. Nearly two years after the initial moves against DDT, the pesticide is still being legally manufactured and used in the US, chiefly by the Montrose Chemical Corporation and some thirty other companies which buy from Montrose and process DDT for use. Eighty per cent of the DDT manufactured in the United States is exported for use against malaria, however, and this activity is not being contested.

What are the chances that DDT will be acquitted by the public hearing? At present, it is difficult to judge, but protagonists for the chemical will argue that apart from the value of DDT for controlling pests on cotton crops, a decision against the pesticide could have international repercussions which may slow down the eradication of malaria in underdeveloped countries. Already, Dr Marshall Laird, a member of the World Health Organization, has warned the public hearing of this possibility. The hand of the DDT protagonists will also be strengthened by the difficulties in controlling the recent spread of mosquitoes of equine encephalomyelitis in some Southern states. But on the other hand, four government committees which have studied DDT between 1963 and 1969 all recommended phasing out its use, and the Mark commission recommended elimination by December 1971 of all uses of DDT not essential to public health.

NEWS AND VIEWS

Rutherford and Geiger Re-evaluated

ADVANCES in nuclear physics in the past decade have arisen chiefly from developments in instrumentation coupled with the higher energy accelerators that became available in the 1960s. It is more than possible that the suggestion by Dr Peter Rice-Evans on page 625 of this issue of *Nature* will eventually be developed into a technique that will have universal application, although the theoretical predictions have yet to be tested. There are certain to be many Jeremiahs who will deem the idea for a new high energy particle detector as unpractical, but there will be few who will argue that the idea is not worth testing.

Rice-Evans's suggestion is based on the proportional counter concept first developed by Rutherford and Geiger in 1908. The novelty of the present approach is that the proportional counter be used in reverse with the outer cylinder being held at a positive potential with respect to the centre wire. The electrons liberated by the passage of an ionizing particle will thus be accelerated towards the outer cylinder and not towards the centre as in a conventional proportional counter. In this way a pulse with a much faster rise can be expected and the instrument becomes, in principle, a powerful tool that can be used to determine the position of an ionizing particle with a higher degree of precision than is currently possible.

To appreciate the advantages to high energy physics if this detector ever became a practical proposition it only needs to be realized that there is a great deal of current concern that the resolutions currently available with wide plane spark chambers, streamer chambers and multi-wire proportional counters will not be adequate to meet the requirements of the experiments that will be carried out with the new generation of synchrotrons typified by the one being built at Batavia in Illinois, and the 300 GeV machine planned for CERN. The higher energy of the accelerated beam means that the analysed particles will also have a higher energy and will thus suffer smaller deflexions in analysing magnets than particles accelerated in machines of lower energies. There is a need therefore for detectors with good spatial resolution and none of the detectors in current use meets the requirements.

How does the proposed reverse proportional counter supply the necessary high resolution? In such a set up the avalanche produced by an ionizing particle entering the chamber will be outwardly directed, and pulses will only arise in the wire when electrons are liberated near the wire surface in the region of high field necessary for avalanche growth. This is to be compared with a conventional proportional counter where a particle is detected irrespective of where in the chamber the event occurs. The difference emphasizes that the proportional counter was initially designed to maximize the efficiency rather than the spatial resolution as in the present proposed use. The analysis of Rice-Evans shows that a pulse produced by an ion within the critical distance needed to form the avalanche achieves 22 per cent of its maximum height within 10^{10} s of the event occurring. This fast rise is ideally suited for obtaining a fast pulse from a differentiation network to trigger the electronic circuits that are necessary in high energy physics experiments. Thus to obtain a pulse from the chamber an electron must be formed near the wire surface within the critical avalanche radius. It is here that the crucial question of the efficiency of the proposed device arises. To obtain maximum efficiency the radius of the wire needs to be as large as possible, but large wire radius is incompatible with the good spatial resolution that the detector sets out to achieve in the first place. Thus the ever present problem of achieving a compromise between efficiency and resolution would seem to limit the usefulness of the detector.

Calculations by Rice-Evans show that for a typical chamber the region of interaction is limited to a cylinder about the central wire of radius approximately twice that of the wire itself. The efficiency of producing an electron within this region depends on whether the interaction occurs in the wire or in the gas of the chamber, and the probability for these processes is estimated to differ by an order of magnitude—the interaction in the wire being most likely to produce an electron. Thus, the detector radius is effectively the same as the wire radius, and to achieve both high efficiency and high resolution a matrix of many wires will be needed. Such systems are already in use with multi-wire proportional counters.

It is now time to carry out an experimental study with the reverse proportional counter. The problems to be overcome are certain to be many, but only time will tell whether they are insurmountable. Multi-wire proportional counters were first used in 1956, but have only recently achieved universal usage. If the suggested detector is to be used with the new generation of high energy accelerators, however, it has to be developed at a faster pace. The decisions to be made before starting experimental work are many and involve such fundamentals as the material to be used for the wire, what gas to be used in the chamber—or whether a noble liquid could be used instead. The dimensions of the chamber have also to be detected, but of more fundamental importance is the question of how spurious positive ions might affect the performance. Such ions might cause ionization of the gas in the high field region or they might cause secondary electrons to be freed from the surface of the wire. A possible way to circumvent these problems would be to introduce a vapour to neutralize the spurious gas ions and so quench the discharge.

The possibility of the detector being a viable proposition depends entirely on how the expected one to several thousand signal to noise ratio that will arise from particles forming ions outside the avalanche region can be overcome. For a chamber of the dimensions given by Rice-Evans the ratio is one to 5,000 as only 1.5 per cent of the particles will pass through the avalanche region.

The chances of a successful detector being developed in the face of all these uncertainties must be rather small, but several innovations that have in the past seemed to the scientists of the day to have even less chances of developing into viable entities are now accepted techniques. Such a detector or series of detectors must be built and tested.

Earth Sciences Converge in Moscow

from a Correspondent

AT the fifteenth general assembly of the International Union of Geodesy and Geophysics (IUGG) held in Moscow during the first fortnight of August it was clear that at long last, and very belatedly, Earth scientists of various disciplines are beginning to talk each other's language. All interests were catered for at the specialized symposia organized by the various member associations within the union. What follows is a selection of items from some of these symposia; a further report will appear in the forthcoming issue of *Nature Physical Science*.

East Africa, and particularly its rift system, has been the site of major British scientific endeavour for many years and this was evident at the East African Rift symposium where eleven of the contributions were by scientists from the United Kingdom. Eight other countries were also represented and this was the first time so many scientists working in East Africa have gathered together.

Seven speakers dealt with the Afar triangle (the junction of the East African rifts with the Red Sea and Gulf of Aden). The structure of the region was ably reviewed by Mohr (Smithsonian Institution) and the volcanology was discussed by Tazieff (CNRS). On display were two maps of the region, a preliminary Bouguer gravity map by Makris (Hamburg) and a very impressive aeromagnetic map by Hall (Newcastle upon Tyne). Hall's map was coloured in such a way that the quiet zones could be readily distinguished from the regions of large magnetic anomalies similar to those found over the seafloor spreading zones of the Gulf of Aden and the Red Sea. Geologists and geophysicists all agreed that sialic crust must be absent beneath much of this region. This view was confirmed by Jobert (Paris) who described some seismic refraction experiments carried out by dropping bombs in the sea and recording on land in the French territory of Afar and Issas. All his profiles showed the presence of oceanic crust. Only the exact proportion of oceanic to continental crust remains uncertain.

From Afar, the theme of the meeting moved to East Africa. A general review of continental rift zones was given by Milanovsky (Moscow). Knopoff (Los Angeles) then presented evidence which convincingly demonstrated the presence of low seismic velocities beneath the East African rift on the basis of surface wave phase velocities at the Addis Ababa and Nairobi stations. There then followed two geological contributions on the rift volcanics in Kenya; Williams (Nairobi) reviewed the distribution in space and time of the various volcanic rocks, contrasting those occurring on the rift floor and shoulders, and McCall (Victoria) described a special field-geochemical study of the Silali volcano. McCall took the view that the eastern rift is an "aborted" ocean which has never opened up; the rocks are from primary mantle derived material and the magma chambers probably reached to within a kilometre or so of the rift valley floor. Such a view is consistent with the mapping and interpretation of the positive gravity anomaly along the rift axis by Searle (now at Addis Ababa) to which many contributors made reference.

The afternoon session opened with an account by Logachev (Irkutsk) of Soviet expeditions between 1967 and 1969. Soviet scientists have an impressive collection of geological data and have made more than 250 potassium–argon age determinations for 170 different localities in Kenya, Tanzania and Uganda. On the basis of this work, it is thought the process of rifting can be divided into six stages—23–16; 23.5–12; 10–5; 5–2; 2–0.7; and 0.7–0 million years—and each of these represents definite stages of magmatism and structural development. Rykounov (Moscow) described Soviet microearthquake studies in the southern part of the Gregory rift and the northern part of the western rift. In northern Tanzania, he and his colleagues have obtained a crustal structure consisting of 18 km with a P-wave velocity of $5.8\ \mathrm{km\ s^{-1}}$ underlain by 19 km with a P-wave velocity of $6.5\ \mathrm{km\ s^{-1}}$.

Fairhead and Girdler (Newcastle upon Tyne) discussed the relocation of epicentres using the joint epicentre technique. Their plots of epicentres on new fault maps prepared by Baker showed a remarkable correlation between earthquakes and recent geological features. They have also calculated travel time corrections for fifteen siesmic recording stations throughout Africa and have tentatively mapped regions in which P-waves are slowing down beneath the rift system north of the equator. From a study of all available fault plane solutions they suggest that this region is associated with a W.N.W.–E.S.E. stress pattern and with a consequent thinning of the lithosphere.

A notable feature of this meeting on the East African Rift was a consensus of opinion that there must be some separation of the lithospheric plates beneath Afar but none beneath East Africa. It seems that the lithosphere in East Africa has thinned but not broken apart.

At first sight, the choice of ocean floor spreading as a major topic for discussion in an international symposium seems somewhat outmoded, for the major impact of this all-embracing theory came 3–4 years ago and is accepted, in varying degrees, by most Earth scientists. Several speakers dealt with the internal processes that cause the seafloor to spread and the plates to move. Models presented by Turcotte (Cornell) and McKenzie (Cambridge) involved the evaluation of solid state thermal convection as the major driving mechanism. Both suggested that the convective process extends deeper into the Earth than the low velocity layer and that total mantle convection could not be ruled out.

Although much of the ocean floor has been investigated by geophysical methods (one eminent US oceanographic geophysicist bemoaned the fact that there was little of the ocean floor left to study), a group of speakers presented the results of regional studies in localized areas; for example, Talwanie and Pitman (Lamont–Doherty Observatory) discussed the Norwegian–Greenland Sea, Krause and Shilling (Rhode Island) gave an account of the Azores area and Barker (Birmingham) described the active spreading centre in the Scotia Sea.

Although there is now a vast amount of data on the physics, chemistry and shape of the Moon, the "vulcanists" and "impacters" at the symposium on this subject seem to be as widely divided as ever. Those selenologists who proposed an internal origin for the Moon's physical phenomena seemed to be dominant, if only because they were thicker on the ground than their opponents.

Because it is to be replaced by the Inter-Union Commission for Geodynamics, the Upper Mantle Project made its last international appearance in a 4½ day symposium.

The lasting impression of the IUGG assembly is that the next decade should be one of great stimulation and interest as continental geological and geophysical data are reappraised in the light of the seafloor spreading–plate tectonics concept. It is to be hoped that the newly formed Inter-Union Commission for Geodynamics will make a major contribution by guiding these studies.

ADENOVIRUSES

Endonuclease Activity

from our Cell Biology Correspondent

REPORTS of the discovery of this or that enzyme in particles of one or another of the enveloped viruses are these days mundane events, unless, of course, the enzyme happens to be as unusual as reverse transcriptase. By contrast the discovery of enzyme activities associated with the virions of encapsidated viruses is still a comparatively rare event. One such is Burlingham, Doerfler, Pettersson and Philipson's report (*J. Mol. Biol.*, **60**, 45; 1971) that the endonuclease activity which appears in cells infected with adenovirus 2 or 12 and is also associated with purified virions seems to reside in the penton base subunits of the capsids of these viruses.

This new endonuclease activity first came to light when Burlingham and Doerfler (*J. Virol.*, **7**, 707; 1971) were studying the replication of adenovirus 2 and 12 DNA in KB cells and a variety of other permissive and nonpermissive hosts. Burlingham and Doerfler were able to identify three classes of viral DNA in infected cells; molecules which cosediment with DNA extracted from virions and appear to be uncoated parental viral genomes; molecules of adenovirus DNA which are considerably smaller than intact genomes; and finally DNA molecules which are much larger than genomes and may well be parts of the host genome carrying integrated adenovirus DNA. Kinetic studies of the appearance of these three classes of DNA during the course of adenovirus replication lead Burlingham and Doerfler to the conclusion that many adenovirus genomes are cleaved by an endonuclease which appears in the cells after infection and that the cleaved molecules may well be those which are integrated into the host genome. Furthermore, although the experimental details have yet to be published, Burlingham and Doerfler have apparently obtained evidence that the endonuclease is not only a virus-coded protein but also remains associated with pure virions.

Collaborating with Pettersson and Philipson, who with their colleagues at Uppsala have extensively analysed the polypeptides in the three structural units of adenovirus capsids (the hexons, penton bases and penton fibres), Burlingham and Doerfler have tested the capsid subunits for endonuclease activity. Only the penton bases, purified from infected KB cells rather than from pure virions for technical reasons, have this activity, and both selective digestion of the penton bases with trypsin and antiserum to penton base inactivates it. The endonuclease activity is specific for DNA; its activity is twenty-fold greater with native DNA than with denatured DNA as substrate and it cleaves both strands of double-stranded DNA by some "double-hit" mechanism. Moreover, the homogeneity of the cleavage products produced by the endonuclease with adenovirus DNA as substrate suggests it attacks specific sites which are probably rich in G.C base pairs because poly dG.poly dC is a strong competitive inhibitor whereas poly d(AT) has little effect, and actinomycin D partially inhibits the activity. Assuming that penton bases in capsids have the same activity as those extractable from infected cells, Doerfler and Burlingham have provided an interesting example of a viral protein which as well as forming part of a capsid also plays a part in the replication of the viral genome.

Also in the current issue of the *Journal of Molecular Biology* (**60**, 185; 1971) Landgraf-Leurs and Green report their various attempts to exploit the differential binding of synthetic RNAs by the DNA of adenoviruses 2, 7 and 12 for the separation of the complementary strands of these genomes. Apparently poly(U.G) molecules with judiciously selected ratios of U and G bind to different extents to the two strands of these three genomes and separation of complementary strands is possible. To prove their point Landgraf-Leurs and Green report the separation on a preparative scale of the strands of adenovirus 2 DNA and self annealing and hybridization experiments show there is no detectable cross contamination.

PHOTOMORPHOGENESIS

Ach Implicated Again

Gressel, Strausbauch and Galun from the Weizmann Institute provide on page 648 of this issue of *Nature* what may be the first evidence that acetylcholine has a part to play not only in plant photoresponses initiated by red light, but also where blue light provides the stimulus. They have shown that sporulation in the fungus *Trichoderma viride*, which is induced normally

---

Drug Resistance and Ribosomal RNA

ANTIBIOTICS such as streptomycin, spectinomycin, erythromycin and fusidic acid all impair to greater or lesser extents the faithful translation of messenger RNAs in *Escherichia coli*. Resistant mutants can, however, be isolated and, as the pioneering work of Nomura and his associates first established, the loci controlling such resistance specify structural proteins of ribosomes or, in the case of fusidic acid resistance, as other groups have shown, the locus specifying the translocation factor G. But it would be wrong to run away with the idea that mutations leading to resistance to antibiotics which affect translation are confined to genes that specify proteins which are part of the translation machinery. For Helser, Davies and Dahlberg report in next Wednesday's *Nature New Biology* that a mutation which results in resistance to the drug kasugamycin causes a failure in the methylation of 16S ribosomal RNA.

Sparling has shown that kasugamycin interacts with the 30S ribosomal subunit of wild type *E. coli* ribosomes. With the expertise required for the reconstruction of ribosomes from their constituent RNAs and proteins, which has been chiefly developed by Nomura, Helser and his colleagues, at hand at the University of Wisconsin, set about reconstructing 30S ribosomes with proteins and RNAs from either wild type of kasugamycin resistant cells. They quickly discovered that resistance to this drug is not the consequence of a mutated 30S ribosomal protein. They therefore compared fingerprints of the 16S ribosomal RNAs of resistant and sensitive strains, and sure enough found a difference. An oligonucleotide sequence dimethyl-AACCUG present in digests of wild type 16S RNA is replaced by the unmethylated oligonucleotide AACCUG in digests of the 16S RNA from resistant strains. Using RNAs labelled in their methyl groups they confirmed this difference, and further reconstruction experiments proved that resistance or sensitivity to kasugamycin depends on the source of the 16S ribosomal RNA and not on the source of ribosomal proteins.

Apparently therefore the mutation to kasugamycin resistance results in the failure to dimethylate an adenine residue near the 3′ terminus of the 16S ribosomal RNA. Helser *et al.* favour the idea that the mutated locus specifies a methylase responsible for adding the two methyl groups to this adenine residue, although alternative explanations of their findings have not been rigorously excluded. As they note, if the kasugamycin resistance locus does specify a methylase probably more than one species of methylase is involved in methylating ribosomal RNA. For even in the kasugamycin resistant strains the 23S RNA of the large ribosomal subunit is methylated normally.

In short, Helser *et al.* have proved that the function of ribosomes can be altered by mutations which affect ribosomal RNA and they have provided circumstantial evidence suggesting that more than one methylase may be involved in the specific methylation of ribosomal RNA.

by small amounts of blue light, can be initiated by treating the fungus with a mixture of acetylcholine and the acetylcholinesterase inhibitor, eserine.

That acetylcholine, known chiefly for its role in the transmission of nerve impulses in animals, may be active in plant photomorphogenesis is a recent and, on the face of things, bizarre idea. But acetylcholine is known from animal studies to mediate certain processes involving changes in membrane permeability, and it is just these sorts of changes which many plant physiologists believe to be the basis of some aspects of photomorphogenesis.

M. J. Jaffe showed last year that mung bean seedlings not only contain appreciable quantities of a substance which the results of a series of tests suggested strongly to be acetylcholine, but also that the levels of acetylcholine in the plant changed characteristically after exposure to red light. Furthermore, acetylcholine, when given to dark grown seedlings, mimicked the effect of red light in certain photomorphogenic responses. One of these responses was the odd electrical phenomenon discovered by Tanada, where mung bean root tips cling to a negatively charged wet glass surface in red light and release in far-red light (see *Nature New Biology*, **230**, 257; 1971). The implication is that acetylcholine is involved in electrical changes in the plant which presumably follow the photoconversion of phytochrome.

The results that Gressel *et al.* have obtained for *Trichoderma* are not so satisfying as Jaffe's for mung bean, perhaps, but they do imply that there may be a fundamental similarity underlying some red and blue light induced photoresponses. *Trichoderma* sporulates in culture after exposure to blue light but not in darkness. Acetylcholine alone cannot replace blue light in this system, but Gressel *et al.* were able to induce sporulation in the dark using acetylcholine in combination with eserine. The relative amounts needed were specific; sporulation equivalent to 95 per cent of the control value was obtained when 10×10^{-5} M acetylcholine was used with 5×10^{-6} M eserine. Curare, an acetylcholine antagonist, suppressed sporulation in the light control material.

Gressel and his colleagues, however, have not indicated very clearly how they think their data applies to the natural regulation of photomorphogenesis. Is, for example, acetylcholine found naturally in *Trichoderma*, and if so what changes take place after exposure to blue or red light? Does addition of acetylcholine and eserine to the fungus really step round the mechanism controlling sporulation or merely provide the operating factors for altering membrane permeability? The authors tentatively suggest that should acetylcholine prove common to the mechanism of both blue and red light photomorphogenesis, their modes of action will have to be thought out anew. Very true; but these latest results say less about this prospect than raise questions about the basic nature of the changes which lead to sporulation in blue light sensitive fungi.

RHIPIDISTIA

Links with Tetrapods

from our Vertebrate Palaeontology Correspondent

IN the study of the course of vertebrate evolution, one of the most important groups of animals are the extinct rhipidistian fishes. Rhipidistia first appear in the fossil record in the early Devonian and persist into the Permian and it is generally agreed that this group gave rise to all the tetrapods—amphibia, reptiles, birds and mammals. Important information on the anatomy of these forms is contained in two recent articles written jointly by S. M. Andrews and T. W. Westoll (*Trans. Roy. Soc. Edinb.*, **68**, Nos. 9 and 12; 1971).

As would be expected vertebrate palaeontologists have devoted considerable time to the Rhipidistia, but detailed study has been largely confined to description of the skull and speculations about their relationships have been based almost exclusively on cranial anatomy. This is very strikingly the case with *Eusthenopteron*. This fish is known chiefly from the early Upper Devonian of Canada and has been a focus of attention since the early years of this century. The skull is probably better known than that of any other fossil vertebrate, and has been studied particularly by Jarwik who has used the technique of serial grinding originally perfected in Sweden by Stensiö. Although there have been many partial accounts of the rest of the skeleton, a comprehensive, detailed and accurate description of the rest of the skeleton has been lacking, so that the work of Andrews and Westoll on the postcranial skeleton of *Eusthenopteron* is welcome.

In their first article, a description of the paired pectoral fins is accompanied by a detailed analysis of the probable muscle origins and insertions of the fins and shoulder girdle. The resemblance of the proximal part of the fin to the primitive tetrapod limb was already known in general terms; detailed analysis now shows that the range of movements of the early tetrapod humerus is almost identical to that of the first axial element of the fin of *Eusthenopteron*. This part of the rhipidistian fin seems in

Structural Gene of Diphtheria Toxin

IN next Wednesday's *Nature New Biology*, Uchida, Gill and Pappenheimer report an elegant series of experiments which prove that the structural gene for diphtheria toxin is carried by the lysogenic bacteriophage β and some of its close relatives. It has, of course, been known for several years that strains of the diphtheria bacillus *Corynebacterium diphtheriae* which do not make the toxin are rendered toxinogenic when they are lysogenized by bacteriophage β and that the yield of toxin depends on the strain of bacterium and its physiological state. Moreover, the *tox* character of phage β maps at a single locus.

In an attempt to decide between the two alternative explanations of these findings, namely that the *tox* gene specifies the toxin protein or that it regulates the expression of a structural gene for the toxin in the bacterial chromosome, Uchida *et al.* devised an interesting selection procedure which enabled them to isolate a mutant of phage β. Cells of non-toxinogenic strains of *C. diphtheriae* lysogenized by this mutant phage make a protein which cross reacts with anti-toxin antibody but is not toxic and lacks the C terminal sequence of the normal toxin molecule. Clearly, therefore, the *tox* gene specifies the toxin protein itself and the mutation Uchida *et al.* have isolated is either a deletion or a nonsense mutation which prematurely terminates the synthesis of toxin protein.

These findings lead Uchida *et al.* to speculate about the evolution of the *tox* gene. Diphtheria toxin, the substrate of which is restricted to eukaryotic cells, has no role in the replication of phage β and its relatives; it can, for example, be totally deleted without altering the rate of phage replication or the yield of phage. Neither does the toxin have any apparent role in the life cycle of the diphtheria bacillus growing in culture.

Why then has the gene been retained? Uchida *et al.* suggest that the ability to make toxin must have some survival value for the bacillus, and therefore its lysogenic phages, in its natural habitat—the throats of animals and man—and they point out that widespread immunization with diptheria toxoid has resulted in the eradication of toxinogenic strains of *C. diphtheriae* from many human populations. As for the origin of the toxin gene, Uchida and his colleagues speculate that because the only known substrate of the toxin is found exclusively in eukaryotic cells, the toxin gene may have once been a eukaryotic gene which the bacilli or their phage picked up and modified to their own advantage.

fact to be a true tetrapod humerus.

Other resemblances to the anatomy of early tetrapods are also confirmed. *Eusthenopteron* has two-headed dorsal ribs, albeit with very little blade, throughout the trunk. Furthermore, the pelvic girdle probably had a ligamentous attachment to the vertebral column in the sacral region. This condition would be the expected forerunner of the tetrapod sacrum with a bone-to-bone articulation of the girdle to one or more pairs of specialized sacral ribs.

The most interesting differences between the postcranial skeleton of *Eusthenopteron* and those of other rhipidistians, described in the second article, concern the structure of the vertebrae. The vertebrae of *Eusthenopteron*, inadequately described before this account, have been cited in the decades since the war as the ideal forerunners of the tetrapod condition. The other rhipidistians, however, have a bewildering variety of vertebral types, and as more becomes known of the condition in very early, pre-Coal Measure tetrapods the orthodox theory of vertebral evolution in early land vertebrates becomes more and more unsatisfactory.

Description of the skeletons of rhipidistians other than *Eusthenopteron* also allows a more rational classification within the group and one is presented by Andrews and Westoll. These two articles are a remarkable achievement and will long remain an essential source of information for students of vertebrate evolution.

CONTINENTAL DRIFT

Initiation of Rifting

from our Structural Biology Correspondent

KNOWLEDGE of the timing of the opening of the Atlantic Ocean and of the mechanism involved is extremely hard to come by. The lack of clear magnetic anomaly patterns in the "quiet" magnetic zone on both sides of the Atlantic floor has required an extrapolation backwards of spreading rates from the well established younger central zone of the Atlantic in order to gain approximate dates for the opening. This method gives a wide range of dates for the commencement of opening from Late Permian onwards, depending on the spreading rate favoured.

Another method which has been fairly successfully used has been to determine two sets of magnetic pole positions from continental rocks to either side of the Atlantic during successive stages of Earth history from the Carboniferous onwards. By rotating one of these sets of poles by the equivalent of the amount required to close the Atlantic, it is possible to see at what stage the two sets of pole positions fail to coincide, thus giving the first point of Atlantic spreading. Larson and La Fontain (*Earth Planet. Sci. Lett.*, **8**, 341; 1971) found by this method that the Atlantic started to open at the close of the Triassic.

On purely geological reasoning, Hallam (*J. Geol.*, **79**, 129; 1971) suggests that spreading started during Lower to Middle Jurassic at the time of the breakdown to a deeper water environment of the widespread and long-lived carbonate platform of the Mediterranean region. He further suggests that downwarping of the southern North Atlantic borders with associated faulting and volcanic activity during the Upper Triassic indicates a period of rifting occurring some 50 million years before the actual separation of the continents. This model is in good accord with a Rift Valley/Red Sea pattern of oceanic opening.

Further corroboration of Hallam's initial tensional phase before spreading has recently been supplied by May (*Bull. Geol. Soc. Amer.*, **82**, 1285; 1971). He has found that Late Triassic doleritic dyke swarms in eastern North America, West Africa and north-eastern South America fall into a regular radiating pattern if these continents are restored to their relative pre-drift positions (essentially according to the Dietz *et al.* reconstruction (*Bull. Geol. Soc. Amer.*, **81**, 1915; 1970). The pattern is roughly convergent on the Blake plateau, the Bahama platform and the western Senegal basin, and individually the dykes bear no particular relationship to the structures of the continental rocks through which they intrude. It thus seems that the stress field responsible for the dyke intrusions was unrelated to continental crust tectonics but rather, as the dolerites themselves are of typical oceanic composition, to activity within the upper mantle.

May is able to show that the intrusion of the dykes was related to a tensional

The Extent of Reverse Transcription

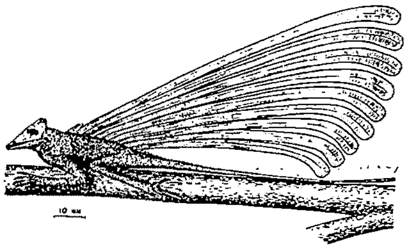
IT is hard to believe that the chief role of reverse transcriptase in tumour viruses is something other than the synthesis of a double stranded DNA provirus as Temin has proposed. And Duesberg and Canaani have already reported, as expected, that the reverse transcriptase in Rous sarcoma virus transcribes *in vitro* all the sequences present in the viral RNA genome, albeit as a series of small DNA molecules rather than a single DNA molecule as big as the viral genome. In next Wednesday's *Nature New Biology*, however, Varmus, Levinson and Bishop describe a more detailed analysis of the products of reverse transcription of Rous sarcoma RNA which suggests that at least *in vitro* some sequences of the viral genome are preferentially transcribed into double stranded DNA.

Reverse transcription takes place in two steps; the first is the synthesis of a single DNA strand complementary to the viral RNA genome to yield a RNA.DNA hybrid; the second involves the synthesis of a double stranded DNA using this hybrid as template. When Duesberg and Canaani measured the extent of transcription *in vitro* of Rous sarcoma virus RNA they hybridized the total DNA, made, both single and double stranded, to viral RNA.

What Varmus *et al.* have now done is measure the extent of reverse transcription *in vitro* into double stranded DNA. After separating the single stranded from double stranded DNA made by Rous sarcoma virus reverse transcriptase they measured the rates of reassociation, after denaturation, of the double stranded product. Most of this DNA rapidly reassociated, a small proportion slowly reassociated and some failed to reassociate. By making various assumptions Varmus *et al.* estimated the molecular weight of the rapidly reassociating fraction and what fraction of the total Rous sarcoma genome it corresponds to. They claim that from 85–90 per cent of the double stranded DNA is probably transcribed from about only 5 per cent of the viral genome assuming its molecular weight to be 10×10^6.

But does the slowly reassociating fraction of the double stranded DNA contain sequences corresponding to the entire viral genome? Varmus *et al.* claim, from measurements of the rate at which it reassociates, that this double stranded DNA corresponds to only about 20 per cent, rather than 100 per cent of the genome. Moreover, they report experiments which they interpret as showing that at least 30 per cent of the DNA sequences found in RNA.DNA hybrids are not transcribed further into detectable amounts of double stranded DNA. They mention, however, unpublished experiments of Martin and his colleagues who apparently also find a major rapidly reassociating fraction and a smaller slowly reassociating fraction of double stranded product but who believe the smaller population of molecules include all the sequences of the complete viral genome. It is perhaps too early to decide which interpretation is correct, but it seems clear that a small part of the viral genome is transcribed *in vitro* into double stranded DNA much more often than into the complete genome.

Triassic Glider



THIS illustration shows a reconstruction of the lower Triassic reptile, *Longisquama insignis*, the remains of which were recovered by A. G. Sharov of the Soviet Academy of Sciences in the river clay deposits at Fergami in the Turkestan Ridge, 60 km from Leninabad (*Priroda*, No. 7, 108; 1971). The spinal appendages in this pseudosuchian were plate-like and were arranged in pairs down the spine. In addition to the skeleton, Sharov also found the scaly cover of the body and extremities.

phase affecting a broad area of crust before the drifting of the continents. He constructed two-dimensional stress trajectories (orthogonal lines representing variation in maximum and minimum stress directions) by drawing lines normal to the dyke trends (minimum stress) and lines parallel to the dyke trends (maximum stress). From these trajectories it is not only possible to determine surfaces of regional tension but also surfaces of regional shear and it was these surfaces that were later utilized to form the spreading axis of the Atlantic. The continental margins of North America north of the Bahamas and of West Africa north of Senegal broadly follow tension lines as does the southern coast of West Africa off Liberia. But from the Ivory Coast west to Nigeria the continental margin follows a direction of shear possibly related to the Romanche fracture zone.

Furthermore, the stress trajectories show a concentration of stress in an area around the Bahamas Ridge—a point not made by May but apparent from his construction. This concentration of stress in the upper mantle resulted in the tensional phase over a wide area of the overlying crust with the consequent dyke intrusion in the Late Triassic. At a later date the continental crust split, possibly initially at the point of stress concentration and subsequently along incipient lines of tension and shear. With the onset of rifting the stress on the continent was relieved and further displacements of the mantle resulted only in the production of new oceanic crust in the rift.

SENSORY PHYSIOLOGY

Olfaction and Taste

from a Correspondent

MOST of the research discussed at the fourth international symposium on olfaction and taste, held at Starnberg, Munich, between August 2 and 4, illustrated the progress which has been made in the understanding of the morphology and electrophysiology of single sensory cells or sensory epithelia, with relatively little emphasis on the central processing of chemosensory information or the influence of chemical stimuli on behaviour.

To elucidate the transduction mechanism by which chemical stimuli induce electrical changes in cells, it is convenient to be able to obtain reasonably large quantities of receptor membrane for analysis of the composition and conformation of the receptor surface. Dr J.-P. Changeux (Institut Pasteur, Paris) has used cell fragments of the electroplaque of the electric eel as a bulk preparation of cholinergic receptors and has been able to extract protein fractions which seem to bind acetylcholine, carbamylcholine and decamethonium.

The ubiquitous *Escherichia coli*, which is also readily available in bulk, has been used by Professor J. Adler (University of Wisconsin) to investigate the molecular basis of chemotaxis. Several species of receptor molecule on the receptor surface of the bacterium interact selectively with one of a group of substances to influence the direction of movement of the organism.

In spite of the difficulty of preparing pure extracts for analysis, sugar binding proteins from the sensory cells have been identified in cow tongue by Dr M. Sato (Kumamoto University) and in rat tongue by Dr K. Hansen (Zoologisches Institut, Heidelburg). In some particularly small scale experiments with blowflies, Dr H. Morita (Kyushu University) has found α-glucosidase activity associated with single contact receptor endings. Much more work will be needed, however, before it is understood how such interactions may be associated with changes in the ionic permeability of sensory cell membranes. Dr U. Thurm (Ruhr University, Bochum) has demonstrated that part of the total potential difference across insect sensory epithelium is dependent on metabolic energy. No further evidence was presented, however, to

Skiddaw Slates and Borrowdale Volcanics

THE rocks of many areas have been greatly faulted and cleaved as a result of Earth movements following their deposition. Over the years much geological work has been carried out in the Lake District, as elsewhere, to unravel the types and ages of Earth movements which have disturbed the country rocks. At the base of the stratigraphical succession of the sedimentary rocks of the Lake District are the Skiddaw Slates, of Ordovician age, on which rest the Borrowdale Volcanic rocks, also considered to be Ordovician. These in turn are succeeded by the Coniston Limestone Group (Ordovician) on which lie the Silurian flags, grits and shales.

The junction between the Skiddaw Slates and the overlying Borrowdale Volcanic rocks has been variously described as conformable, an unconformity, or faulted. A considerable obstacle to a confident opinion on this problem is the rarity of clear sections of the junction, because the surface of the country is widely covered by glacial and other drift deposits.

The Coniston Limestone Group succeeds the Borrowdale Volcanic rocks and it is now widely thought that the junction between these two groups of strata is markedly unconformable.

A thick succession of shales, flagstones, grits and greywacke lie above the Coniston Limestone Group and these belong to the Silurian System. These beds are succeeded with marked unconformity by the local representatives of the Old Red Sandstone (Devonian) or Carboniferous Limestone Series.

Recent wide scale mapping, involving the collection and plotting of large numbers of dips, strike lines and faults, suggests that the structure is more complicated than previously supposed. In particular, the relationship between the Borrowdale Volcanic rocks and the underlying Skiddaw Slates has been examined in great detail where exposed, but opinion still differs as to whether the junction is conformable, unconformable or faulted and, if the last-named, the nature of the faulting involved. Some workers, indeed, claim to recognize six groups of minor structures in the Black Combe area which, together with south-west Cumberland, Borrowdale (Keswick) and Cross Fell, has recently been re-examined.

In next Monday's *Nature Physical Science*, Helm and Roberts describe in detail the latest information about the Skiddaw Slate–Borrowdale Volcanic junction. But there is still much doubt about the detailed succession and the faulting and folding in the Skiddaw Slates.

clarify the issue of whether energy amplification is necessary at some stage in chemosensory transduction. Many of the speakers implied that it is, and this is clearly a crucial problem.

The low threshold for the response of many insect antennae to sex attractants is now well known. Dr R. A. Steinbrecht (Max Planck Institute für Verhaltensphysiologie, Seewiesen) explained how the silk moth antenna collects molecules of sex attractant, bombykol, and how these molecules reach the receptor cell membrane, and Drs K. E. Kaissling and G. Kasang (also of Seewiesen) discussed its remarkable isomeric specificity and its ability to resolve brief pulses of sex attractant applied in series. The properties of this receptor mechanism are rivalled in specificity by those of the antenna of the moth *Plusia ni* in response to its sex pheromone, cis-7-dodecenyl acetate. Dr L. K. Gaston (University of California, Riverside) spoke of the enormous differences in responses by this preparation when stimulated by different isomers of the compound, and similar ones of differing hydrocarbon chain length. Other speakers, including Dr J. Jakinovich (University of Michigan, Ann Arbor) on fly chemoreceptor specificity in response to different isomers of inositol, and Dr W. A. Kafka (Seewiesen) on the relative responses of locust olfactory cells to different isomers of methylbutanoic acid, revealed astonishingly narrow specificities. But few clues have emerged about the structure of the receptor sites for any of these pheromone molecules.

Studies were also presented on aspects of response characterization of sensory cells of vertebrate nasal and lingual epithelia, on the innervation of these cells, and on the way in which their responses may be influenced through intravascular stimuli. Perfused cyclamates can generate responses which summate with those to sweet tasting substances applied externally to the rat tongue (Dr R. M. Bradley, Nuffield Institute for Medical Research, Oxford). Another interesting study presented by Dr B. Oakley (University of Michigan, Ann Arbor) indicates that sets of taste papillae of the rat tongue, which show similar response characteristics, become associated with branches of the same afferent nerve fibre. This may imply that nerve fibres growing out into the tongue "search for" and recognize papillae of similar specificity pattern.

The proceedings of the symposium are due to appear in a volume of collected papers in about six months time, and it was agreed that the next symposium in this series would be held in Melbourne, Australia, about three years hence.

THE EARTH

Chondritic Again

from our Geomagnetism Correspondent

THE idea that the principal minor element in the Earth's fluid outer core may be sulphur rather than either nickel or silicon—and thus that the Earth as a whole may be chondritic after all—has now received further substantial support from Hall and Murthy (*Earth Planet. Sci. Lett.*, **11,** 239; 1971). This thesis, which Murthy and Hall propounded last year (*Phys. Earth Planet. Interiors,* **2,** 276; 1970) on the basis of an analysis of the relative abundance of volatile elements in the crust and mantle, was more recently taken up by Lewis (*Earth Planet. Sci. Lett.*, **11,** 130; 1971) who adduced several pieces of indirect evidence for it and discussed in some detail its important consequences. But Lewis's admitted difficulty was the lack of the thermodynamic data needed to make the case really convincing. It is this deficiency which Hall and Murthy have now remedied to a limited extent.

The classical view of the formation of the Earth's core is that an iron–nickel mixture segregated from a silicate matrix. It is Hall and Murthy's contention, however, that the first melt to form during the early history of the Earth was an FeS–Fe eutectic. These different processes are clearly likely to have different effects on the distribution of elements throughout the Earth and thus imply different courses for the Earth's subsequent history. So how would the elements be distributed if an Fe–S liquid had separated to form the Earth's core? In an attempt to answer this question Hall and Murthy have taken seventeen "diagnostic" elements, including the alkali metals Li, Na, K, Rb, and Cs, the alkali earths Be, Mg, Ca, Sr, and Ba, the transition metals Mn and Fe, and other critical elements such as Cu, Zn, U, Th, and Pb, and have tried to determine thermodynamically how they would become distributed between a predominantly Mg–Fe silicate matrix and an Fe–S melt during the Earth's early history.

As a starting point Hall and Murthy present thermodynamic data for the simple reaction $MeO+FeS=MeS+FeO$ where Me is an electropositive element. It is quite clear from the table they have compiled that K, Rb and Cs have high negative free energies of reaction at both 298 K and 1,000 K and thus preferentially form sulphides. These data thus support Lewis's contention that if an Fe–S liquid segregates into the core it will take with it a great deal of the available K, Rb and Cs, so that these elements will accordingly be depleted in the crust relative to a chon-

---

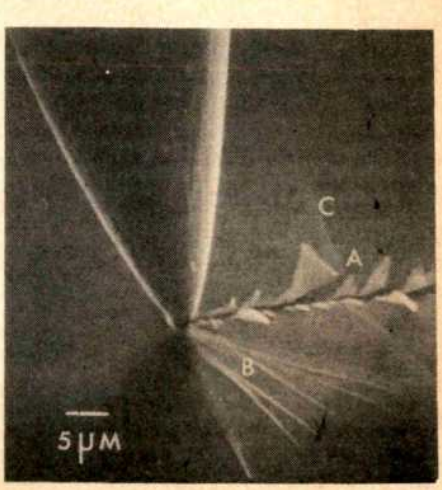
The Micro-friction of Graphite

NEW frictional studies of graphite at low loads (1 to 400 mg) have revealed much greater differences between the coefficient of friction of the basal and edge planes than expected. In next Monday's *Nature Physical Science,* Skinner, Gane and Tabor report their measurements of the coefficient of friction, using a scanning electron microscope and a delicately loaded tungsten stylus with a tip radius of about 1 μm.

They have found that the coefficient of friction on the edge plane is between fifteen and sixty times greater than the coefficient measured on the basal plane (0.3 compared with about 0.005 to 0.02); this is a much larger anisotropy than has been measured previously. Skinner *et al.* suggest that this is because their experiment involved not only small loads but also almost perfect surfaces which were carefully selected under the electron microscope.

At loads less than 40 mg, the low coefficients of friction of the basal plane were usually accompanied by only slight surface damage and, in some cases, by no damage at all. Sometimes the cleaving of a flake was observed as in the figure and very occasionally a peeling action took place; this was associated with much higher coefficients of friction (about 0.4).

At greater loads the basal planes were often too weak to support the stylus and even when they did, the stylus was found to penetrate further and further into the specimen until the experiment had to be stopped.



Scanning electron micrograph of a stylus sliding on the basal plane of graphite. ***A***, Flaking of the surface; ***B***, peeling; ***C***, a cleavage step.

dritic composition. On the other hand, it would be unrealistic to suppose that such a simple reaction is applicable to the whole Earth and thus that the oxide-sulphide equilibrium is anything but vaguely indicative of real conditions. This view is supported by the data. For example, sodium can also be seen to possess a high negative free energy of reaction, which fact would suggest that sodium was also withdrawn into the core and correspondingly depleted in the crust. But this is patently not so.

As Goldschmidt pointed out many years ago (in *Geochemistry*, Clarendon Press, Oxford, 1954), different reactions are applicable to different parts of the Earth. Thus Hall and Murthy go on to consider more appropriate equilibria. For the crust they examine reactions of the type $MeSO_4$ with FeS and $MeCO_3$ with FeS, which are consistent with the typically high oxidation states of crustal material. By contrast, for the mantle, which at the time of Fe–S segregation would have been in a reducing environment, the corresponding reactants with FeS are of the type $MeSiO_3$ and Me_2SiO_4. For each of the seventeen elements and for each reaction Hall and Murthy have computed heats of reaction (which dominate the free energies of reaction).

The results are striking. Elements like Be and Th (and thus by implication U) are strongly lithophilic under both crustal and mantle conditions. By contrast, elements such as Cu, Hg, Ag, Pb and Zn are strongly chalcophilic under crustal conditions and remain so throughout the mantle. But the critical elements K, Rb and Cs which are strongly lithophilic under crustal conditions rapidly change to being chalcophile under mantle conditions. In the presence of an Fe–S liquid these elements would thus have been drawn into the liquid and thus into the core. Sodium, on the other hand, remains lithophilic in the primitive mantle—and so the anomaly implied by the oxide–sulphide equilibrium disappears. In short, notwithstanding the fact that the reactions considered must be oversimplified compared with those applicable to the real Earth, the thermodynamic data obtained by Hall and Murthy are consistent with known abundances of the relevant alkalis in the crust and mantle.

As Hall and Murthy admit, however, this apparent success conceals some real objections. For example, their calculations have been made for an aluminium-free system ; and it is well known that Na and K have different terrestrial relationships with Al. Under crustal conditions both Na and K form alumino-silicates, especially felspars. But in the mantle, where pressures are higher, felspars are unstable and change to denser phases such as jadeite, the Na alumino-silicate. Potassium, on the other hand, does not apparently produce a high pressure form equivalent to jadeite for Na. If such a form really cannot exist, that fact alone may account to some extent for the different behaviours of Na and K in mantle regions. Ignorance of mantle conditions at the time of core formation precludes any detailed assessment of the role of Al at present, although, whatever the cause, the difference in behaviour between Na and K seems established. And further support for it comes from crystal chemistry. Potassium (and Cs and Rb) ions are larger than Na (and Li) ions and are thus less likely to fit into the mantle pyroxene structure but more likely to be absorbed into an available liquid. Thus Hall and Murthy suggest that "the incompatible nature of K, Hb, and Cs which has been reflected in their selective enrichment into basaltic melts through geological time must also be reflected in their enrichment into an Fe–S liquid during core formation in the early history of the Earth".

The distribution of elements implied by the early existence of an Fe–S melt, and, in particular, the removal of K into the core, would, of course, have affected the Earth's thermal history—and this implies that many conventional thermal models of the Earth must be revised. The apparent near-constancy of K/U ratios for a variety of terrestrial rocks has led many people to suppose that such a ratio (about 1×10^4) is a fixed feature of the Earth, the critical variable being the original total radioactivities in various parts of the Earth. But what Hall and Murthy have done is to show that this assumed "coherence" between K and U is not necessarily valid and that, on the contrary, it is quite possible that K separated from U (and Th) during the early history of the Earth.

Finally, the fact that Rb behaves like K in the presence of an Fe–S liquid would explain why Rb/Sr ratios in the Earth's crust are lower than chondritic. In short, if Hall and Murthy are right about the presence of an Fe–S melt during the early history of the Earth, the principal objections to the chondritic Earth are apparently removed.

Dynamic Stereochemical Model for ATP Synthesis

THERE have been many models of the mechanism by which chloroplasts and mitochondria can utilize redox energy associated with electron flow for photo and oxidative phosphorylation. In recent years the chemiosmotic scheme and chemical hypothesis (see Greville in *Current Topics in Bioenergetics* (edited by D. R. Sanadi, **3**, Academic Press, New York and London, 1969) have commanded the most attention. These schemes, however, tend not to deal with the detailed molecular processes which must be involved.

In next Wednesday's *Nature New Biology*, A. Bennun presents an hypothesis which attempts to relate molecular conformational changes within proteins with the process by which electron flow gives rise to ATP synthesis. He considers that the proteins involved in the energy transduction are coupling factors isolated from either chloroplasts (CF_1) or mitochondria (F_1) which show ATPase activity under appropriate conditions. These factors are thought to undergo dynamic stereochemical reactions induced by electron transport which involve charge dependent conformational changes.

Bennun has used kinetic data from chloroplast studies (Bennun and Avron, *Biochim. Biophys. Acta*, **79**, 646 ; 1964) to produce a scheme which involves two stages. The first stage is the formation of a proton dependent electrostatic complex, which is produced, it is argued, when an electron carrier, closely associated with CF_1 or F_1, is reduced. Because of the change in electric field the redistribution of the electrons within the coupling factors produces new dipolar forms. This complex, however, is not thought to be capable of directly inducing phosphorylation until the second stage, which is brought about by a loss of a proton and the dissipation of the partial charges within the protein. Bennun argues that the disappearance of the dipolar structure of CF_1 or F_1 leads to the formation of a phosphorylated high energy intermediate which then gives rise to ATP. The formation of these complexes is suggested to occur within the membrane which, as Bennun points out, would tend to result in structural restriction of the conformational changes associated with charge redistribution. Such restrictions could then act as effective energy sources.

Bennun takes his model one step further in attempting to explain the mechanism by which membrane located ATPase catalyses the hydrolysis of ATP and utilizes the energy for active transport of ions. Essentially the reverse process to that already described is thought to occur. The protons released could conceivably conserve their energy by binding to the relevant proteins which may be the ATPase itself or some other membrane protein. This binding could induce conformational changes involving stretching dipoles similar to that suggested for the coupling factors, and may occur as a result of changes in the degree of hydration of the protein.

Supermassive Objects in Astrophysics

VIRGINIA TRIMBLE
Institute of Theoretical Astronomy, Cambridge.

The scientific interests of William A. Fowler cover an unusually wide range. The symposium held at the Institute of Theoretical Astronomy, Cambridge, on July 19 to 21, 1971, in honour of his sixtieth birthday was designed to explore some of the topics with which he has been concerned and the relasionships among them.

IT was the extraordinarily large amounts of energy needed to explain radio sources and quasars that first led Hoyle and Fowler[1,2] and Hoyle, Fowler, Burbidge and Burbidge[3] to suggest that single objects much more massive than stars might be important in astrophysics. Since then, supermassive stars have been in and out of fashion several times as sources of energy and sites of nucleosynthesis, each time motivating a variety of theoretical studies and suggesting new ways of looking at observational data. Speakers at the symposium repeatedly emphasized the importance of the interplay of observations, theory and laboratory experiments in shaping our understanding of massive objects as well as ordinary stars and the processes occurring in them.

Black and White Holes

In accordance with the Fowler dictum of always searching for the objects with the largest possible binding energy, a session was devoted to collapsed configurations. The work of Penrose[4,5], Israel[6], Carter[7] and Hawking (to be published) has led to a picture of gravitational collapse in which the formation of a closed, trapped surface (roughly, one from which no light ray or other signal can ever escape to infinity) is followed inevitably by the formation of a singularity in the curvature of space–time. This singularity is probably shielded from view by a horizon, forming a "black hole", although the existence of what Penrose calls naked singularities cannot be excluded. Their work has also shown that space–time outside the singularity probably comes to look (asymtotically as time goes on) like either the Schwartzschild solution or the Kerr solution to Einstein's field equations. These solutions are characterized respectively by the mass of the singularity and by its mass and angular momentum. The singularity may also be charged but all other properties (lepton number, baryon number, multipole moments of the electromagnetic field and so forth) are probably lost or radiated away during the collapse. Thus, in the words of J. A. Wheeler, a black hole has no hair.

B. Carter (University of Cambridge) reviewed recently proven theorems directed towards changing the probabilities in the above picture to certainties. Not all loopholes have yet been closed.

S. Hawking (University of Cambridge) has shown[8] that there is a limit to the fraction of the mass energy of a singularity that can be radiated in all forms from a collision with another singularity. This limit is always less than 50%. Thus black hole collisions cannot be a 100% efficient source of gravitational radiation in a particular bandwidth, even if each object is involved in a series of collisions. This increases the amount of mass that must be involved in producing the pulses of gravitational radiation reported by Weber[9].

Despite the horizon surrounding a Kerr-type singularity, it is possible to extract that part of its energy associated with rotation rather than rest mass. J. A. Wheeler (Princeton University) suggested this as one of the most promising ways of finding observational evidence for black holes. (Searches for gravitational lens effects produced by them, X-rays resulting from matter accreting on them, gravitational radiation emitted at their formation, and binary systems with black hole components have also been proposed, and in some cases attempted.) Wheeler considered the fate of a white dwarf or neutron star falling into the neighbourhood of a black hole of mass $\sim 10^5\ M_\odot$. Tidal forces may fracture the star into two or a few pieces in that region known as the ergosphere (where the dragging of inertial frames by the rotating singularity is so rapid that no particle can remain at rest as seen by an observer at infinity). Then (as discussed by Penrose[10] for a single incident particle) while one fragment falls into the singularity, another can come out with more total energy than the incident star originally had. About 30% of the total mass-energy of a black hole with the maximum possible angular momentum for its mass could be extracted in this fashion.

W. D. Arnett (Rice University and Cambridge) provided a certain amount of discouragement by reporting a series of calculations of the late phases of evolution of a star of mass 30 to 100 $M_\odot$. In no case was a dense remnant left which was significantly larger than the Chandrasekhar limit for stable white dwarf stars. Thus only if the upper limit to neutron star masses is smaller than that for white dwarf masses would any of these remnants ever collapse to a black hole.

White holes are the time reversals of black holes, that is, mass energy expanding out of a singularity. They can thus provide literally unlimited amounts of energy. The apparently complete arbitrariness of the time at which the expansion starts was partially responsible for the lack of enthusiasm for white holes expressed by many of the symposium participants.

Supermassive Stars and Disks

The relativists participating in the symposium clearly regarded collapse to a singularity as a desirable event. The astrophysicists, on the other hand, were principally concerned with preventing supermassive stars from collapsing, or at any rate from collapsing too quickly, so that their energy could be extracted in some useful form. The problem is that there are, once one has taken relativistic effects into account, no stable, cold, slowly rotating configurations for masses greater than one or two $M_\odot$. And even the "hottest" configuration of a spherical supermassive star burning nuclear fuel is not stable against collapse above about 10^6 $M_\odot$—whether the star is assumed to be static or undergoing relaxation oscillations. Possible additional sources of support to balance gravitation in still larger objects are magnetic fields and rotation. The latter is particularly useful: there is no limit to the amount of mass that can be put into an infinitely thin, rotating disk. The problem in this case is that the disk seems to fragment into a number of small objects, and this process and subsequent events are very difficult to calculate. F. Hoyle (University of Cambridge) and E. Salpeter (Cornell University) each reviewed a probable "life history" of a supermassive object from formation to fragmentation and/or ultimate collapse.

Very massive stars may have formed out of the general interstellar medium early in the history of the galaxy, but this does not seem to be occurring at the present time. The most likely (or at any rate least unlikely) point of origin for a supermassive star now is at the centre of a galaxy or star cluster. Here, the gas lost by stars (either peacefully or through supernova explosions) will collect and condense, provided it can dissipate enough of its angular momentum. If the gas falls in sufficiently quickly, creating turbulence, dissipation of its dynamical energy will heat the gas and raise its entropy. If opacities are high enough to trap all the energy, pressure will suffice to support a spherical equilibrium configuration (which may or may not be stable) of mass up to 10^8 $M_\odot$. Slower collapse will lead to a rotating disk because the entropy of a given mass of interstellar medium is not as great as that of a spherical supermassive star of the same mass.

The supermassive star continues to lose energy by radiation and to contract. Its centre heats up and, for masses up to 10^6 $M_\odot$ (if there is no rotation) or 10^8 $M_\odot$ (for the maximum amount of uniform rotation), nuclear reactions "turn on" before the star reaches the critical radius at which its binding energy ceases to increase making collapse inevitable.

I. Appenzeller and K. Fricke (University of Göttingen), R. Talbot (Rice University and Cambridge) and R. Tayler (University of Sussex) presented results of calculations of nuclear burning in stars of mass 100 to 10^7 $M_\odot$. The German group finds that the relaxation oscillations proposed by Fowler[11] will probably occur and prevent collapse in stars of masses $\gtrsim 0.75 \times 10^6$ $M_\odot$. The star first contracts: Its central temperature and density, and thus the rate of nuclear energy generation, increase rapidly until the star is lifted back out again and the energy generated is released as thermal and non-thermal radiation and kinetic energy of matter blown off. The star then contracts again and so forth. The oscillation period is about one year, which might be relevant to periodicities perhaps observed for quasars. Objects more massive than 10^6 $M_\odot$ will not have their collapse halted by this process.

For stars with masses of 10^2 to 10^3 $M_\odot$, R. Talbot and Papeloizou (University of Sussex) find that pulsational instability almost certainly does not blow the star apart because pulsation energy is dissipated in shock waves and fed into stable harmonics of the unstable fundamental mode. Rather, material seems to be lost gradually. As this process continues, layers are lost which were previously in the star's convective core. They thus contain material in which nuclear reactions have occurred. Stars of this type early in the history of the galaxy, could have contributed an appreciable fraction of the helium as well as heavier elements we now observe. (Stars less massive than 50 $M_\odot$ or so appear to contribute very little helium.)

These objects can yield, at best, 0.01 of their rest mass energy in useful form, because that is the limit of efficiency of nuclear processes. To do better, we must consider more condensed configurations, whose binding energy is a significant fraction of Mc^2. Because of the collapse that sets in, for a massive spherical object below $r \sim 3 \times 10^5 \left(\frac{M}{M_\odot}\right)^{3/2}$ cm (where the binding energy is still $\ll Mc^2$), this means considering a rotating disk or a dense swarm of smaller objects.

The binding energy of a uniformly rotating, thin disk—most of the results thus far obtained will carry over to differentially rotating disks—increases monotonically with its degree of compactness to a maximum of about 0.37 Mc^2 at the point where the redshift of light from its centre is infinite[12,13]. Such a disk is therefore probably stable against overall gravitational collapse and thus useful for astrophysical purposes.

J. Bardeen (University of Washington) discussed the optical appearance of such a disk. As it contracts, it develops an ergosphere from which energy can be extracted. The effect of the rapid rotation on photons emitted is roughly to blueshift those emitted in the forward direction of the rotation and redshift those emitted in the backward direction. This effect is superimposed on the gravitational redshift produced by any massive object. Thus, although some photons are received at infinity with a blueshift, the total luminosity of the object goes to zero as the redshift from its centre goes to infinity. Light from the disk has a unique redshift only if it is uniformly rotating and seen face on. Most of the photons which reach a distant observer are, however, emitted in the equatorial plane of the disk, making such a disk by itself unpromising as a quasar model.

A. G. W. Cameron (Yeshiva University) discussed the probable behaviour of a gas disk inside a dense star cluster. The winding up of magnetic field lines by differential rotation may lead to the ejection of jets of material along its rotation axis by a mechanism analogous to that found by Leblanc and Wilson[14] for supernovae. Second, even a small amount of gas in this form can turn cosmic ray energy into thermal radiation very efficiently. Finally, accretion of the gas onto stars, by raising their masses, may appreciably enhance the supernova rate in the cluster.

Contributions by F. Pacini (Frascati) and by L. M. Ozernoi and V. V. Usov (Lebedev Institute, Moscow) dealt with massive rotating objects with magnetic fields. These might be expected to exhibit behaviour somewhat like that of pulsars, which somehow convert rotational energy to a useful form very efficiently. Some rough analogies between the properties of quasars and those of the Crab Nebula add interest to this type of model.

The contraction of a mass of 10^9 $M_\odot$ from an initial radius of 10^{21} cm, initial rotation period of 3×10^8 yr, and initial magnetic field strength of 10^{-5} to 10^{-6} gauss (reasonable properties for the centre of a galaxy) to a radius of 10^{16} cm yields a rotation frequency $\Omega \sim 10^{-7}$ s^{-1} and a magnetic field strength $B \sim 10^4$ to 10^5 gauss if angular momentum and magnetic flux are conserved. The rotation energy of such an object is $\sim 10^{60}$ erg and its magnetic energy $\sim 10^{56}$ erg. The magnetized disk then radiates by non-thermal as well as thermal mechanisms[15,16], contracts, and speeds up its rotation.

If the dominant form of energy loss is by magnetic dipole radiation at the rotation frequency, or particle ejection, then the luminosity of the object increases as $(1-t/t_0)^{-4/3}$ and its rotation frequency increases as $(1-t/t_0)^{-1/2}$. Such low frequency electromagnetic radiation cannot, of course, propagate in any reasonable surrounding medium, but there is, in any case, of order

$$L = 10^{47} \left(\frac{B}{10^5}\right)^2 \left(\frac{R}{10^{16}}\right)^6 \left(\frac{\Omega}{10^{-7}}\right)^4 \text{ erg s}^{-1}$$

of energy made available in some form. L may be decreased by a factor $\sin^2 \theta$ if magnetic and rotation axes are not perpendicular.

The time dependence of the luminosity is more complicated if there are other important energy losses or if the magnetic axis gradually becomes aligned with the rotation axis but the observed number of quasars, active galactic nuclei and the like as a function of luminosity is not inconsistent with the prediction of even a simple model. The luminosity of the disk stops increasing and drops rapidly to zero as seen by an observer at infinity when the disk has shrunk to such an extent that relativistic effects become important.

R. Wagoner (Cornell University and Cambridge) discussed the fate of the electromagnetic moments of a disk having a distribution of azimuthal currents and charges (such that the net charge of the disk is zero) on it, when the disk collapses as a result of the extraction of angular momentum from it. The magnetic dipole and octapole and electric quadrupole moments all appear to vanish as $(Z_c+1)^{-2}$ as Z_c, the redshift of light coming from the centre of the disk, goes to infinity. There is no reason to believe that other multipoles will not do the same. Thus, only visible objects seem able to radiate by the pulsar mechanism.

A black hole continues to exert a gravitational force on the outside world and thus can have a rotating disk around it, made up of material which either did not collapse or was accreted after the collapse. Lynden-Bell has considered such disks as the energy sources for quasars and galactic nuclei[17,18]. Viscosity in the disk will cause the gas to spiral gradually into the black hole. By the time a particle reaches the last stable orbit outside the black hole, it will have lost 42% of its rest mass energy at infinity. This is the most efficient way of extracting energy from ordinary matter so far found.

The thin, uniformly rotating disk considered by Salpeter, Bardeen and Wagoner[12,13,19] seem to be stable against collapse (though not, of course, against contraction as they radiate energy away). They are not, however, stable against fragmentation. Although gravitational forces are balanced by rotation and thermal forces (gas and radiation pressure) for the disk as a whole, gravitation predominates strongly for a piece of the disk having a size much greater than the thickness of the disk but much less than its radius, when this piece is considered by itself. As the disk loses energy, it becomes flatter, and, when it reaches an axial ratio of 0.05 (for a uniformly rotating disk), it will probably break into two or a few pieces. These, in turn, radiate away energy and flatten until they are unstable to breakup, and so forth.

Although the swarm of fragments continues to occupy about the same volume as the original disk did, they are individually much denser than it was. Thus binding energy has been extracted from them. Each generation of fragments is able to radiate the same fraction of its total energy. In many generations, the process can therefore become quite efficient.

The smaller objects will differ from the original disk in having higher equilibrium central temperatures, so that nuclear burning will turn on at some point, delaying the contraction and fragmentation of the blobs. They will also be increasingly efficient emitters of magnetic dipole radiation (provided some magnetic field was initially present) if total angular momentum and magnetic flux are conserved as they fragment. Gravitational radiation will also become increasingly important as fragmentation progresses if the mass distribution maintains its asymmetry.

There are many complications in this process, for example, variations in the angular momentum distribution, possible collisions of blobs, possible recoalescence of blobs, dissipation of angular momentum by viscosity, dissipation of asymmetries in the mass distribution by gravitational radiation, and possible ways of extracting the thermal energy of the gas. It is just possible that the fragmentation could end in a swarm of (essentially) mass points whose gravitational potential is sufficiently flat at the centre to produce a fairly constant gravitational redshift from a central gas cloud, thus permitting objects with large redshift to be closer to us than their cosmological distances, as suggested by Fowler and Hoyle[20].

F. Hoyle (Cambridge) pointed out that, despite the wide range of phenomena possible in this scheme in which objects proceed toward more and more condensed states, it may not suffice. We may yet have to consider matter evolving through expansion especially to explain violent events in galactic nuclei and the formation of large double radio sources.

Observations

The need for large amounts of energy ($\sim 10^{62}$ erg) to account for the observed properties of radio sources first suggested to Hoyle and Fowler that supermassive objects might be important in astrophysical contexts. Observations since that time have, with about equal frequency, suggested new theoretical approaches to the problems of quasars and galactic nuclei and created difficulties for old ones.

G. Burbidge (University of California, La Jolla) and R. Weymann (Arizona) reviewed some of these problems, ranging from very general questions of energetics to specific difficulties, such as whether or not emission line profiles of Seyfert galaxies can be understood in the context of a gas disk rotating around a condensed central mass.

A compilation of the various kinds of radiation and mass loss which we see going on[21] suggests that the release of gravitational energy in the nuclei of galaxies may be the most important source of energy in the universe. Some more or less continuous process must be involved, since nearly all nuclei show a degree of activity. This continuous activity might be easier to understand as the result matter expanding from, rather than contracting to, a condensed state or singularity.

It also appears difficult to explain the ejection of coherent, massive objects from galactic nuclei on the basis of the conventional contraction hypothesis (although either Wheeler's ergosphere mechanism or ejection of bits of Salpeter's fragmented disk could impart large velocities to bodies of mass $\lesssim 1\ M_\odot$). Among possible evidence for such massive coherent ejecta are tiny radio source components far from their parent galaxies (including those in Cygnus A, ref. 22), the bright optical blobs in the jet of M87, and the Seyfert-like galaxies VV 144 and I Zw 95 which are connected by jets or bridges to nearby, smaller, more normal looking objects. On a less spectacular scale, asymmetries and transient doubling of Seyfert galaxy emission lines can be attributed to the ejection of blobs of gas of mass 0.1 to 1.0 $M_\odot$ with velocities of 1,000 to 5,000 km s^{-1}.

Actual or potential observations can also have a bearing on whether a single, supermassive object or a swarm of smaller ones is more likely to be responsible for active nuclei. Genuine periodicity in the variations of the brightness of quasars and so on would be strong evidence for a single, coherent rotating object (although differential rotation is likely to shear off a "hot spot", so that a given pattern of variability would only last for a few periods[23]). The large amplitudes of quasar light variations already suggest that one or a few objects are responsible for most of the continuum light output at a given time. A series of radio outbursts along the same axis or a single outburst involving more than $\sim 10^{52}$ erg (the binding energy of a neutron star) would also be evidence for a single object. A single, supermassive magnetic rotator might be detectable as a low frequency, very small radio source in the way that the Crab Nebula pulsar was first seen[24]. Finally, an outburst from a single central object might be expected to disturb and blow away the thermal gas cloud which produces the emission lines in an active nucleus. The Seyfert galaxy NGC 3516, in which a broad Hβ line disappeared sometime between 1942 and 1967 (ref. 25) and reappeared between 1967 and 1969 may have done something of the sort.

In 3C 120, on the other hand, the broad hydrogen lines appear not to have been disturbed by the sharp radio outbursts which have occurred in the past few years. This would seem to be evidence for a group of smaller objects, only one of which blew up (or perhaps for a single object with strong directivity). If successive violent events in a number of small objects are responsible for the luminosity of a small, bright Seyfert nucleus like that of NGC 4151, then the centre of light ought to move around. This also applies to the radio positions of unresolved quasars.

The amount of energy and mass required to explain violent

Pure Chemistry Library

activity in quasars and galactic nuclei depends, of course, on their distances. Distances to peculiar objects are measured either by direct application of the Hubble–Lundmark relation or from their physical association with other objects whose distances we think we know. The problems arise when these conflict. For ordinary galaxies, selected by some appropriate luminosity criterion, the redshift–magnitude relation is fitted extremely well by a straight line. For quasi-stellar objects, on the other hand, it is a scatter diagram[26]; thus we lack strong evidence that redshift is a measure of distance for these objects.

Several cases have been found in which two objects with different redshifts appear to be connected by bridges, jets or arms. These include NGC 4319+MK 205, NGC 7603+ a nearby compact galaxy, and IC 1746+ an adjacent quasi-stellar object. In other cases (for example, VV 172 and Seyfert's Sextet) objects with different redshifts are not connected but are clustered at least in the plane of the sky. Finally, Burbidge, Burbidge, Solomon and Strittmatter (to be published) have found that four of the 3C quasars are within 6′ of spiral galaxies in de Vaucouleur's catalogue. Only 0.2 such close association would be expected by chance. On the other hand, one or more QSOs appear to be associated with normal galaxies having the same redshift[27]. Some of the participants were sufficiently impressed to express the view that conventional physics may be inadequate to explain these data.

The optical continuum of an active nucleus should reveal something about the objects producing it; if we see the surface of a supermassive star or disk directly, then the continuum spectrum ought to be basically thermal whereas a non-thermal spectrum would imply that the energy of the central object is being transformed into relativistic particle and magnetic field energy.

As observed, the optical continuum of active objects typically looks non-thermal. It may, however, be necessary to correct the continuum for the reddening found by Wampler[28] for Seyfert emission lines. This will be the case if the material producing the lines covers all or most of the continuum source. After making such a correction, Pagel (Royal Greenwich Observatory) and Kaneko[29] find that the emitted spectrum in fact looks like the Rayleigh–Jeans part of a thermal continuum, with $T \gtrsim 60{,}000$ K, rising into the ultraviolet.

As for line spectra, Seyfert and Markarian type nuclei are easier to understand than QSOs. The galactic nuclei typically show relatively sharp forbidden emission lines and hydrogen Balmer lines with sharp cores and much broader ($\Delta V \sim 5{,}000$ km s^{-1}) wings. Along a sequence from Seyfert galaxies to QSOs, the ratios [OIII]/Hβ and Hβ (core)/Hβ (wings) tend to drop (to zero in some cases).

Photoionization appears able to account for all the lines observed in Seyfert nuclei, as the presence of [FeXIV] in NGC 4151 has not been confirmed[30]. The forbidden lines are necessarily produced in a low density region, while the broad Balmer lines are most easily understood in terms of a combination of electron scattering in dense, small cores ($N_e \sim 10^8$ to 10^9; $R \sim 10^{16}$ cm) and ejection of line-radiating blobs at velocities of 1,000 to 5,000 km s^{-1}. A model with rotational broadening due to an optically thin disk rotating around a central mass $\gtrsim 3 \times 10^8\ M_\odot$ is also possible, but only a very narrow range of parameters can account for the observed profiles. No abundance anomalies are required to explain Seyfert galaxy line spectra, but abundance variations by factors of 2 or 3 are quite possible.

In quasars, on the other hand, no model appears to account satisfactorily for the line profiles. If resonance lines are trapped efficiently enough to yield the observed widths, then they should be strongly self-reversed. And sufficient gas motions to smear out the self-reversal would also make the forbidden lines broad.

As far as abundances are concerned, it is still not clear whether the weakness of helium recombination lines relative to those of hydrogen in 3C 273 is an abundance effect. It might, rather, be due to a low degree of helium ionization[31]. A better upper limit to, or detection of, HeI (5876 Å) would resolve the question.

Objects like NGC 1068, whose Balmer lines do not have broad wings, may have had their dense gas swept out by violent events at their centres. If the events take the form of ejection of relativistic particles, Rayleigh–Taylor instability at the particle–gas interface would produce filamentary structure like that seen in NGC 1275. The gas would also be given the sorts of velocities found in transient line doubling and would eventually produce larger regions of more tenuous gas. These are seen (with diameters ~100 pc) in Seyferts and perhaps in QSOs if the relatively sharp QSO absorption lines are produced in the objects themselves.

We may be observing one object in transition between the broad, supernova-like absorption bands seen in PHL 5200 (ref. 32) and the sharp absorption lines, often at several very different redshifts, seen in other QSOs. The object PHL 957 (ref. 33) has an emission redshift of 2.679 (as measured on Lick spectrograms; E. M. Burbidge, private communication) and at least eight separate absorption redshifts. One of these is represented by extremely broad Ly α and Ly β lines at $Z=2.308$. This is the largest difference so far found between an emission and an absorption redshift for broad lines, and neither the large ΔZ nor the broadness of the lines can be understood in terms of intergalactic absorption. The object is thus evidence for a high velocity explosion in a high density region of the QSO.

Nucleosynthesis and Reaction Rates

The rates of nuclear reactions are crucial to the determination of what nuclear fuels are burned, at what temperatures and densities they burn, and what the reaction products are, both in supermassive and ordinary stars. C. A. Barnes (California Institute of Technology) pointed out that the completeness of our knowledge declines rapidly with increasing atomic weight of the reactants. All the cross sections needed to deal with hydrogen burning seem to be well established but at the other end of the scale, it is not even obvious what nuclear information is required in order to treat silicon burning. The abundances of the products of silicon burning may, in some cases, depend on nuclear reaction rates and, in others, on equilibrium considerations and binding energies.

In the intermediate range, after the conversion of hydrogen to helium, the next nuclear reactions in stars are (*a*) the combination of three alpha particles to form carbon-12, (*b*) the addition of another alpha particle to form oxygen-16, and (*c*) successive heavy ion reactions to form neon-20, magnesium-24 and so on. Since it appears that the rates of the reactions of type (*c*) are much slower than (*a*) and (*b*), the relative abundances of carbon and oxygen depend almost exclusively on the rates of (*a*) and (*b*). Recent information[34–36] (and unpublished work by Barnes, Dyer, Kavanagh, Mann, McCaslin, Morgan, Nichols, Thompson and Weisser at California Institute of Technology) leads to the conclusion that the time is rapidly approaching when a comparison of the observed carbon to oxygen ratio with that predicted from laboratory measurements will provide a critical test of prevailing theories of helium-burning nucleosynthesis. At the same time, the carbon–oxygen ratio at the conclusion of helium burning appears to be of great importance for later, explosive stages of stellar evolution, possibly even determining whether or not a remnant is left after such an explosion or supernova.

A knowledge of the nuclear reactions that occur in stars of various masses and the birth rates of these stars as a function of time in the Galaxy could be combined to give the history of the nuclear composition of the Galaxy provided we knew how much of each star is thrown back into the interstellar medium for reuse and how much is trapped in white dwarfs, neutron stars, and black holes. Calculating this history therefore requires three input parameters, all of which are extremely uncertain; nor are the data on stellar compositions as a function of age and position in the galaxy, with which the calculations are to be compared, as well established as we would like. Nevertheless, such calculations have been attempted by W. D. Arnett (Rice University and Cambridge) and A. G. W. Cameron (Yeshiva University) and their colleagues. The two groups have made rather different assumptions, especially about the fractions of a star going into the various possible reaction products (unprocessed hydrogen + proton enriched nuclei, helium, carbon and oxygen, iron, neutron enriched nuclei, white dwarfs, neutron stars and black holes). Each group has succeeded in matching the observations of metal abundance as a function of stellar age. Cameron has also taken account of the constraints on the production of heavy nuclei provided by solar system data on the abundances of radioactive nuclei and their decay products. Arnett's work also reproduces the distribution of stellar abundances as a function of position in the galaxy.

Insight is provided by these calculations into some details of observed stellar abundances. For instance, the systematic tendency of the ratio N/Fe to be very low whenever Fe/H is low (B. Pagel), follows from the fact that nitrogen is largely produced in CNO cycle burning of hydrogen, which requires that carbon and oxygen have been produced in a previous generation of stars. Thus no nitrogen is formed until the second generation.

It is necessary in these nucleosynthesis calculations to assume that a larger fraction of star forming material went into massive stars in the past than it does now. Otherwise, according to Cameron, almost 25% of the one solar mass stars now seen would have metal abundances less than 10% of the solar value, which is not the case. Thus galaxies should have been much bluer and much brighter in the past.

There is, however, some observational evidence that the birthrate function, $\Phi(M)$, does not change its shape as a function of time. W. L. W. Sargent (California Institute of Technology), L. Searle, and W. Bagnuolo have found that the present *UBV* colours of Irregular and Sc galaxies can be understood in terms of a birthrate function given by $\Phi(M,t)=A(t)M^{-n}$. If a typical galaxy is 10^{10} yr old, then $n=1.35$ for Sc and 1.1 for Irregular type galaxies. For the bluest galaxies of each type, $A(t)$ has been essentially constant, whereas for the reddest ones (for example, Magellanic Cloud type Irregulars), $A(t)$ is now about 0.1 of its value 10^{10} yr ago.

Even at the present time and for our own Galaxy, $\Phi(M)$ is not well known, especially for very high and very low masses. The power law M^{-n}, must cut off (for $n>1$) below some minimum mass; otherwise the integral mass involved would be infinite. Where this cutoff occurs is important because low mass stars lock up both processed and unprocessed material and prevent it from taking further part in galactic nucleosynthesis. Because objects with $M \lesssim 0.1\ M_\odot$ never burn hydrogen, they are almost undetectable except as astrometric companions to nearby dwarf stars (for example, Barnard's star). This means that the low mass cutoff may be unobservable. The very massive stars, which contribute the largest fraction of heavy elements, are also a problem. N. J. Woolf (Minnesota) noted that the brightest (and therefore presumably most massive) single star known (6 Cyg No. 12, $M_{\mathrm{bol}} \lesssim -10$) is hidden behind a dense envelope, seriously hampering our determination of the space density of such stars. A large, wide field telescope in the southern hemisphere should make it possible to determine the birthrate function for stars more massive than 1 to 2 $M_\odot$ in the Magellanic Clouds where the effects of dust obscuration are much less severe than in the Galaxy.

Finally, it is not even clear that our Galaxy is a closed system as assumed in all calculations of galactic nuclear evolution. It is quite possible that the clouds of neutral hydrogen considered by Oort[38,39] to be falling into the galactic plane at high velocities represent intergalactic matter being added to the interstellar medium at a rate of 0.1 to 1 $M_\odot$ yr^{-1}. Such matter would presumably consist of only hydrogen and perhaps helium and would thus significantly dilute the heavier elements produced in stars.

Concluding Remarks

A number of problems remain, particularly in understanding how supermassive objects are formed. Nor is there yet any really firm observational evidence for their existence. But when supermassive stars were first proposed as a source of large amounts of energy, they seemed to be pulsationally unstable. Subsequent work, some of it discussed at this symposium, has shown that this is probably not the case. Thus we are perhaps justified in joining the Fathers of the Subject, Hoyle and Fowler[40], in turning a blind eye, a deaf ear, and a cold shoulder to written, oral and implied criticism respectively.

We thank Drs M. J. Rees, W. L. W. Sargent, R. V. Wagoner, S. W. Hawking, E. M. Burbidge, G. Steigman and W. D. Arnett for comments; the speakers who supplied preprints or outlines of their talks, and especially Professor William A. Fowler for starting it all.

[1] Hoyle, F., and Fowler, W., *Mon. Not. Roy. Astron. Soc.*, **125**, 169 (1963).

[2] Hoyle, F., and Fowler, W., *Nature*, **197**, 533 (1963).

[3] Hoyle, F., Fowler, W., Burbidge, G., and Burbidge E., *Astrophys. J.*, **139**, 909 (1964).

[4] Penrose, R., *Phys. Rev. Lett.*, **14**, 57 (1965).

[5] Penrose, R., and Hawking, S. W., *Proc. Roy. Soc.*, A, **314**, 529 (1970).

[6] Israel, W., *Phys, Rev.* **164**, 1776 (1967).

[7] Carter, B., *Phys. Rev. Lett.*, **26**, 331 (1971).

[8] Hawking, S. W., *Phys. Rev. Lett.*, **26**, 1344 (1971).

[9] Weber, J., *Phys. Rev. Lett.*, **25**, 180 (1970).

[10] Penrose, R., *Nuovo Cimento*, serie 1, **1**, 252 (1969).

[11] Fowler, W., in *High Energy Astrophysics*, Proc. Internat. School Phys. "Enrico Fermi" Course 35 (edit. by Gratton, L.), 322 (Academic Press, New York, 1966).

[12] Bardeen, J., and Wagoner, R., *Astrophys. J.*, **158**, L65 (1969).

[13] Salpeter, E., and Wagoner, R., *Astrophys. J.*, **164**, 557 (1970).

[14] Leblanc, J. M., and Wilson, J. R., *Astrophys. J.*, **161**, 541 (1970).

[15] Woltjer, L., *Pont. Acad. Sci., Scripta Varia*, **35**, 477 (1971).

[16] Morrison, P., and Cavaliere, A., *Pont. Acad. Sci. Scr. Var.*, **35**, 489 (1971).

[17] Lynden-Bell, D., *Nature*, **223**, 690 (1969).

[18] Lynden-Bell, D., *Pont. Acad. Sci. Scr. Var.*, **35**, 527 (1971).

[19] Bardeen, J., and Wagoner, R., *Astrophys. J.*, **167**, 359 (1971).

[20] Hoyle, F., and Fowler, W., *Nature*, **213**, 373 (1967).

[21] Burbidge, G., *Pont. Acad. Sci. Scr. Var.*, **35**, 427 (1971).

[22] Hogg, D., MacDonald, G., Conway, R., and Wade, C., *Astron. J.*, **74**, 1206 (1969).

[23] Fowler, W. A., in *The Crab Nebula*, IAU Symposium No. 46 (edit. by Davies, R. D., and Smith, F. G.) (D. Reidel, Dordrecht, 1971).

[24] Hewish, A., and Okoye, S., *Nature*, **203**, 171 (1964).

[25] Andrillat, Y., and Souffrin, S., *Astrophys. Lett.*, **1**, 111 (1968).

[26] Sandage, A., *Pont. Acad. Sci. Scr. Var.*, **35**, 289, 615 (1971).

[27] Gunn, J., *Astrophys. J.*, **164**, L113 (1971).

[28] Wampler, E., *Astrophys. J.*, **154**, L53 (1968).

[29] Kaneko, N., *Proc. Astron. Soc. Japan* (in the press, 1971).

[30] Weedman, D., *Astrophys. J.*, **167**, L23 (1971).

[31] Williams, R., *Astrophys. J.*, **167**, L27 (1971).

[32] Lynds, C. R., *Astrophys. J.*, **147**, 396 (1967).

[33] Lowrance, J., Morton, D., Zucchino, P., Oke, J., and Schmidt, M., *Bull. Amer. Astron. Soc.*, **3**, 238 (1971).

[34] Austin, S., Trentleman, G., and Kashy, E., *Bull. Amer. Phys. Soc.*, **15**, No. 1 (1970).

[35] Stocker, H., Browne, C. P., and Rollefson, A. A., *Bull. Amer. Phys. Soc.*, 16, No. 4 (1971).

[36] Jaczcack, R. J., Macklin, R. L., and Gibbons, J. H., *Phys. Rev. C2*, **63**, 2452 (1970).

[37] Burbidge, E. M., Burbidge, G. R., Fowler, W. A., and Hoyle, F., *Rev. Mod. Phys.*, **29**, 547 (1957).

[38] Oort, J., *Bull. Assoc. Netherlands*, **18**, 421 (1966).

[39] Oort, J., in *Radio Astronomy and the Galactic System* IAU Symp. 31 (edit. by van Woerden, H.), 279 (Academic Press, 1967).

[40] Hoyle, F., and Fowler, W., in *Quasi-Stellar Sources and Gravitational Collapse* (edit. by Robinson, I., Schild, A., and Schucking, E.), 17 (University of Chicago Press, 1965).

South Labrador Sea and the Evolution of the North Atlantic

A. S. LAUGHTON
National Institute of Oceanography, Wormley, Godalming, Surrey

Basement morphology and magnetic anomalies in the South Labrador Sea have preserved the history of the changes from a two-plate configuration of sea floor spreading to a three-plate configuration after the split between Greenland and Rockall Plateau and back to a two-plate configuration when the Labrador Sea stopped widening.

BEFORE the separation of Rockall Plateau and North-West Europe from Greenland and the growth of the Reykjanes Ridge, the Labrador Sea was the northern part of the Atlantic Ocean. Although it is floored by sediments[1,2] which almost totally obscure the basement topography except south of Greenland, a buried mid-ocean ridge has been located beneath the sediments by seismic and magnetic methods[2-6]. The identification of the magnetic anomalies on the Heirtzler time scale[7], and hence the history of the sea during the Mesozoic and Tertiary has, however, been uncertain[2,6,8].

This article reports a new interpretation of the South Labrador Sea based on data obtained during leg 12 of the Deep Sea Drilling Project in D.V. Glomar Challenger and earlier data.

Basement Morphology

Fig. 1 shows what is now known of the basement in the southern Labrador Sea, and includes some features typical of seafloor spreading, such as mid-ocean ridges, median valleys and transform faults. At 57° N, 48° W, Le Pichon *et al.*[2] have identified a buried median valley which is consistent with the magnetic data in the area. Further east, at 57° N, 43° W, two lines of basement ridges are separated by an east–west valley filled with sediment, which appears to be a median valley paralleled by magnetic anomalies[9]. On this meridian, this is the only place between the Gibbs Fracture Zone and Cape Farewell where the basement topography suggests a spreading centre (see Fig. 11 of ref. 10). The adjacent ridges both seem to have been uplifted and tilted away from the axis preserving a sediment cover of 0.2 s on their outer flanks. These two sections of median valley are not colinear and are separated by a broad ridge complex about 70 km wide striking north-east–south-west. The ridge can best be interpreted as a fracture zone or transform fault offsetting the axis of the median valley. The strike of the transform fault is in general agreement with the pole of rotation of 61° N, 92° W calculated for the last phase of the opening of the Labrador Sea by Le Pichon *et al.*[2].

South of Greenland, Le Pichon *et al.* found basement ridges which they interpeted as a transform fault (the Farewell Transform Fault) associated with the first phase of opening of the Labrador Sea. Further south, they also associated the buried part of the Gibbs Fracture between 45° and 47° W with this phase, and from these two features the pole for the first phase of opening was computed to be at 80° N, 89° W.

The remaining structural trend is normal to the west end of the Gibbs Fracture Zone, and is perhaps truncated by it, suggesting that the ridges on this trend are the remains of structure parallel to the spreading axis during the first phase of opening of the Labrador Sea. Magnetic analyses indicate that the ridges may be the extinct spreading axis. Further north-west, the ridge at 55.7° N, 48.5° W, is parallel to the median valley to the north of it and probably belongs to the second phase of opening.

All the basement trends in Fig. 1, except the exposed part of the Gibbs Fracture Zone, can be correlated with either the spreading axes or transform faults associated with the two phases of opening of the Labrador Sea. The Gibbs Fracture Zone[11] east of 43° W is associated with spreading about the Mid-Atlantic Ridge further east.

The semicircle formed by the basement ridges has been a barrier to deep water circulation and the transport of sediment. It encloses a large body of sediment typified by topographic

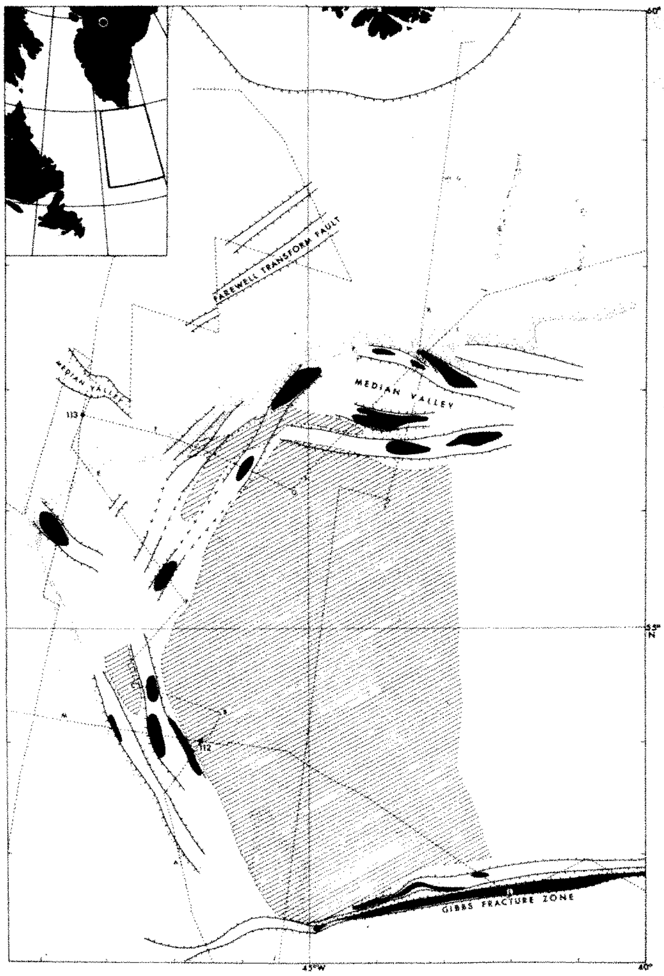


Fig. 1 Basement structure and sediment cover of the South Labrador Sea derived from seismic reflexion profiles and from bathymetry. Ticked lines = basement scarps; dense hachure = basement exposures on sea floor; stippled boundary = contact between terrigenous sediments and bottom current controlled sediment body; light hachure = area of oligocene mid-sediment reflector.

ridges several hundred kilometres long, a wavy surface, marginal channels and moats, and no conformity with the basement. It is relatively transparent except for a mid-sediment reflector (shaded in Fig. 1) shown by drilling to be Oligocene. These characteristics suggest that the sediments were laid down under the influence of the near bottom currents[8,10,12] and the pattern of ridges indicates an anticlockwise bottom circulation fed from the east through the Gibbs Fracture Zone. From the extent of the reflector and the conformity with it of overlying beds, the deep circulation in the southern Labrador Sea seems to have remained relatively constant since the mid-Tertiary in spite of the considerable change in width of the Atlantic.

Some of the sediments have overflowed the basement barriers and to the west and south are lying underneath the terrigenous sediments which have accumulated at a high rate due to the proximity of land and which were sampled at site 113; their extent is shown in Fig. 1 by the stipple boundary. Another sedimentary supply by ocean currents along the south-east coast of Greenland gave rise to the Eirik Ridge[12] which lies above the Farewell Transform Fault. Contrary to the suggestion of Jones *et al.*[10], this does not seem to be related to the sediment body at 55° N, 45° W.

Magnetic Data

There is a symmetrical pattern of magnetic anomalies, which trends north-west–south-east in the Central Labrador Sea and east–west south of Cape Farewell[1,2,4–6,9,13,14], offset by two fracture zones trending north-east–south-west. The axis of symmetry in the central Labrador Sea is magnetically smooth, but two groups of high amplitude anomalies (500 gamma peak-to-peak) lie halfway between the axis and the continental slope. South of Cape Farewell, the southerly group of these anomalies bends sharply southwards parallel to the Mid-Atlantic ridge. At 52.5° N they are offset eastwards by the Gibbs Fracture Zone. The interpretation of the tectonic history of the Labrador Sea depends critically on the age of these anomalies and of the axis.

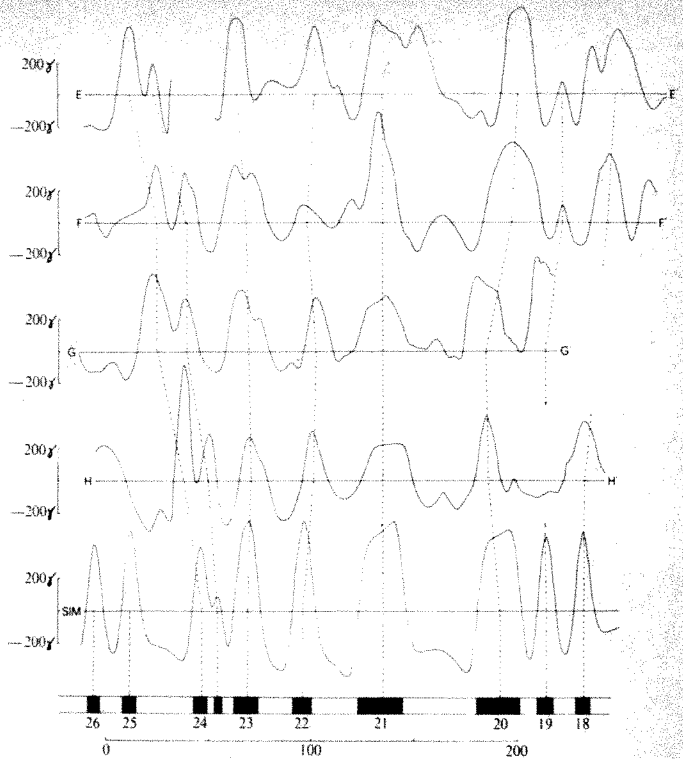


Fig. 3 Magnetic anomalies along tracks EE′, FF′, GG′ and HH′ (Fig. 2) projected perpendicular to anomaly trend 170°, compared with simulated anomaly using Heirtzler *et al.*[7] time scale for anomalies 18 to 26. (Magnetized layer 4.5 to 6.5 km below sea level; effective susceptibility ±0.01 c.g.s. units; black blocks normally magnetized. Spreading rate of 1.26 cm yr^{-1}.)

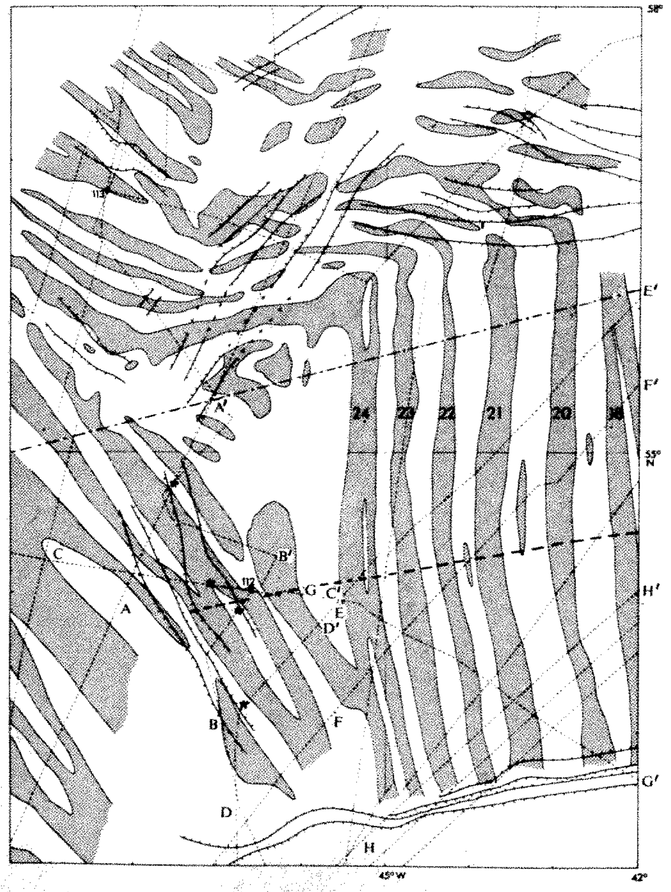


Fig. 2 Magnetic anomaly chart of South Labrador Sea. North of dot-dash line, contours are modified from Vogt *et al.*[15]. South of this line, control is by tracks shown. Shaded area is positive. Anomaly numbers are from Fig. 3.

In the South Labrador Sea, the magnetic anomalies (Fig. 2) relate closely to basement trends and reflect the various phases of opening of the Labrador Sea and the North Atlantic. North of 56° N, the contours (modified from Vogt *et al.*[15]) show the trends parallel to the median valleys, and the symmetry about them, and the offset due to the transform fault. The strong north–south lineations between 42° and 45° W can be matched with a simulated anomaly pattern for anomalies 18 to 24 (ref. 7) with a spreading rate of 1.3 cm yr^{-1} parallel to the Gibbs Fracture Zone (Fig. 3). Some dislocation of this pattern may, however, be present along the heavy dashed line in Fig. 2, which is a westward continuation of the fracture zone crossing the Mid-Atlantic Ridge at 53.5° N (ref. 16).

West of 45° W, the magnetic anomaly pattern trends north-west–south-east, parallel to the basement ridges near site 112. The correlations in this area are less certain than the north–south ones, but the trend is clear and continues to the north-west into the area of better mapped anomalies in the Labrador Sea. Near site 112, the lack of continuity may be due to the fracture zone. The same north-west–south-east trend is repeated towards the south-west as far as the continental margin. Further evidence of a change of pattern west of anomaly 24 comes from the absence of anomalies 25 and 26 on the projected profiles in Fig. 3. A triangular area centred on 55° N, 46° W, for which there are no data, is enclosed by the three prominent trends.

Data Interpretation

We can now follow the evolution of the northern part of the North Atlantic with some certainty by working backwards using the above magnetic data together with other magnetic data from several sources (refs. 14, 17–20 and data from the USNOO) and the fracture zones (Fig. 4). The principal fracture zone controlling the North Atlantic opening is the Gibbs Fracture Zone, known also as the Charlie or 53° N Fracture Zone (refs. 11, 16, 21 and data from the NIO and the USNOO). South of this are numerous fracture zones offsetting the ridge axis to 28° W and indicated by earthquake epicentres. These have been lumped together as one fracture zone at 50° N.

The symmetry of magnetic anomalies 19 to 24 about the Mid-Atlantic Ridge between 53° and 56° N confirms the identification of the north–south anomalies south of Greenland. The continuity of anomaly 20 between a north–south orientation and an east–west orientation at 56.5° N, 43° W (Fig. 2), its proximity to the median valley, and the apparent absence of such a right angle for anomaly 19, suggests that the Greenland and Labrador plates have not separated appreciably since anomaly 19 (47 m.y. ago). On this assumption, the European plate can be closed into the Labrador–Greenland plate using the Gibbs Fracture Zone as a control guide to give the 47 m.y. (Middle Eocene) palaeogeography (Fig. 5). A good fit of anomaly 19 is obtained. A consequence of the immobility of Labrador–Greenland plate boundary after 47 m.y. is that the median valley south of Cape Farewell should not extend further east than 41.5° W; this can be tested by further surveys.

Before 47 m.y., Greenland and Labrador have to be closed along the lines indicated by the fracture zones offsetting the Mid-Labrador Sea Ridge axis, while the closure of the Reykjanes Ridge still continues. The anomaly 24 or 60 m.y. (Palaeocene) palaeogeography (Fig. 6) differs slightly from that presented by Vogt *et al.*[9] in the greater counterclockwise rotation of the European plate. Rockall Plateau, shown by holes 116 and 117 to have subsided from above sea level since the Palaeocene, is not close up against the continental slope of Greenland at this time although anomaly 24 is very close to Rockall Plateau. The original Greenland continental margin may, perhaps, have been downwarped after the breakaway of Rockall Plateau or else an Upper Cretaceous geosynclinal sedimentary basin may have existed before the split. In the central Labrador Sea, the two prominent magnetic anomaly groups are apparently older than 60 m.y.

At 60 m.y., the whole anomaly pattern changed from the two plate geometry because of the separation of the European plate from Greenland. All magnetic anomalies older than 60 m.y. have a trend more north-west–south-east than those of 60 m.y. and younger. These trends were directed parallel to the Labrador continental margin and approximately parallel to that of the Greenland–European plate margin. The most prominent of these trends are those near site 112 which are now median to the Palaeocene ocean and offset from the central Labrador Sea anomalies by the Farewell Transform Fault and its counterpart on the Labrador margin[2]. This transform fault may be part of the line of weakness continuing north-east into the region between Rockall Plateau and Greenland which subsequently split to give the Reykjanes Ridge. The anomalies near site 112 and the associated basement ridges may be part of the fossil ridge axis resulting from the final phases of the pre-Palaeocene spreading which took place before a shift of stress pattern occurred beneath the plates. The initiation of spreading between Rockall Plateau and Greenland profoundly modified the geometry by the introduction of a triple junction and switched the spreading axis near site 112 from north-west–south-east to north–south.

If this theory is correct, the age of the seafloor near site 112 should increase towards the north-east, up to the angle of anomaly 24, and the magnetic anomaly pattern across the ridge should be symmetrical. Some profiles, projected at right angles to the ridge, are presented in Fig. 8. Some crude symmetry can be seen about an axis between two positive peaks. Only profile AA′ is long enough without course alterations to test the symmetry in detail beyond the positive peaks. The symmetry axes are indicated in Fig. 2 by asterisks, and do not lie in a straight line along the ridge. Either the detailed contouring of the ridge (both by basement topography and by anomaly) is wrong or there are undetected fracture zones offsetting the old axis.

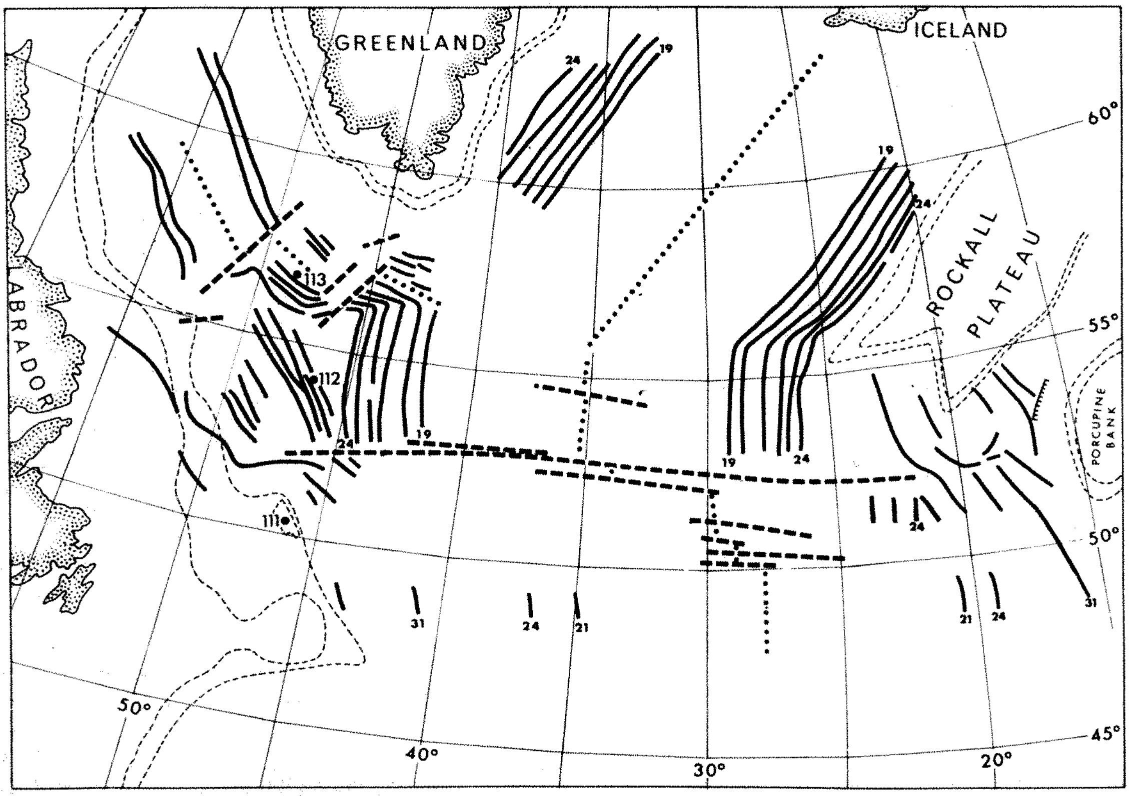


Fig. 4 Magnetic anomaly trends in the North Atlantic. - - -, Top and bottom of continental slope; ———, positive magnetic anomalies from number 19 and older; — — —, fracture zones; . . ., present and extinct spreading axes.

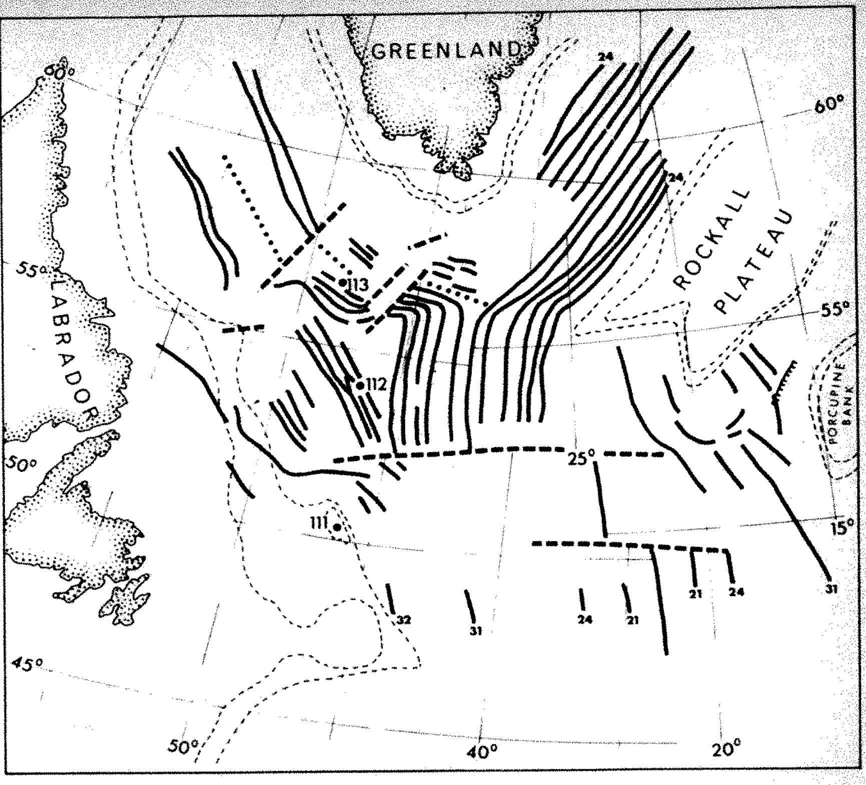


Fig. 5 Palaeogeographic reconstruction at anomaly 19 (47 m.y., Middle Eocene).

The age of the basement sampled at site 112 is calculated to be about 65 m.y. from the ages of the sediments sampled; that is, about anomaly 26. The magnetic profiles are, however, not long enough to compare a synthetic model with the observed profiles and thus to test this age against the Heirtzler time scale. The absence of large anomalies to the north-east of 112 agrees with the suggestion that the positive peak near the site is anomaly 26, in which case the central negative anomaly would be at 64 m.y. The switch of spreading axis therefore probably took place during the period 64 to 60 m.y. ago.

If the western end of the Gibbs Fracture Zone is a transform fault[2], the pre-Palaeocene spreading centre to the south of it must be further east. The eastward end of this transform fault might be found in the south-eastern margin of Rockall Plateau and the anomalies perpendicular to this may be parallel to this spreading centre. A second, parallel fracture zone may be linked to the south end of the Jean Charcot fault[22] in Rockall Trough. The suggestion that the south end of Rockall Trough has resulted from north-east–south-west transform faults bounding north-west–south-east magnetic anomalies is contrary to previous ideas that a spreading axis lay parallel to the trough.

Further evidence for the switch in orientation of the spreading axis comes from the magnetic anomaly pattern immediately south of the east end of the Gibbs Fracture Zone at 51° N, 22° W (Fig. 4). Anomaly 24 runs north–south along 22° W whereas, immediately to the east of this, the anomaly trend is north-west–south-east. This north-west–south-east anomaly is associated with a pronounced basement ridge similar in many ways to that at site 112, which may therefore be a remnant of the pre-Palaeocene spreading centre to the south of the Rockall Trough transform fault. Similar remnants have been reported to the south of the Gibbs Fracture Zone in the western Atlantic by Le Pichon (personal communication).

Reconstruction

A tentative reconstruction (Fig. 7) can be made for anomaly 31 (72 m.y. or Late Cretaceous) using (*a*) the three suggested transform faults, and (*b*) the position of spreading centres of (i) the central Labrador Sea in the same position as after 60 m.y., (ii) the southern Labrador Sea on the ridge near site 112 and (iii) the Atlantic on a median line between anomalies 31 on either side. The continuity of anomaly 31 south of Rockall Plateau[20] suggests that the transform faults between 50° and 53° N did not exist at this time and that the spreading axis was therefore inclined to the 60 m.y. year spreading axis.

It is difficult to take this reconstruction further back in time. But clearly Greenland can be made to fit into Labrador in the position indicated by Bullard *et al.*[23]. With Rockall Plateau attached to Greenland, no problems of overlap would

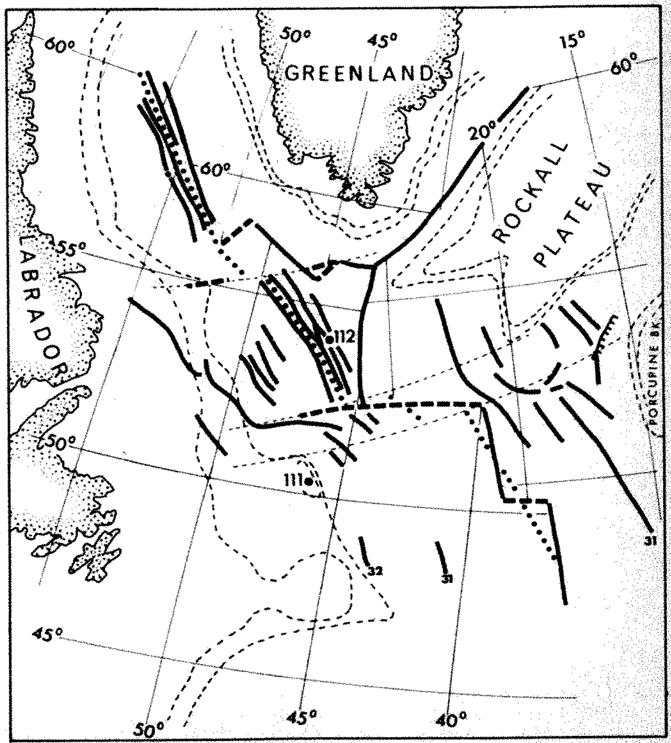


Fig. 6 Palaeogeographic reconstruction at anomaly 24 (60 m.y., Palaeocene). — — —, Possible small circles of transform faults; . . . , spreading axes before 60 m.y. ago.

ensue, but the east–west and north–south scarps at the south-west end of Rockall Plateau would not be explained. The wide separation of anomalies between the two ends of the two transform faults associated with Rockall Trough suggests that they are older than anomaly 32 (76 m.y.) and would imply that the true continental margin off Labrador at 51° N is towards the shelf break. It does not explain the continuation of Rockall Trough to the north-east. South of this, both Porcupine Bank in the east, and Orphan Knoll and Flemish Cap in the west appear to be partially detached pieces of continent which need to be restored to their original position before this region can be reconstructed. Anomaly 32 (76 m.y.) places a minimum age on the separation of Europe from Canada. Linear extrapolation of the spreading rate between anomaly 31 and 32 back to the Labrador shelf edge at 49° N indicates that its age is 82 m.y.

The stratigraphic history of Orphan Knoll (site 111) shows that during the Late Cretaceous (100 to 70 m.y. ago) the sediments deposited there were neritic. By the Maestrichtian (70 m.y.), Orphan Knoll had subsided to about 200 m depth, whereas before 100 m.y. it was probably non-marine. This is consistent with an age of splitting of Europe from Labrador of about 80 m.y. Appreciable subsidence of Orphan Knoll to oceanic depths did not, however, take place until the early or middle Tertiary, by which time a considerable width of North Atlantic Ocean had been formed. The adjustment of the continental margins to an equilibrium state after the initiation of an oceanic split seems to take several tens of millions of years, a time constant that may reflect the creep of lower crust or upper mantle in response to non-isostatic conditions. Alternatively the late subsidence may be the result of later tectonic activity.

This reconstruction follows closely that suggested by Le Pichon *et al.*[2], but differs in suggesting that Rockall Trough did not open as a spreading axis, but as part of an earlier transform fault. A slight revision of Le Pichon's timing gives the sequence of events: (1) 82 m.y.: European plate (with Rockall Plateau and Greenland) breaks from Labrador pulling off fragments of Porcupine Bank, Flemish Cap and Orphan Knoll; (2) 82 m.y. to 60 m.y. (anomaly 24): Labrador Sea and North Atlantic open; (3) 60 m.y. (anomaly 24): Rockall Plateau breaks from Greenland initiating a triple junction and a shift of spreading axes and fracture zones to accommodate the new geometry; (4) 60 m.y. to 47 m.y. (anomalies 24 to 19): simultaneous opening of Labrador Sea, Reykjanes Ridge and North Atlantic; (5) 47 m.y. (anomaly 19): Greenland stops moving relative to Labrador, and Labrador Sea growth finishes; (6) 47 m.y. to present (anomalies 19 to 0): Reykjanes Ridge and North Atlantic grow as European plate separates from Greenland–Labrador plate[15].

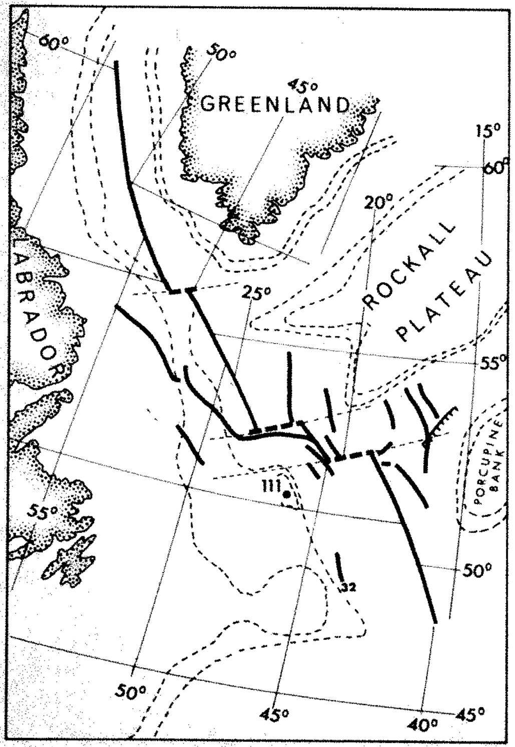


Fig. 7 Palaeogeographic reconstruction at anomaly 31 (72 m.y., Late Cretaceous). ┬┬┬┬, Jean Charcot fault[22].

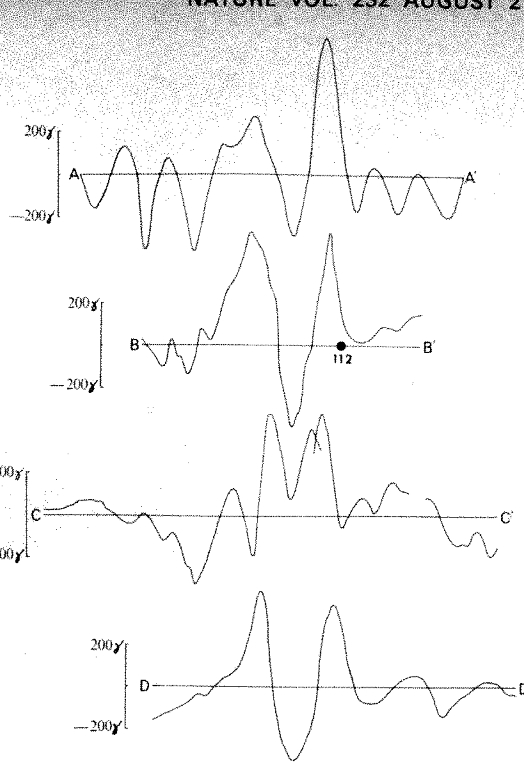


Fig. 8 Magnetic anomaly profiles AA′, BB′, CC′ and DD′ (Fig. 2) across ridge near site 112, projected perpendicular to trend 330°.

These ideas arose out of discussions with the scientific party of leg 12 of the Deep Sea Drilling Project, whom I thank. I also thank many institutions and scientists who contributed unpublished data and manuscripts to leg 12. Especially I would like to acknowledge the magnetic data prepared by R. H. Higgs and P. R. Vogt of the US Naval Oceanographic Office. P. R. Vogt, W. C. Pitman and X. Le Pichon provided helpful criticisms.

Received May 26; revised July 26, 1971.

[1] Johnson, G. L., Closuit, A. W., and Pew, J. A., *Arctic*, **22** (1), 56–68 (1969).
[2] Le Pichon, X., Hyndman, R., and Pautot, G., *J. Geophys. Res.*, **76**, 4724 (1971).
[3] Drake, C. L., Campbell, N. J., Sander, G., and Nafe, J. E., *Nature*, **200**, 1085 (1963).
[4] Manchester, K. S., thesis (Dalhousie Univ. 1964).
[5] Godby, E. A., Baker, R. C., Bower, M. E., and Hood, P. J., *J. Geophys. Res.*, **71**, 51 (1966).
[6] Mayhew, M. A., thesis (Columbia Univ., 1969).
[7] Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman III, W. C. and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).
[8] Johnson, G. L., Vogt, P. R., and Schneider, E. D., *Deutsches Hyd. Zeit.* (in the press).
[9] Vogt, P. R., Avery, O. E., Schneider, E. D., Anderson, C. N., and Bracey, D. R., *Tectonophysics*, **8**, 285 (1969).
[10] Jones, E. J. W., Ewing, M., Ewing, J. I., and Eittreim, S. L., *J. Geophys. Res.*, **75**, 1655 (1970).
[11] Olivet, J.-L., Sichler, B., Thonon, P., Le Pichon, X., Martinais, J., and Pautot, G., *CR Acad. Sci.*, **271**(D), 949 (1970).
[12] Johnson, G. L., and Schneider, E. D., *Earth Planet. Sci. Lett.*, **6**, 416 (1969).
[13] Mayhew, M. A., Drake, C. L., and Nafe, J. E., *Canad. J. Earth. Sci.*, **7**, 199 (1970).

[14] Avery, O. E., Vogt, P. R., and Higgs, R. H., *Trans. Amer. Geophys. Union*, **50** (4), 184 (1969).
[15] Vogt, P. R., Avery, O. E., Morgan, W. J., Johnson, G. L., Schneider, E. D., and Higgs, R. H., *Trans. Amer. Geophys. Union*, **50** (4), 184 (1969).
[16] Johnson, G. L., *Earth Planet. Sci. Lett.*, **2**, 445 (1967).
[17] Godby, E. A., Hood, P. J., and Bower, M. E., *J. Geophys. Res.*, **73**, 7637 (1968).
[18] Pitman, W. C., and Talwani, M., *Geol. Soc. Amer. Bull.* (in the press).
[19] Fenwick, D. K. B., Keen, M. J., Keen, C., and Lambert, A., *Canad. J. Earth Sci.*, **5**, 483 (1968).
[20] Williams, C. A., and McKenzie, D. P., *Nature*, **232**, 168 (1971).
[21] Fleming, H. S., Cherkis, N. Z., and Heirtzler, J. R., *Mar. Geophys. Res.*, **1**, 37 (1970).
[22] Le Pichon, X., Cressard, A., Mascle, J., Pautot, G., and Sichler, B., *Res. Sci. de la campagne du NO Jean Charcot en Atlantique Nord*, publication No. 2 (August–September 1969).
[23] Bullard, E. C., Everett, J. E., and Smith, A. G., *Phil. Trans. Roy. Soc.*, A, **258**, 41 (1965).

C-Type RNA Tumour Virus Genome Expression in Wild House Mice

MURRAY B. GARDNER, J. EARLE OFFICER & ROBERT W. RONGEY
Department of Pathology, University of Southern California School of Medicine, Los Angeles, California

JOHN D. ESTES, H. C. TURNER & ROBERT J. HUEBNER
Viral Carcinogenesis Branch, National Cancer Institute, Bethesda, Maryland

The presence of the indigenous latent C-type RNA tumour virus genome can be demonstrated in wild house mice by detection of gs antigen and C-type particles. There seems to be strong suppression of the activity of this viral genome in this feral species.

ALTHOUGH laboratory strains of mice have been subjected to extensive cancer virus investigations, wild house mice (*Mus musculus*) have received limited attention[1-4]. There are no reports of this isolation of murine leukaemia and sarcoma viruses nor of C-type virus particles from wild house mice, but recently our associates have reported the presence of group specific (gs) antigens of the murine C-type viruses in the tissues of wild mice bred in captivity[5]. Since the genome of this species has been demonstrated in all laboratory strains of mice tested[5-7], we set out to look for the murine C-type viral genome in feral wild house mice.

In 1968, we began a study in Los Angeles County (*a*) to detect and determine the natural history of the C-type tumour virus expression in wild mice and (*b*) to study the effect of chemical carcinogens on the expression of the viral genome. Here we report our findings on the prevalence of detectable gs antigen by complement fixation and of C-type particles by electron microscopy in tissues from recently trapped wild mice and from mice kept in captivity for 16–26 months, some of which have recently developed spontaneous tumours, and from young and old mice following tumour induction by 3-methylcholanthrene (MC).

Treatment of Mice

About 600 wild mice were trapped alive in urban Los Angeles, weighed and individually housed in mason jars. Several hundred mice were killed without treatment and 180 were set aside without treatment to live out their life span (geriatric colony). The remainder were equally divided by sex into three treatment groups. One group of 150 mice was inoculated subcutaneously with 300 μg and another group of 50 mice with 150 μg of MC dissolved in 0.05 ml. of trioctanoin. A control group of 146 mice received a 0.05 ml. subcutaneous dose of trioctanoin alone. Sera, collected from all mice by retro-orbital puncture immediately after capture and at the time of tumour collection, were tested for antibodies in complement fixation against eleven murine viral antigens*. Ten per cent sonicated aqueous extracts of (*a*) spleen from the untreated mice, (*b*) spleen from the control trioctanoin injected mice and (*c*) tumour and spleen from mice with induced or spontaneous tumours were tested for complement fixing murine gs antigen by the microtitre technique[8] using, as antiserum, a 1 : 20 dilution of serum pools from Fisher rats bearing transplanted sarcomas induced by the Moloney strain of murine sarcoma virus (MSV)[9].

Sections of tumour and spleen were also prepared for light and electron microscopy. At the time of collection of each MC-induced tumour a trioctanoin injected control mouse was killed and the tissues similarly processed. The time of initial appearance of MC tumours was noted but tumours were allowed to reach 25–35 mm in size before the animal was killed unless they showed evidence of ulceration or necrosis. The average weight of the mice at the time of trapping was 14 g, ranging from 8–19 g.

Methylcholanthrene Indices

No significant differences were observed in tumour incidence or latent period before tumour appearance between the 150 μg and 300 μg MC treatment groups, and so these two groups are combined in Table 1. After intervals of 12–15 months, ninety-eight of 135 (73%) surviving mice which had been inoculated with MC had developed tumours at the inoculation site. The latent period varied from 75–458 days with an average of 143 days. Most of the tumours were fibrosarcomas; several had

---

* Lymphocytic choriomeningitis, murine adenovirus, polyoma, murine hepatitis virus, reovirus-3, K virus, Theiler's GD7 virus, Kilham rat virus, syncytial virus-5, Moloney sarcoma virus (MSV) and Sendai virus.

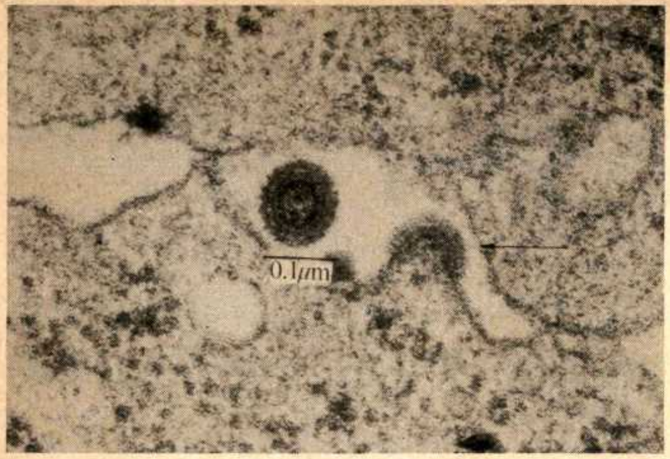


Fig. 1 One budding (arrow) and one extracellular 100-nm C-type virus particle in spleen from a wild house mouse bearing an MC-induced sarcoma. The sarcoma from this mouse also contained C-type particles. (Uranyl acetate; × 105,750.)

features of rhabdomyosarcomas and eleven were squamous cell carcinomas. In two mice both a carcinoma and a sarcoma occurred at the inoculation site. Tumours were not observed by gross or microscopic examination in 146 surviving trioctanoin injected control mice observed up to 18 months.

All inoculated mice remained free of antibodies to the eleven indigenous murine viruses. When we used a highly specific but narrow range MSV rat serum pool (No. 12), selected to contain antibodies reactive only with the species specific major (gs-1) antigen[10], we found antigen in low titre (1 : 2–1 : 4) in six of fifty-eight (10%) tumours tested and in none of the corresponding spleens; but spleen extracts from seven of fifty (14%) recently trapped untreated wild mice also contained gs antigen. In the trioctanoin inoculated control group, two of sixty-one spleen extracts contained gs antigen. Many of these same tissue extracts were thawed after storage for 1 yr and retested with a more broadly reactive MSV rat serum pool (No. 21, see below); positive CF reactions were observed in fourteen of forty-four (32%) MC tumours, ten of forty-four (23%) of the corresponding spleens, none out of twenty-five of the spleens from trioctanoin control mice and thirteen of forty-two (31%) of spleens from untreated control mice (not shown in Table 1).

C-type particles (Fig. 1) were found by electron microscopy in both the MC tumour and corresponding spleen from eight of sixty-six (12%) mice examined. Seven of these tumours were fibrosarcomas, one a squamous cell carcinoma, and all contained detectable gs antigen using the narrow range serum pool (No. 12). C-type particles were not found in any of twenty spleens from the trioctanoin control group. A few were observed in three of twenty-one spleens randomly chosen from the freshly trapped untreated group (see below). Intracisternal A-type particles (Fig. 2) were found in nineteen MC induced tumours, six of which were squamous cell carcinomas and thirteen of which were fibrosarcomas; no C-type particles were detected in these tumours.

In a second experiment (Table 2), twenty old wild mice from the geriatric colony, which had been kept in captivity without treatment for 15 months, were inoculated subcutaneously with 200 μg of MC. Nine of sixteen surviving mice developed sarcomas at the inoculation site after an average latent period of 100 days, when the mice were estimated to be 19–20 months of age. One mouse without a tumour at the inoculation site had a granulosa cell tumour of the ovary.

At this time an MSV rat serum pool of intermediate sensitivity (No. 18) was used for complement fixation tests; this serum pool contained antibodies against the species specific major component (gs-1) and, at lower titres, antibodies against an interspecies cross reacting component (gs-3)[11] of the gs antigen complex. Five of nine (55%) tumour extracts from these old mice contained gs antigen compared with four of thirty-one (13%) MC-induced tumour extracts from young adult mice tested with the same rat serum pool No. 18 (Table 1); several of the tumours from older mice were positive at titres higher (1 : 4–1 : 16) than those observed in younger mice with tumours induced by MC. In the older mice C-type particles were present in two of eight MC-induced sarcomas and in spleens of the same animals. Complement fixation antigen was detected in two of seven spleen extracts and C-type particles were seen in two of eight spleens from normal old mice which had been kept in captivity without treatment for 20 months. All of the old mice also remained free of antibodies to the eleven indigenous viruses described above.

Spontaneous Tumours in Old Wild Mice

Tumours have occurred in sixteen of 150 surviving old mice that had been under observation without treatment for 19–26 months (Table 3). Eight of these tumours were lymphomas, four were fibrosarcomas and four were large pulmonary adenomas. The lymphomas were lymphoblastic and characterized primarily by splenomegaly and generalized lymphadenopathy without thymic involvement. Two of the lymphomas arose in mice subjected 1–2 yr previously to unsuccessful transplants of MC-induced wild mouse sarcomas; the remaining tumours were unquestionably spontaneous. Using more broadly reactive MSV serum pools (Nos. 21 and 25) which contained high titres of antibodies to species specific, interspecies gs antigen[12,13] and possibly additional antigens, complement fixing reactions were detected in high titre (1 : 4 or above) in the spleen and/or tumour in all eight lymphomatous mice, and in lower titre (1 : 2–1 : 4) in two of the fibrosarcomas and in each of the pulmonary adenomas. C-type particles were observed in five

Table 1 Tumour Incidence, Murine C-Type Tumour Virus gs Antigen and C-Type Particle Expression in Young Adult Wild Mice Untreated and after Inoculation with Methylcholanthrene

Treatment	Tumours †	Tissue	CF gs antigen * MSV-12	CF gs antigen * MSV-18	C-type particles	A-type particles
Methylcholanthrene, 150 or 300 μg subcutaneously	98/135 ‡	Tumour	6/58 §	4/31	8/66 ‖	19/66 ¶
		Spleen	0/60	2/31	9/61	5/61
Control—trioctanoin	0/146	Spleen	2/61		0/20	2/20
No treatment—killed immediately	0/121	Spleen	7/50		3/21	1/21

* The MSV rat serum pools utilized differed in sensitivity but not specificity for the murine C-type tumour virus gs antigen. MSV-12 was least sensitive and MSV-18 was of intermediate sensitivity (see text). The mice tested with MSV-18 were different from those tested with MSV-12.

† Number of tumours/number of surviving mice.

‡ Eighty-seven sarcomas and eleven squamous cell carcinomas (including two cases of combined carcinoma and sarcoma); period of observation 12–18 months. Latent period range 75–458 days with an average tumour latent period of 143 days.

§ Number positive/number tested free of anticomplementary activity.

‖ All eight tumours and six of the corresponding spleens were positive for complement fixing antigen; seven of these tumours were fibrosarcoma and one a squamous cell carcinoma.

¶ Six of the nineteen tumours with A-type particles were squamous cell carcinomas; three of the five spleens with A-type particles were from mice with squamous cell carcinomas.

Table 2 Tumour Incidence, Murine C-Type Virus gs Antigen and C-Type Particle Expression in Old Wild Mice Untreated and following Inoculation with Methylcholanthrene

Treatment	Tumours †	Tissue	CF gs antigen * MSV-18	C-type particles	A-type particles
Methylcholanthrene 200 μg subcutaneously	10/16	Tumour	5/9	2/8‡	2/8
		Spleen	3/9	2/10	1/10
Methylcholanthrene 200 μg subcutaneously—non-tumour group	0/6§	Spleen	0/7	1/6	0/6
No treatment—age-matched controls from geriatric colony	0/8‖	Spleen	2/7	2/8¶	2/8

* MSV-18 was of intermediate sensitivity for the complement fixing gs antigen (see text).

† Number of tumours/number of surviving mice.

‡ C-type and A-type particles were in different tumours. The two tumours and one of the two spleens with C-type particles were complement fixing antigen positive.

§ The six surviving MC-inoculated mice without tumours were killed 148 days after inoculation.

‖ These eight mice were killed after 18 months in captivity.

¶ These two spleens had both C-type and A-type particles but were complement fixing antigen negative.

Table 3 Spontaneous Tumours in Wild Mice

Type of tumour †	Tissue	CF gs antigen * MSV 21 and 25	C-type particles	A-type particles
Lymphoma	Lymph node tumour	4/4‡	4/4	0/4
	Spleen	8/8	3/5	1/5
Sarcoma	Tumour	3/4	0/5	1/5
	Spleen	1/5	0/4	1/4
Pulmonary adenoma	Tumour	4/4	0/3	1/3
	Spleen	1/4	0/2	0/2

* MSV-21 and MSV-25 were of greatest sensitivity for the murine C-type tumour virus gs antigen (see text).

† The lymphomas were lymphoblastic, nonthymic and primarily involved the spleen. The fibrosarcomas were located in the thigh, low back, face and liver. The pulmonary adenomas each occupied nearly one entire pulmonary lobe. The period under observation before these tumours occurred was 19–26 months.

‡ Number positive/number tested.

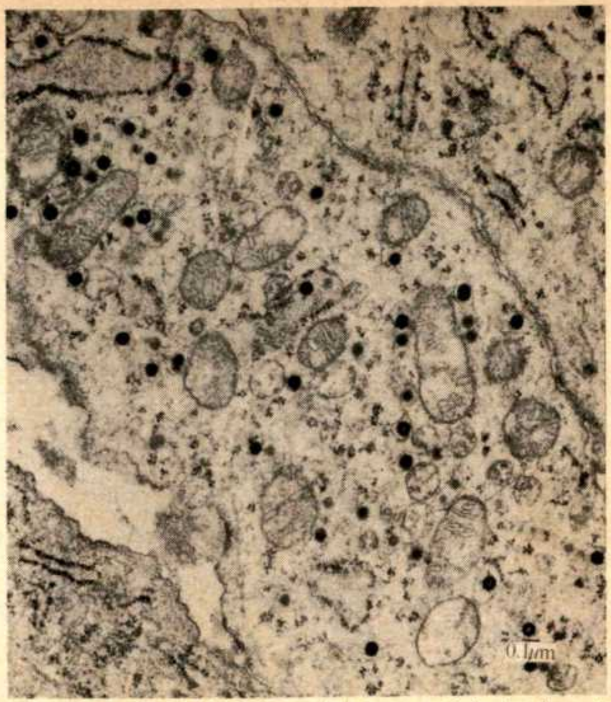


Fig. 2 Many 70-nm intracisternal A-type particles in the cytoplasm of an MC-induced sarcoma cell. (Uranyl acetate; ×22,800.)

of six lymphomatous mice examined but not in the sarcomas or pulmonary adenomas. At the time of tumour harvest, antibodies against polyoma and the other indigenous viral antigens were absent from the sera of the seven mice so tested (lymphoma —4; sarcoma—1; pulmonary adenoma—2). Polyoma virus particles were not seen in any of the MC induced or spontaneous tumours.

Virus Particles and CF Antigen

In a recent preliminary survey of several tissues from freshly trapped untreated wild mice, C-type particles were seen in two of twenty livers, one of six kidneys, six of ten large intestinal mucosae and five of eight uterine endometria. In one embryo a few C-type particles were noted budding from nucleated erythrocytes in the hematopoietic liver. Intracisternal A-type particles were also seen on occasion in this untreated group in postnatal spleen, liver, intestine, skeletal muscle, breast, uterus, placenta and in embryonic liver and skeletal muscle. B-type particles were seen in normal lactating breast tissue from two of four pregnant mice examined. In general, the prevalence of complement fixing antigen in these tissues, using the most broadly reactive MSV rat serum pools (Nos. 21 and 25), was much greater than visualization of C-type particles and those tissues showing C-type particles were usually, although not invariably, positive for this antigen (M. B. G., J. D. E., and R. J. H., unpublished observations).

Implications of the Findings

These findings demonstrate the presence of the C-type RNA tumour virus genome in tissues of feral wild house mice unexposed to laboratory infection or to manipulated breeding programmes. It seems clear that the inbreeding of laboratory mice has produced several strains in which the indigenous RNA tumour virus genome was more frequently expressed in terms of gs antigen, C-type particles and neoplasia than in the natural outbred species[14]; further, several inbred strains have been derived which have segregated for lesser expressions of this genome[14,15]. The percentage of young adult wild mice showing detectable gs antigen in the MC-induced tumours and corresponding spleens and in untreated mouse spleens and other tissues depended on the sensitivity of the MSV rat serum pools used for the complement fixation procedure. With the most narrowly reactive MSV rat serum pool, the gs antigen could be detected in only a small percentage (14%) of spleens from untreated young adult mice or in their MC-induced tumours (10%). This corresponded closely to the prevalence of detectable C-type particles by electron microscopy in the spleens (14%) of untreated young adult wild mice and in the MC induced tumours (12%) and corresponding spleens (15%) of this age group. With the more broadly reactive MSV rat serum pool, capable of detecting a greater proportion of the gs antigenic complex, antigens were detected in larger quantities in MC-induced tumours (32%) and corresponding spleens (23%). The relatively low prevalence of detectable gs antigen in MC-induced tumours using the most sensitive MSV rat serum pool so far available is probably explained, in part, by hypothetical strong cellular repressor mechanisms for RNA tumour virus genome expression in mesenchymal tissues of wild house mice[5]. The comparatively low prevalence of gs antigen detection in spleens and MC-induced tumours (compared with laboratory strains) could also be partly attributed to some degradation of the gs antigen resulting from prolonged storage and repeat thawings of the tumour extracts before retesting with the more sensitive sera. The detection of occasional C-type particles by the electron microscope in both tumour and spleen from only 12% of the mice with tumours also indicates that this latent genome is infrequently activated by MC to form visible virions. Infectious virus, however, has not yet been isolated from any of the MC-induced or spontaneous tumours, including those containing C-type particles, in spite of the use of the most effective virus isolation procedures[7,16,17]*. The findings so far also indicate that in freshly trapped wild mice a few C-type particles may often be seen in various tissues. The extent to which natural activation of this latent murine RNA tumour virus genome to form gs antigen or C-type virions may depend on environmental carcinogens, indigenous DNA viruses such as polyoma, and/or ageing is under study.

In old mice there seems to be less suppression of viral genome activity as suggested (*a*) by the shorter latent period between MC injection and tumour formation, (*b*) by the higher prevalence and titres of gs antigen in tumours and spleens following MC inoculation, and (*c*) by higher incidences of spontaneous tumours in old compared with younger mice and the presence of gs antigen in almost all tumours of the older mice. Whitmire *et al.* also observed that 18 month old BALB/c mice treated with MC developed tumour and gs antigen much more often than younger mice treated with the same dosage of chemical (C. E. Whitmore, R. A. Salemo, L. S. Rabstein, R. J. H., and H. C. T., unpublished).

The absence of demonstrable antibodies to murine RNA tumour virus gs antigen (a 20% clarified extract of a Fisher rat MSV sarcoma) in all mice tested, including those with detectable gs antigen in tumour or spleen extracts, suggests that wild mice, like laboratory mice[18], are immunologically tolerant to this ubiquitous antigen. This was also demonstrated by the absence of such antibodies in wild mice bearing MSV-induced tumours[19]. Such tolerance is perhaps best explained by vertical transmission and embryonic expression of the viral gs antigen which was demonstrated in the tissues of 16–18 day old wild mouse embryos trapped in Maryland[5,19]. The absence of antibodies against polyoma antigen in mice bearing MC-induced and spontaneous tumours suggests that polyoma virus was not responsible for these tumours.

Virus Particles

The significance of the intracisternal A-type particles is unknown. These particles have been seen in several mouse tumours[20], including MC-induced sarcomas[21] and carcinomas[22]. They have also been observed in muscle, liver and other tissues of embryos of a number of laboratory strains (M. L. Vernon, W. Lane, and R. J. H., unpublished observations) and in wild mouse embryos (M. B. G., R. W. R., and J. D. E., unpublished observations). They have not been shown to have biological activity[23], nor do they bear any known developmental or immunologic relationship to C-type particles[21,23]. This point is borne out in the present study where A-type particles were found more often in gs antigen negative normal tissues and tumours while C-type particles were seen mainly in the tumours containing antigen.

The finding of B-type particles in the lactating breast tissue of two of four feral mice examined supports previous experimental evidence that wild mice carry the mammary tumour agent[24]; but this particular RNA tumour virus has not been previously demonstrated in wild mice obtained directly from the feral state. Recent evidence[25] suggests that the B-type virus genome is similar to the C-type virus genome in being vertically inherited and largely repressed in most strains of mice by host cell genetic mechanisms.

It is doubtful that MC tumour induction *per se* could be attributed to superinfection of the tumour by infectious C-type virus particles. Such particles were seldom found in the wild mice treated with MC and, as in laboratory mice, there seems to be no evidence that the virus is communicable in nature. Thus, an MC-induced immunological inability to neutralize infectious C-type virus is probably not an important mechanism in the pathogenesis of MC-induced tumours in wild mice. MC-induced suppression of immune surveillance mechanisms[26], however, may contribute to the proliferative growth of antigenically altered tumour cells. There is much evidence[14,27] to support a role for C-type virus genome activation as a determinant of chemical or spontaneous oncogenesis in laboratory strains of mice. The selective activation of this genome to form gs antigen in the subcutaneous fibrosarcomas induced by MC in laboratory mice but not in adjacent mesenchymal tissue and muscle suggests that activation of this sarcoma oncogene in the tumour cells is often accompanied by activation of the gs antigen of the virogene[5,14,27]. Our study suggests that in wild mice there is a similar causative relationship between chemical and spontaneous oncogenesis, ageing and activation of the latent C-type RNA tumour virus genome.

We thank Rene Germaine for technical assistance. This project was supported by a US National Cancer Institute research contract.

Received May 21, 1971.

[1] Rowe, W. P., Hartley, J. W., and Huebner, R. J., *A Symposium on the Problems of Laboratory Animal Disease* (Academic Press, London and New York, 1961).
[2] Rowe, W. P., Huebner, R. J., and Hartley, J. W., in *Perspectives in Virology*, **2**, 177 (1961).
[3] Huebner, R. J., Rowe, W. P., Hartley, J. W., and Lane, W. T., *Ciba Foundation Symposium on Tumour Viruses of Murine Origin*, 314 (1962).
[4] Andervont, H. B., and Dunn, T. B., *J. US Nat. Cancer Inst.*, **28**, 1153 (1962).
[5] Huebner, R. J., Kelloff, G. J., Sarma, P. S., Lane, W. T., Turner, H. C., Gilden, R. V., Oroszlan, S., Meier, H., Myers, D. D., and Peters, R. L., *Proc. US Nat. Acad. Sci.*, **67**, 366 (1970).
[6] Huebner, R. J., and Todaro, G. J., *Proc. US Nat. Acad. Sci.*, **64**, 1086 (1969).
[7] Hartley, J. W., Rowe, W. P., Capps, W. I., and Huebner, R. J., *J. Virol.*, **3**, 126 (1969).
[8] Huebner, R. J., Rowe, W. P., Turner, H. C., and Lane, W. T., *Proc. US Nat. Acad. Sci.*, **50**, 379 (1963).
[9] Huebner, R. J., *Proc. US Nat. Acad. Sci.*, **58**, 835 (1967).
[10] Oroszlan, S., Fisher, C. L., Stanley, T. B., and Gilden, R. V., *J. Gen. Virol.*, **8**, 1 (1970).
[11] Geering, G., Aoki, T., and Old, J. J., *Nature*, **226**, 265 (1970).
[12] Sarma, P. S., Gilden, R. V., Huebner, R. J., Turner, H. C., and Log, T. S., *Nature* (in the press).
[13] Gilden, R. V., Oroszlan, S., and Huebner, R. J., *Nature* (in the press).
[14] Huebner, R. J., Todaro, G. J., Sarma, P. S., Hartley, J. W., Freeman, A. E., Peters, R. L., Whitmire, C. E., Meier, H., and Gilden, R. V., *Proc. Second Intern. Symp. Tumor Viruses* (in the press).
[15] Myers, D. D., Meier, H., and Huebner, R. J., *Life Sci.*, **9**, 1071 (1970).
[16] Huebner, R. J., Hartley, J. W., Rowe, W. P., Lane, W. T., and Capps, W. I., *Proc. US Nat. Acad. Sci.*, **56**, 1164 (1966).
[17] Klement, V., Rowe, W. P., Hartley, J. W., and Pugh, W. E., *Proc. US Nat. Acad. Sci.*, **63**, 753 (1969).
[18] Huebner, R. J., and Igel, H. J., *Perspectives in Virology* (in the press).
[19] Huebner, R. J., Sarma, P. S., Kelloff, G. J., Gilden, R. V., Meier, H., Myers, D., and Peters, R. L., *Ann. NY Acad. Sci.*, **181**, 246 (1971).
[20] Dalton, A. J., Law, L. W., Moloney, J. B., and Manaker, R. A., *J. US Nat. Cancer Inst.*, **27**, 747 (1961).
[21] DeHarven, E., *Sixth Intern. Congr. Electron Microsc.* (Kyoto, Japan, 1966).
[22] Kakafuda, T., Roberts, E., and Suntzeff, V., *Cancer Res.*, **30**, 1011 (1970).
[23] Hall, W. T., Hartley, J. W., and Sanford, K. K., *Virology*, **2**, 238 (1968).
[24] Andervont, H. B., and Dunn, T. B., *Acta Unio Intern. Contra Cancerum*, **12**, 530 (1956).
[25] Bentvelzen, P., Daams, J. H., Hageman, P., and Calafat, J., *Proc. US Nat. Acad. Sci.*, **67**, 377 (1970).
[26] Prehn, R. T., *J. US Nat. Cancer Inst.*, **31**, 791 (1963).
[27] Huebner, R. J., Freeman, A. E., Whitmire, C. E., Price, P. J., Rhim, J. S., Kelloff, G. J., Gilden, R. V., and Meier, H., *Twenty-fourth Ann. Symp. Fundamental Cancer Research* (Houston, Texas, 1971).

---

* We have recent evidence from ^{3}H-uridine particle labelling and positive XC cell tests[17] suggesting the presence of infectious C-type leukaemia virus in several normal wild mouse embryo cultures after short term *in vitro* propagation (J. E. O., Hartley, and Freeman, unpublished observations).

LETTERS TO NATURE

PHYSICAL SCIENCES

Wobble Excitation by Earthquakes in Real Earth Models

THERE have been several accounts recently[1–4] describing connexions between polar motion and earthquakes. Because a simple correlation is of little significance unless a physical mechanism can be produced to explain it, the most important point discussed in these articles is the question of the efficacy of the mass displacements associated with major earthquakes in exciting the observed polar motion. We wish to report here calculations based on realistic Earth models. The elasticity theory of dislocations[5], used to model earthquake displacement fields, has been generalized to take account of self-gravitation, the radial variation of elastic properties, density and gravity, initial hydrostatic stress and the presence of the liquid core. We have already reported initial results, giving the effect of such realistic Earth model displacement fields on the rotation[6]. Results of a later independent study have been reported by Dahlen[7] and our complete study will be published elsewhere[8].

The calculation of the polar shift and excited Chandler wobble (the free polar motion) is considerably simplified because they depend only on four spheroidal displacement fields of second degree. This obviates the necessity of summing slowly convergent series to obtain the entire displacement field. Fig. 1 shows the radial coefficients of one of the displacement fields affecting polar motion. The six variables shown are the same as those used in free oscillation theory, representing radial displacement, change in normal stress, transverse displacement, transverse shear stress, decrease in gravitational potential and change in radial gravitational flux density, respectively.

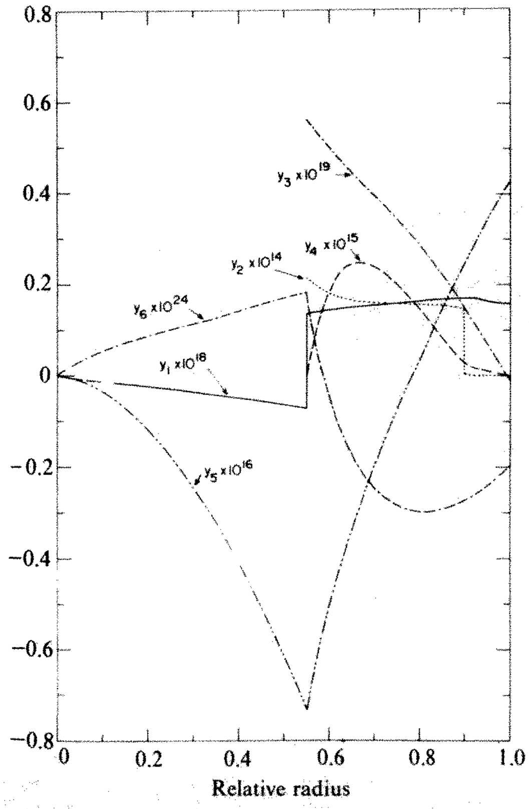


Fig. 1 Radial coefficients of one of the four spheroidal deformational modes affecting polar motion. The units are c.g.s. divided by the slip-fault area product (cm^3). This might be 10^{18} cm^3 for a major earthquake. Focal depth is 0.1 Earth radii.

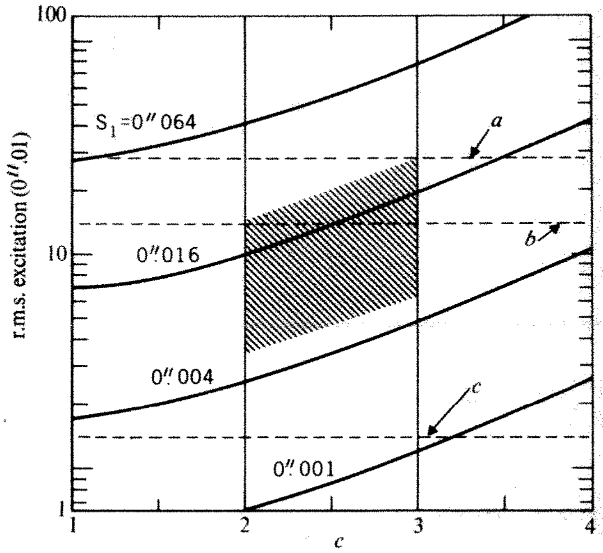


Fig. 2 Root mean square Chandler amplitude in terms of S_1, the average polar shift for an earthquake of Richter magnitude 8.5, and the parameter *c*. The shaded area indicates the degree of uncertainty in the theoretically computed value. *a*, Double observed excitation; *b*, observed excitation; *c*, 1/10 observed excitation.

For the first time in a static problem, the conditions at the core–mantle boundary have been treated without the assumption of the Adams and Williamson relation in the core. This relation takes the density profile to be that for a homogeneous material under compression in an adiabatic temperature field. Jeffreys and Vicente[9] have shown that without the assumption of the Adams and Williamson relation in the core, application of the usual conditions at the core–mantle boundary leads to an insoluble problem. We have shown that in the static case, the conditions at the boundary must be modified; the modification allows the problem to be solved.

We compute that the Chile earthquake of 1960 shifted the pole 1.03 centiseconds of arc towards 269° E, and that the Alaska earthquake of 1964 shifted the pole 1.20 centiseconds of arc toward 51° E. These provide equal and opposite contributions to Chandler wobble excitation. We have used the fault parameters of Plafker and Savage[10] for the Chile earthquake and those of Hastie and Savage[11] for the Alaska earthquake. Both earthquakes were of Richter magnitude $M = 8.5$ but were rather unfavourably oriented for affecting polar motion. For shallow focus earthquakes of Richter magnitude 8.5, the average polar shift is estimated to be between 0.5 and 2.5 centiseconds of arc. The dependence of average polar shift (S_1) on Richter magnitude is given by[12]

$$S_1 \times 10^{(M-8.5)c}$$

where c may have a value from 2.0 to 3.0. The range of the resulting r.m.s. Chandler wobble excitation is shown in Fig. 2.

Ben-Menahem and Israel[2] used a much simpler uniform Earth model and estimated that 30% of the Chandler wobble excitation may be attributed to seismic activity; this is evidently a lower limit. Our theory adequately accounts for the full excitation, but as most geophysical theories tend to be more precise than accurate we must await better measurements of both polar motion and earthquake deformation fields before the argument can finally be decided.

D. E. SMYLIE *

Department of Geophysics,
University of British Columbia,
Vancouver

L. MANSINHA *

Department of Geophysics,
University of Western Ontario,
London

* On leave at Code 550, Goddard Space Flight Center, Greenbelt, Maryland, 20771.

Received April 2, 1971.

[1] Smylie, D. E., and Mansinha, L., *J. Geophys. Res.*, **73**, 7661 (1968).

[2] Ben-Menahem, A., and Israel, M., *Geophys. J. Roy. Astron. Soc.*, **19**, 367 (1970).

[3] Myerson, R., *J. Geophys. Res.*, **75**, 6612 (1970).

[4] Whitten, C. A., *Earthquake Research in ESSA 1969–70*, 7 (US Government Printing Office, 1970).

[5] Steketee, J. A., *Canad. J. Phys.*, **36**, 192 (1958).

[6] Smylie, D. E., and Mansinha, L., *Trans. Amer. Geophys. Union*, **50**, 645 (1969).

[7] Dahlen, F. A., *Proc. Intern. Symp. Geophysical Theory and Computers* (Stockholm, 1970).

[8] Smylie, D. E., and Mansinha, L., *Geophys. J. Roy. Astron. Soc.*, (in the press).

[9] Jeffreys, H., and Vicente, R. O., *Memoires de l'Académie Royale de Belgique*, **37**, 5 (1966).

[10] Plafker, G., and Savage, J. C., *Bull. Geol. Soc. Amer.*, **81**, 10001 (1970).

[11] Hastie, L., and Savage, J. C., *Trans. Amer. Geophys. Union*, **50**, 237 (1969).

[12] Mansinha, L., and Smylie, D. E., *J. Geophys. Res.*, **72**, 4731 (1967).

Optical Variability of a Bright New Quasi-stellar Source

BROWNE[1] has identified the radio source 1215+30 of the Bologna B2 survey with a remarkably bright quasi-stellar source of magnitude approximately 14.5. We report here that its optical flux is variable by more than two magnitudes, with discernible brightness changes over time scales of hours, as well as secular trends on a time scale of years.

The light curve of B2 1215+30 (=ON325; J. D. Kraus, private communication) is given in Table 1. The data were obtained by visual estimates from fifty-eight Harvard plates taken between 1926 and 1950 with the f/5 16 inch Metcalf doublet. Table 1 lists (1) the plate number, (2) date, (3) exposure in minutes, (4) object brightness with respect to comparison stars A, B, C and D (identified in Table 2), (5) control brightness estimates of star B with respect to A and C, and (6) of star C with respect to B and D. The sequence A to D is in order of increasing brightness. The relative magnitudes are recorded in standard notation (for example, C9D denotes a magnitude brighter than C by 9/10 of the magnitude interval between C and D). Abbreviations used in Table 1 are bt, brighter than; ft, fainter than; nv, not visible, and s, slightly.

Table 2 gives the coordinates of the comparison stars in centimetres on the finding chart of Browne[1] taking the source as origin, with east to the left and north to the top of the chart. A transfer of magnitudes from nearby selected area 56

Table 1 Brightness of B2 1215+30

Plate	Date	Exp	QSS	B	C
21893	4–12–26	60	C9D	A3C	B7D
21910	5–4–26	61	B5C	A3C	flaw
22369	2–4–27	68	B8C	A2C	B7D
22395	2–27–27	62	B7C	A3C	B8D
22411	3–3–27	70	B5C	A3C	B6D
22579	6–18–27	62	C9D	ABnv	Bnv
23007	12–21–27	70	C	A3C	B6D
23083	1–20–28	68	C5D	A2C	B7D
23116	1–26–28	66	C9D	A2C	B7D
23203	2–21–28	63	sbt D	A2C	B6D
23213	2–24–28	108	D	A3C	B7D
23290	3–14–28	63	sbtD	A2C	B6D
23355	4–10–28	67	sbtD	Anv	B7D
23362	4–13–28	84	sbtD	Anv	B7D
23370	4–16–28	84	sbtD	A2C	B7D
23421	5–16–28	84	C2D	ABnv	Bnv
24124	3–9–29	69	ft B	Anv	—
24165	3–29–29	64	ft B	Anv	—
24174	4–3–29	58	ft B	Anv	—
24207	4–30–29	64	B	Anv	B7D
24221	5–7–29	77	B2C	A2C	B7D
24232	5–10–29	60	ft C	ABnv	Bnv
24699	1–4–30	77	B	Anv	B7D
24800	3–4–30	68	ft C	ABnv	Bnv
24831	3–21–30	83	B2C	A2C	B7D
24858	4–2–30	72	B2C	A2C	B7D
24890	4–22–30	74	ft C	ABnv	Bnv
24905	4–28–30	103	B2C	Anv	B7D
24928	5–16–30	64	ft C	ABnv	Bnv
24938	5–23–30	54	ft C	ABnv	Bnv
25297	1–13–31	96	B	Anv	B6D
25321	1–22–31	94	B	Anv	B6D
25327	1–24–31	97	B1C	Anv	B5D
25410	3–18–31	102	ft B	Anv	B7D
25440	4–11–31	92	B	Anv	B7D
25450	4–15–31	95	ft A	A2C	B7D
25808	12–15–31	43	ft C	ABnv	Bnv
25873	2–13–32	90	B	Anv	B5D
25944	3–12–32	114	B2C	Anv	B6D
26044	4–28–32	95	B1C	Anv	B6D
26363	2–24–33	45	B7C	A2C	B7D
26390	3–16–33	60	B4C	A3C	B6D
26395	3–23–33	60	B8C	A3C	B7D
26397	3–23–33	60	B4C	A3C	B7D
26401	3–24–33	45	B7C	A3C	B6D
26409	3–27–33	48	B8C	A3C	B6D
26412	3–27–33	60	B7C	A2C	B5D
26443	4–20–33	50	B8C	A3C	B7D
26445	4–20–33	50	B4C	A3C	B6D
26450	4–22–33	45	B6C	Anv	B7D
26452	4–22–33	45	B4C	A2C	B7D
31365	2–24–41	190	A	A3C	B6D
31433	4–17–41	70	A6B	A4C	B6D
31436	4–19–41	180	A4B	A3C	B8D
31437	4–20–41	120	A8B	A4C	B7D
31484	6–23–41	120	B2C	A3C	B8D
36940	11–6–50	13	ft C	ABnv	Bnv
36951	11–14–50	16	B7C	A4C	B7D

Table 2 Position of B2 1215+30

Star	A	B	C	D	QSS
Position (cm)	1.35 E 2.0 S	1.2 W 2.0 N	0.7 E 3.75 N	2.35 W 0.05 N	0.0 0.0

was made on three good quality 120 min exposure RH plates taken with the f/7, 3 inch Ross–Fecker camera. The range of variability was found to be $\Delta m_{pg} = 2.2$ or possibly slightly greater. The absolute range is less certain but is probably between 13.7 ± 0.2 and 15.8 ± 0.2.

Table 1 shows that B2 1215+30 undergoes brightness fluctuations compared with the constant brightness comparisons. The fluctuations occur over months and years, and are even discernible over time scales of hours. For example, the change of 4/10 of the magnitude range B to C in the pairs of plates MC26395, 26397 and MC26443, 26445 is probably real in each case, and occurs over times of 0.10 and 0.14 days respectively. Further inspection of Table 1 shows that the QSS was systematically brighter in 1927/28 than at other times, and systematically fainter from 1929 to 1932, while in 1933 it seems to be systematically somewhat brighter again. The light curve is consistent with the interpretation that the optical output consists of long and short term variability which is known to be characteristic of compact galaxies and quasi-stellar radio sources.

We thank the director and staff of the Harvard College Observatory for hospitality, and the National Science Foundation for support.

S. C. Lucchetti
P. D. Usher

Astronomy Department,
Pennsylvania State University

Received July 16, 1971.

[1] Browne, I. W. A., *Nature*, **231**, 515 (1971).

Application of Laser Source Mass Spectroscopy to Analysis of Geological Material

The preferred ion source for use in analytical mass spectrography is the spark source in its various forms such as the r.f. spark or the low voltage discharge, but some attention has been devoted in recent years to the possibility of using a focused laser beam to produce ions from the analytical sample[1,2]. The advantage of using such a system lies in its ability to vaporize and ionize non-conducting samples.

Until now, emphasis has generally been placed on the fundamental principles involved, and the use of a laser source has been limited to very specific problems[2]. This communication describes a successful attempt to use a pulsed neodymium laser as a source for the analysis of some elements in geological material.

One reason why lasers have previously been found unpromising as mass spectrographic sources is the slow pulse rates that were available, typically 1 pulse every 30 s. Furthermore severe space-charge broadening effects occurred because often high power Q-switched laser pulses were used, especially in time-of-flight systems.

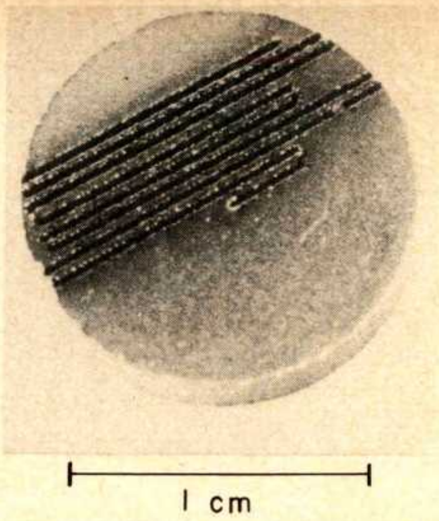


Fig. 2 Sample pellet after laser irradiation.

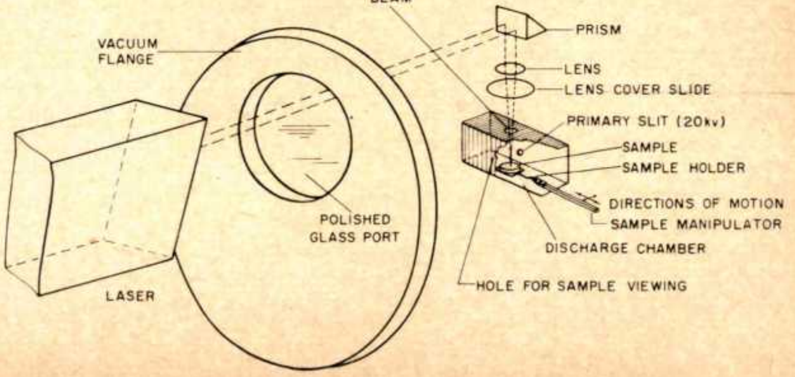
In the study described here, we have used a laser source with a pulse repetition rate of 6.25 s^{-1} in the non-Q-switched mode. Parameters of the laser radiation are given in Table 1. The laser was coupled to a double focusing mass spectrograph (Varian Mat SMIBF) to which some source modifications were made (Fig. 1).

Table 1 Parameters of Laser Radiation

Wavelength	10,600 Å
Mode	Conventional mode
Energy of burst	0.15 J
Total duration of burst	250 μs
Individual spike duration (½ width)	0.1–0.3 μs
Number of spikes per burst	~300
Mean burst power	~0.6 kW
Repetition rate	6.25 s^{-1}

The laser beam was focused on a spot on the sample (in the form of a pressed pellet or a rock chip) in front of the primary slit of the spectrograph. If allowed to, the successive laser pulses would drill into the sample, leading to a rapid diminution in plasma as the vaporization progressed. In the experiments described here, however, the sample was automatically moved laterally in order to present some unvaporized sample surface to each laser pulse. A constant scanning rate of 1 mm min^{-1}, or 3 μm per laser pulse, was found to be satisfactory. A typical sample pellet after irradiation, is shown in Fig. 2.

Fig. 1 Schematic diagram of modified source region of the mass spectrograph.



With the system described the total ion current was of the order 10^{-11} A. An ion-current of this magnitude permits analysis within a reasonably short exposure time. Inspection of the mass spectra revealed an extremely high sensitivity for the alkali metals. Furthermore, the spectral line shape was found to be excellent and much superior to that obtained using spark excitation. Rectilinear line profiles, which have been described as the ideal shape[3], were obtained in all cases using laser excitation. This is presumed to be due to the formation of the plasma envelope at a constant position relative to the primary slit of the mass spectrograph, resulting in an ion beam geometry of constant solid angle.

A problem encountered in pressing pure rock powder such as G1 into a pellet was that the pellet did not bind satisfactorily. To overcome this problem, an admixture of 30% by weight of graphite (to act as binder) was made. We found that the presence of this graphite greatly increased the sensitivity for some elements. Variations in the quantity of graphite showed that in most cases less than 5% by weight of graphite was needed. It is thought that the presence of carbon in the laser plasma creates a reducing environment which assists in the fracture of metal–oxygen bonds.

Table 2 Limits of Detection (p.p.m.) for Typical Elements Found in Rocks*

Element	Without carbon	With carbon
Li	~0.05	~0.05
Na	⩽0.05	~0.05
Mg	100	100
Si	20,000	200
K	⩽0.01	⩽0.01
Ca	20	20
Cr	100	25
Ti	50,000	200
Fe	200	20
Rb	<0.05	<0.1
Sr	50	15
Cs	≤0.1	~0.1

* The following elements were not detected, although present in the sample at greater than 1,000 p.p.m.: carbon, oxygen, fluorine, phosphorus, sulphur, and chlorine. The oxygen was seen as $(M\text{–}O)^+$ complex only.

Assessed limits of detection for some elements in geological samples are given in Table 2. The specificity for the group one elements is noteworthy. We have made use of this characteristic to determine the lithium isotopic ratios in a number of terrestrial and extra-terrestrial samples which have reasonably low lithium levels.

The ^{7}Li/^{6}Li ratio obtained for W1 was 11.1±0.3 in good agreement with the accepted value of 11.15. Values obtained for a number of South African stony meteorites were in the region of 14.0. Further studies of isotopic ratios are being undertaken.

Thus it has been shown that a medium pulse repetition rate laser can be successfully used as a routine source in mass spectrography where good sensitivity for the alkali metals is required. The excellent line shapes that can be obtained and the feasibility of *in situ* analysis of non-conducting samples are important advantages of the system described.

R. H. Scott
P. F. S. Jackson
A. Strasheim

National Physical Research Laboratory,
Council for Scientific and Industrial Research,
Pretoria

Received May 17; revised July 30, 1971.

[1] Honig, R. E., and Woolston, J. J. R., *Appl. Phys. Lett.*, 2, 138 (1963).

[2] Honig, R. E., *Applications of Mass Spectroscopy to the Analysis of Solids: a Review* in *Recent Developments in Mass Spectroscopy*—Proc. Intern. Conf. Mass Spectrosc., Kyoto, 1969 (University Park Press, Tokyo, 1970).

[3] Franzen, J., and Schuy, K. D., *Z. Anal. Chem.*, **225**, 295 (1967).

Transformation of Hematite to Goethite in Soils

In many parts of the world, especially in those that were not glaciated during the Pleistocene epoch, red hematitic soils that formed in a previous climate (different from the present one) still exist. In regions where the present climate is cooler and more humid than the previous one, the red colour seems to be disappearing and being replaced by more yellowish-brown colours that indicate the mineralogical transformation of hematite (α-Fe_2O_3) to goethite (α-FeOOH).

Near to the surface of profiles of these soils the proportion of red coloration decreases during these changes and the proportion of yellowish-brown colouring increases. A yellowish-brown rim forms on red mottles (in soils formed in the so-called mottled zones of former laterites exposed by truncation, for example) and this rim gradually grows inwards leaving only a red core that finally disappears. Hard nodules display a thin yellow skin covering a reddish hematitic interior. I have observed these features in areas as different as the North Island of New Zealand, South and Western Australia, Ceylon, Hong Kong and in south-eastern United States[1]. Other people have observed them in Northern Japan[2] and Natal, South Africa[3].

Both the reason for this transformation and its mechanism are closely connected to the change in the organic matter regime of the soil, which in turn reflects climatic change. A cooler and more humid climate causes a slower decomposition rate of the organic matter. In these conditions, increased amounts of organic compounds migrate into the soil and dissolve the hematite by reducing and/or complexing the iron. When this iron is reprecipitated (sometimes following oxidation) the newly formed oxide is usually goethite.

These observations are supported by laboratory experiments with synthetic iron oxides. In the *p*H range of most soils of humid climate areas (3.5 to 7.5) amorphous ferric hydroxide usually crystallizes *in vitro* to a mixture of goethite (through solution) and hematite (probably through internal dehydration). Hematite formation is stopped if the precipitation of the amorphous hydroxide is inhibited, or, in cases where it is formed, if it is prevented from dehydrating to hematite. This can happen when the iron released on weathering is complexed by organic compounds or when such compounds are adsorbed on the hydroxide. Recent experiments in this laboratory have shown that oxidation of ferric citrate yields pure goethite in a *p*H range where goethite and hematite are formed from ageing of amorphous ferric hydroxide[5]. Thus the higher the concentration of the organic compounds compared with the rate of Fe release from primary silicates, the greater the probability that goethite will be the dominating or only ferric oxide formed. The hematite–goethite ratio in soil therefore reflects the carbon regime and, in turn, the climate[4].

The influence of organic matter is further supported by several observations. In cases where roots penetrate into old red soils, removal of iron normally occurs in the vicinity of the roots, forming a bleached zone around them. Some distance out from the root, the iron reprecipitates and forms a yellow rim that separates the bleached zone from the red soil matrix. Furthermore, a red soil which had come into close contact with a humus-rich surface soil by erosion and deposition showed a red→yellow transformation in the area of contact between the two layers. A similar hematite→goethite transformation through solution can also be observed in hydromorphic soils on red hematite sandstone (Bunter Sandstone, for example) in mid-Europe, where, under anaerobic conditions, hematite has gone into solution and the newly formed oxide is always goethite or lepidocrocite, but never hematite.

No change in climate has so far been definitely established at any of the localities where the colour change was observed. But from the striking similarities between the colour of soil profiles in these areas, and those in areas where such a climatic change could be proved and the soils are definitely palaeosols (for example, Australia, N. Japan), a generalization seems to be justified. Therefore observations on these soils may have implications for palaeoclimatology.

Soils in which hematite (as well as goethite) is presently forming are well aerated and permeable, and slightly acid to calcareous. They occur under a warm subhumid to semiarid mediterrane or even arid climate (for example, Red Brown Earths of Australia[6], Hamra soils of Israel, Red Desert Soils of California[7,8]). This is in accord with the idea of a low ratio between supply of organic compounds and Fe release by weathering favouring hematite formation. Perhaps hematite is also forming in soils under moist tropical climatic conditions, where organic matter decomposes rapidly compared with a high rate of Fe release in acid conditions. This is, however, somewhat uncertain as many soils in these areas are probably non-recent and thus may have been influenced during their formation by other climates. Also at least some of the recent soils in these areas are brownish in colour rather than red.

U. SCHWERTMANN

Institut für Bodenkunde,
Technische Universität Munchen,
805 *Freising-Weihenstephan*

Received March 18; revised June 7, 1971.

[1] Daniels, R. B., *et al.*, *Soil Sci. Soc. Amer. Proc.*, **34**, 648 (1970).
[2] Matsui, T., *Proc. Seventh Cong. Quaternary Soils*, **9**, 222 (1967).
[3] Maud, R. R., *J. Soil. Sci.*, **16**, 60 (1965).
[4] Schwertmann, U., *Fortschr. Mineral*, **46**, 274 (1969).
[5] Fischer, W. R., thesis, Tech. Univ. Munich (1971).
[6] Radoslovich, E. W., *J. Soil. Sci.*, **9**, 242 (1958).
[7] Walker, T. R., *Geol. Soc. Amer. Bull.*, **78**, 353 (1967).
[8] Norris, R. M., *J. Sed. Petrol.*, **39**, 7 (1969).

In Support of a Physical Explanation of Ball Lightning

THE purpose of this communication is to present additional evidence in favour of the exclusion of explanations of ball lightning[1-6] in terms of, (*a*) the retinal afterimage hypothesis, (*b*) the intense point discharge hypothesis and (*c*) the burning material hypothesis. Altschuler[7] lists these in his summary of possible explanations.

During a dinner on the evening of July 6, 1971, at Orselina, near Locarno, Switzerland, I observed a flash of lightning which struck an unidentified target near the roof of a building about 200 m away. An intense point discharge immediately followed the flash and was visible clearly at the target for 1 or 2 s. I immediately told the other people at the dinner (Professor E. H. Schröter and Dr E. Wiehr from Göttingen and Mr C. Kühne from Oberkochen) of my observation because they were not looking out of the window at the time.

This occurrence was unmistakably different from another lightning event, which I observed several years ago and which I consider to have been a rare event of actual ball lightning. At that time a thunderstorm accompanied by heavy rain took place in the area of Neustadt near Coburg, Germany. I was sitting with some other people (among them Mr C. Förster from Berlin) right behind a ground floor window so that I could look at the weather; the field of view out to the street was limited by the frame of the window. Suddenly, at a height of about 16 m above the ground and at a short range of about 24 m, I saw a spherical plasma ball coloured bright yellow-white. This object appeared to have a diameter of 50 to 100 cm. It moved vertically downwards with a speed of about 4 m s^{-1}, and its path ended in the top branches of a tree, at a height of approximately 9 m. On touching the tree the ball instantly disintegrated into eight to twelve smaller spheres. These were the same colour as the large one and each had a diameter to 12 to 15 cm. They fell to the ground, guided by the outer contour of the tree, and moved vertically during the last few metres in the absence of branches. On reaching the ground (an asphalt roadway and a neighbouring footpath) the spheres instantly disappeared. There was no noise apart from that of the rain and no lightning associated with the primary plasma-like sphere. Three to five minutes afterwards, the same phenomenon occurred again in precisely the same way as before, indicating that the conditions needed to produce and guide the primary sphere were still maintained or re-established during the time that had elapsed. Immediately after the rain had stopped, I went out into the street to look for further evidence. There were circular patches of melted asphalt on the wet asphalt cover of the roadway which showed the interference colours of thin layers. Their diameters were each 12 to 15 cm and they obviously marked the impact areas of the smaller spheres. This event was described similarly by all the witnesses.

Because roadway asphalt in general contains B-80 bitumen—a thermoplast from which liquid components disintegrate at about 170° C—one may very roughly calculate the minimum amount of energy needed to produce the observed patches. Assuming that a water layer of thickness 0.5 mm at 20° C was evaporated and that an asphalt layer of thickness 1 mm was then heated to 170° C, and assuming a mean density of 1.0 g cm^{-3} and a mean specific heat $c_p = 0.46$ calorie g^{-1} for B-80, one may easily calculate the energy density of the plasma spheres to be at least 1.9×10^7 J m^{-3}.

A. WITTMANN

Solar Physics Division,
Universitäts-Sternwarte,
Göttingen

Received August 9, 1971.

[1] Argyle, E., *Nature*, **230**, 179 (1971).
[2] Charman, W. N., *Nature*, **230**, 576 (1971).
[3] Davies, P. C. W., *Nature*, **230**, 576 (1971).
[4] Gibbs-Smith, C. H., *Nature*, **232**, 187 (1971).
[5] Jennison, R. C., *Nature*, **230**, 576 (1971).
[6] Wagner, G. A., *Nature*, **232**, 187 (1971).
[7] Altschuler, M. D., *Scientific Study of Unidentified Flying Objects*, Ch. 7 (New York, 1969).

Proposal for a New High Precision Particle Detector

THE precision commonly quoted when measuring the position of a charged particle track is ±0.3 mm for wire plane spark chambers[1], ±0.4 mm for streamer chambers[2] and ±0.7 mm for multiwire proportional chambers[3,4]. With the onset of the new generation of synchrotrons there is a need for detectors with better resolution. This arises from the fact that the accelerated particles will receive only small deflexions in analys-

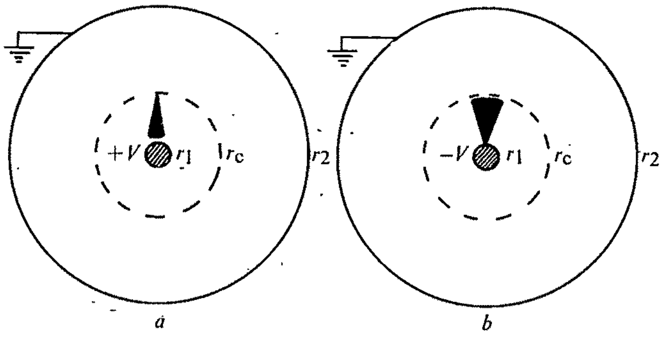


Fig. 1 *a*, Conventional proportional counter; *b*, outwardly directed avalanche chamber. Not to scale.

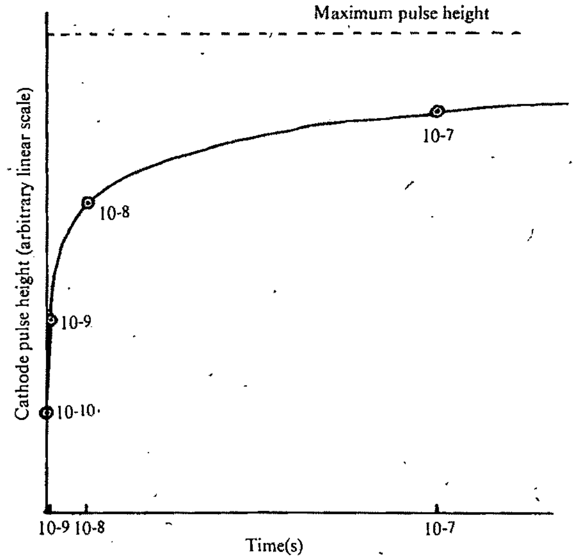


Fig. 2 Pulse formation in the outwardly directed avalanche chamber, calculated for the values mentioned in the text. The ions are collected at the wire cathode at $t_1=4.10^{-9}$ s and the maximum pulse height is obtained at about $t_2=10^{-6}$ s when the electrons arrive at the cylindrical anode.

ing magnets. This article consists of a proposal for a detector that will provide the necessary increase in resolution.

The basic design of the proportional counter has remained unchanged since Rutherford and Geiger[5]. The pulse formation in such a counter has been discussed in detail by Wilkinson[6]. In a radial field where the electric field (E) is related to the applied potential (V) by

$$E = \frac{V}{r \ln r_2/r_1} \tag{1}$$

the electrons, from ion-pairs released by traversing charged particles, drift towards the anode wire (Fig. 1). On arrival at a critical radius

$$r_c = \frac{r_1 V}{V_c} \tag{2}$$

where V_c is the threshold voltage for ionization, secondary ionization starts. An avalanche results, the number of electrons roughly doubling in every mean ionizing path. Of the electrons falling on the wire, half are produced within only one mean path of the wire, and are collected practically instantaneously. The rise-time of the pulse, however, is governed by the drift of the positive ions away from the wire.

I shall consider the opposite design—in which the wire is the cathode. Any free electron at the surface of the wire will be accelerated radially and an outgoing avalanche will be formed (Fig. 1). The multiplication will continue until the critical radius is attained, after which the electrons will drift slowly to the cylindrical anode. The positive ions will accelerate towards the cathode where, on arrival, one assumes that they are merely neutralized. On completion of the avalanche at time $t=0$, one may assert that a shell of positive ions and electrons lies at the critical radius r_c. If the drift velocities be given by

$$\begin{aligned} u_+ &= \frac{dr_+}{dt} = -k_+E \\ u_- &= \frac{dr_-}{dt} = k_-E \end{aligned} \tag{3}$$

where k_+, k_- are the appropriate mobilities, one may show, using Green's theorem, that the growth of the cathode pulse with time is described by

$$P(t) = \frac{Ne}{C \ln r_2/r_1} \ln \left\{ \sqrt{\frac{2k_- Vt + r_c^2 \ln r_2/r_1}{-2k_+ Vt + r_c^2 \ln r_2/r_1}} \right\} \tag{4}$$

where C is the chamber capacity and Ne the charge in the avalanche. The positive ions will all be collected at time

$$t_1 = \frac{(r_c^2 - r_1^2) \ln r_2/r_1}{2k_+ V} \tag{5}$$

and the electrons at

$$t_2 = \frac{(r_2^2 - r_c^2) \ln r_2/r_1}{2k_- V} \tag{6}$$

Let the radius of the wire be 20 µm and that of the cylinder 1 cm. Suppose that the threshold voltage (V_c) is 1,200 V and the operating voltage 2,000 V. Then from equation (2), r_c is 33 µm. The avalanches will thus have a radial distance of 13 µm in which to develop. If the counter is filled with argon at atmospheric pressure, taking[6] $k_+=2.5$ cm^2 V^{-1} s^{-1} and $k_-=2{,}000$ cm^2 V^{-1} s^{-1}, the pulse in Fig. 2 is obtained. Thus 22% of the maximum pulse is attained in 10^{-10} s and 65% in 10^{-8} s. The sharp rise means that a short detectable fast pulse may be obtained with a differentiation network.

If the "outwardly directed avalanche chamber" is to be a viable detector, particles must produce one free electron near the wire surface. The primary specific ionization in argon is about 27 ions/cm. Assuming that an electron produced inside a radius of 26.5 µm will result in a detectable pulse, the available distance is 13 µm—counting both sides of the wire. The chance of an ion-pair being produced in this distance is therefore $(13.10^{-4})(27)=3.5\%$.

When a particle passes through the wire, a secondary electron may be emitted from the surface. This probability, δ, is thought to be independent of energy at high energies. Per-

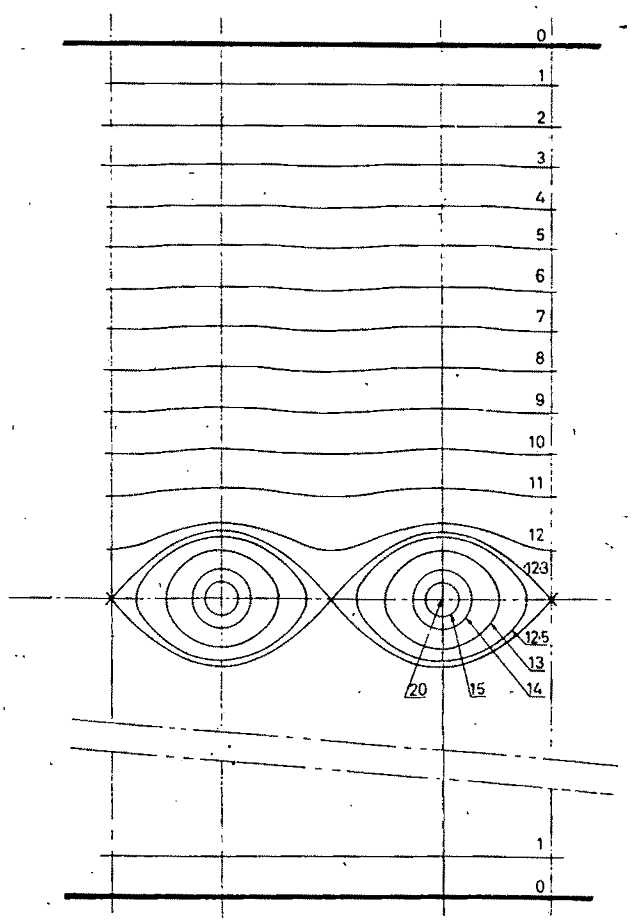


Fig. 3 The equipotentials surrounding the wires of a cathode wire-plane when placed between two plane anodes. It has been possible to take the figure from Charpak *et al.*[3,4] as the electric field will have the same shape whether the wires be cathodes or anodes.

tinent values for δ are difficult to find. A value of $\delta = 2.5\%$, however, has been given for 19.2 GeV/c protons traversing 5 µm aluminium foils[7]. Applying this to entrance and exit surfaces, the probability then becomes 5%. Bruining[8] has mentioned that for 80 kV electrons, the probability increases by a factor 2 when the aluminium thickness increases from 3.6 to 9.4 µm. It seems reasonable therefore to use a factor 2 in applying the 5 µm value to 40 µm wire. Bruining has also commented on the effect of the density of the wire. For 300 kV electrons, the emission from tungsten (density 19.3 g cm^{-3}) was 3.6 times that from aluminium (2.7 g cm^{-3}). Thus if we use stainless steel wires coated with gold (density 19.3 g cm^{-3}) our probability figure would become 36%—and more if the high field of the wire geometry enhances electron emission. Of course, these are rough estimates.

Quenching gas would be required to neutralize spurious ions produced by cosmic rays and non-intersecting particles. External quenching might also be necessary to cope with the action of the avalanche ions. Another possibility is to operate in a pure organic quencher—say, isobutane. Nevertheless, electron emission from the cathode might prove to be a difficult problem.

If successfully developed, the outwardly directed avalanche chamber might be used to assess the spatial distribution of particles in a beam. Alternatively, the wires might be arranged in a Charpak[3,4] multiwire format in which the wires avalanche independently (Fig. 3). It is clear that the high resolution (~30 µm) is bought at a cost of low efficiency—for example, a plane of 30 µm wires, spaced at 1 mm would be 97% transparent. Optimistically, one can envisage matrices comprised of hundreds of planes each with hundreds of wires.

I thank Drs Andreas Weitsch, Branko Bošnjaković, Robert Klanner and C. Schmied for discussing this work; and Professor P. Preiswerk and Dr Werner Kienzle for their hospitality at CERN, and also the Science Research Council for making my visit to CERN possible.

PETER RICE-EVANS

Physics Department,
Bedford College,
University of London,
Regent's Park, London NW1

Received August 24, 1970; revised May 15, 1971.

[1] Perez-Mendez, V., Devlin, T. J., Solomon, J., and Droege, T., *Nucl. Instrum. Methods*, **46**, 197 (1967).
[2] Rice-Evans, P., and Mishra, S. R., *Nucl. Instrum. Methods*, **67**, 337 (1969).
[3] Charpak, G., Bouclier, R., Bressani, T., Favier, J., and Zupančič, C., *Nucl. Instrum. Methods*, **62**, 262 (1968).
[4] Charpak, G., *Ann. Rev. Nucl. Sci.*, **20**, 195 (1970).
[5] Rutherford, E., and Geiger, H., *Proc. Roy. Soc.*, A, **81**, 141 (1908).
[6] Wilkinson, D. H., *Ionization Chambers and Counters* (Cambridge University Press, 1950).
[7] *CPS Users Handbook*, I (CERN, 1969).
[8] Bruining, H., *Physics and Applications of Secondary Electron Emission* (Pergamon, London, 1954).

BIOLOGICAL SCIENCES

Prostaglandin-induced Myometrial Activity inhibited by Progesterone

PROSTAGLANDINS (PGs) are being used increasingly as abortifacients although their precise mechanism of action is uncertain. One of the compounds frequently used is PGF$_{2\alpha}$, which has been shown to stimulate the myometrium *in vitro*[1] and *in vivo*[2]. Whether or not the direct action of this agent on the uterine muscle alone is responsible for the interruption of pregnancy is not clear, but the long interval between infusion and abortion[3] suggests that other mechanisms might be involved; for example, a luteolytic effect[4–6]. The prompt increase in uterine activity after treatment with prostaglandin, however, argues that the direct effect plays some part in the abortifacient action. Little is known of the influence of other myometrial regulatory agents such as the ovarian steroids on the uterine response to PGs. Accordingly we examined the influence of progesterone on myometrial responses to prostaglandin F$_{2\alpha}$ in the rabbit, a species in which progesterone is known to have a blocking action on the uterine muscle[7].

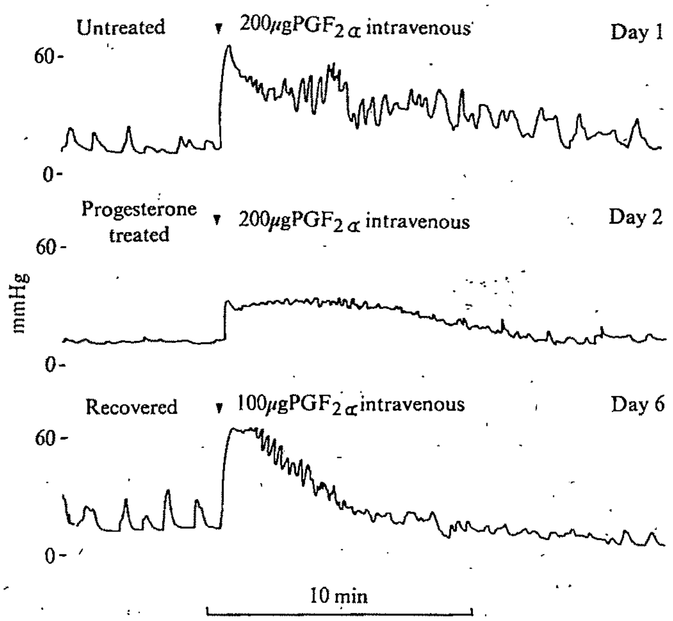


Fig. 1 Intra-uterine pressure recordings from a non-pregnant rabbit showing the response to an intravenous injection of prostaglandin PGF$_{2\alpha}$ before treatment with progesterone (day 1) during the progesterone effect (day 2) and after recovery from progesterone (day 6). NB. Treatment on day 6 was only 100 µg of PGF$_{2\alpha}$ whereas, on days 1 and 2, 200 µg of PGF$_{2\alpha}$ was used.

Adult non-pregnant New Zealand White rabbits were equipped with intra-uterine recording balloons as described by Csapo, Takeda and Wood[8] and intra-uterine pressure monitored via Statham pressure transducers and a Grass 7B polygraph. In twenty-three tests with four rabbits PGF$_{2\alpha}$ was administered by single intravenous injections of either 100 or 200 µg before, 12–14 h after treatment with 10 mg progesterone given subcutaneously when the inhibitory effect of this hormone on spontaneous myometrial pressure cycles was maximal, and also following recovery from the progesterone effect. In all animals the uterine response to both doses of PGF$_{2\alpha}$ was one of an immediate contracture. But, in the progesterone treated animals the amplitude, and with the 200 µg dose the duration, of the contracture was significantly less than in the untreated animals (Table 1). Following prostaglandin treatment rhythmic pressure cycles recurred before the resting pressure returned to the pre-treatment level in intact and recovered animals, but were reduced to a mere ripple (Fig. 1) in the progesterone treated rabbit. Injection of 200 µg of oleic acid intravenously as a control elicited no response from the uterus. In two further animals the effect of progesterone on the uterine response to the intravenous infusion of PGF$_{2\alpha}$ was examined. Whereas a single injection of 100–200 µg PGF$_{2\alpha}$ elicited a marked contracture of high amplitude, infusion of a similar quantity of the substance over a period of 30 min produced no elevation of the resting pressure but increased the frequency of pressure cycles by more than 100% (that is, mean frequency before treatment was 45.6 ± 13.0 s.e. pressure cycles/h, during infusion 94.8 ± 18.4 s.e./h (before against during $P < 0.1$) and after infusion 27.6 ± 7.0 s.e./h (during against after $P < 0.01$.) After

Table 1 Myometrial Response to $PGF_{2\alpha}$ in Untreated, Progesterone-treated and Progesterone-recovered Rabbits (Four Animals)

	Treatment intravenous	Untreated and recovered ±s.e.	Progesterone treated ±s.e.	P
Amplitude of response (mm Hg)	100 μg $PGF_{2\alpha}$	37.8±2.2 (8)	22.4±2.2 (3)	<0.01
	200 μg $PGF_{2\alpha}$	40.8±3.7 (6)	24.8±3.3 (6)	<0.001
P		>0.1	>0.1	
Duration of response (min)	100 μg $PGF_{2\alpha}$	9.5±0.9 (8)	9.7±0.6 (3)	>0.1
	200 μg $PGF_{2\alpha}$	14.5±1.1 (6)	9.9±1.1 (6)	<0.025
P		<0.025	>0.1	

Figures in parentheses = number of tests.

treatment of the animals with 10 mg progesterone, this response was either totally abolished or so reduced as to be almost imperceptible, but returned on recovery from the progesterone block.

These results demonstrate that progesterone inhibits the myometrial response to $PGF_{2\alpha}$ in the rabbit. This might be due to its action in impairing the electro-coupling of cells[7], or in reducing their reactivity by hyperpolarization of their cell membranes[7], or both. Whatever the underlying cause it is clear that $PGF_{2\alpha}$ is a much less effective oxytocic agent in the presence of the progesterone-block. The significance of this in relation to its abortifacient action in women cannot be judged until the role of progesterone itself in maintaining human pregnancy is clarified. If progesterone is a major uterine regulatory agent in the human female, then the results of these experiments suggest that alternatives to a direct action on uterine muscle such as an interference with steroidogenesis should not be overlooked in seeking the mechanism by which prostaglandins induce abortion.

We thank Miss Marilyn Murray for technical assistance, Drs G. W. Duncan and J. E. Pike for prostaglandin and the Milton Fund of Harvard Medical School for financial support.

D. G. PORTER

Department of Anatomy,

H. R. BEHRMAN

Department of Physiology,
Harvard Medical School,
25 Shattuck Street,
Boston,
Massachusetts 02115

Received March 3, 1971.

[1] Sandberg, F., Ingelman-Sundberg, A., and Ryden, G., *Proc. Fifth World Congress on Fertility and Sterility*, 675 (Excerpta Medica Foundation, Amsterdam, 1967).
[2] Karim, S. M., Trussell, R. R., Patel, R. C., and Hillier, K., *Brit. Med. J.*, **2**, 621 (1968).
[3] Wiqvist, N., and Bygdeman, M., *Lancet*, i, 889 (1970).
[4] Kirton, K. T., Pharriss, B. B., and Forbes, A. D., *Proc. Soc. Exp. Biol. Med.*, **133**, 314 (1970).
[5] Behrman, H. R., Yoshinaga, K., and Greep, R. O., *Amer. J. Physiol.* (in the press).
[6] Behrman, H. R., Yoshinaga, K., and Greep, R. O., *Ann. NY Acad. Sci.* (in the press).
[7] Csapo, A. I., *Amer. J. Obstet. Gynecol.*, **85**, 359 (1963).
[8] Csapo, A. I., *Amer. J. Obstet. Gynecol.*, **85**, 813 (1963).

Necessity for Restudying Nucleic Acid and Protein Synthesis in Mammalian Semen

IT is generally accepted that mature spermatozoa cannot synthesize nucleic acids[1]. The only enzyme involved in nucleic acid synthesis which has been identified in semen is polynucleotide phosphorylase[2], but this finding remains unconfirmed, and it has been suggested that the apparent enzyme activity resulted from interactions of other enzymes not involved in nucleic acid synthesis[3].

We have reported the existence of a very potent compound in bull seminal plasma and sperm cell extracts which inhibited tRNA methylation[4,5] by degrading the tRNA substrate into non-dialysable polynucleotide fragments[6]. Evidence strongly suggested that the active compound was not a ribonuclease, but a polysaccharide which formed an irreversible complex with the tRNA before degradation of the tRNA[6]. Data are presented here to demonstrate that very dilute amounts of seminal plasma or sperm cells are sufficient to hydrolyse all species of RNA. Consequently, the activity of exogenously added enzymes which synthesize RNA, or utilize it as a substrate or template, were undetectable in the presence of either of the semen components.

Semen from stud bulls collected over liquid nitrogen was supplied by the Eastern Artificial Insemination Corporation, Ithaca, New York. No antibiotics or preservatives were added to the final pooled sample. R17 viral RNA was provided by Dr James Eley. Ribosomal and transfer RNA samples were purchased from Miles Laboratories and General Biochemical Company, respectively. Bovine pancreatic ribonuclease and all nucleotides were purchased from the Sigma Chemical Company.

Seminal plasma was separated from sperm cells by centrifugation at 850*g* for 10 min. The sperm cells were washed twice with 0.25 M sucrose before use. Trichloroacetic acid was added slowly to 1 : 80 diluted samples of the semen components until solutions were made 7% with respect to the acid. After centrifugation, the supernatants were neutralized with NaOH and dialysed against water for 24 h before use. Final dilutions were made with distilled water. Preparing semen extracts in this manner inactivated an endogenous ribonuclease which preferentially hydrolyses double-stranded RNA (ref. 7 and B. S. and S. M. W., unpublished).

Percentage hydrolysis of the RNA samples was determined by incubating 0.1 mg of each species of RNA with various dilutions of seminal plasma or sperm cells in 1.5 ml. of 0.02 M Tris buffer (*p*H 7.8). After 30 min at 37° C, 0.2 ml. of 50% trichloroacetic acid was added to the assay mixtures to terminate the reactions. The mixtures were then kept at 4° C for 15 min before being centrifuged. The 260 nm absorbtion of the supernatant solutions were then read to determine the amount of acid soluble products. Concurrent blanks were assayed with each individual concentration of seminal plasma and sperm cells.

Tables 1 and 2 show the percentage hydrolysis of the different species of RNA after being incubated with various dilutions of the semen components. Dilutions as low as 1 : 2,500 of seminal plasma, and 1 : 500 of sperm cells, were

Table 1 Hydrolysis (%) of RNA after Incubation with Varying Dilutions of Seminal Plasma

Dilution of seminal plasma	% Hydrolysis of 0.1 mg of			
	Viral RNA	tRNA	Polyuridylic acid	rRNA
1 : 500	100	100	100	100
1 : 1,000	100	100	100	100
1 : 2,500	100	100	100	100
1 : 5,000	100	93	93	81
1 : 10,000	96	89	80	70
1 : 25,000	92	84	70	60
1 : 50,000	85	79	52	33
1 : 100,000	44	40	20	9
1 : 200,000	19	14	4	0
1 : 400,000	10	10	0	0
1 : 750,000	5	1	0	0
1 : 1,000,000	1	0	0	0

The preparation of the seminal plasma and the assay for RNA hydrolysis are described in detail in the text.
The RNA samples used were R17 viral RNA, *E. coli*$_B$ tRNA, and *E. coli*$_B$ rRNA.

Table 2 Hydrolysis (%) of RNA after Incubation with Varying Dilutions of Sperm Cells

Dilution of sperm cells	% Hydrolysis of 0.1 mg of Viral RNA	tRNA	Polyuridylic acid	rRNA
1 : 500	100	100	100	100
1 : 1,000	100	100	96	89
1 : 2,500	100	98	75	71
1 : 5,000	85	79	49	35
1 : 10,000	73	65	20	14
1 : 25,000	68	50	4	3
1 : 50,000	31	26	0	0
1 : 100,000	13	12	0	0
1 : 200,000	1	0	0	0

The preparation of the sperm cells and the assay for RNA hydrolysis are described in detail in the text.

The RNA samples used were R17 viral RNA, *E. coli*$_B$ tRNA, and *E. coli*$_B$ rRNA.

both able to hydrolyse totally 0.1 mg of tRNA, rRNA, polyuridylate or R17 viral RNA. There were no significant differences in the percentage hydrolysis of tRNA samples isolated from different sources.

Because of the degradation of RNA, these dilutions of the semen components completely masked the activity of exogenous enzymes which required RNA as a substrate, such as tRNA methylases[5], rRNA methylases[5] and valine tRNA aminoacylase[8]; and enzymes which synthesize RNA such as DNA-dependent RNA polymerase[9], and uridine diphosphate polymerase[10]. These data were independent of the degree of activity of the exogenous enzyme preparation. Where applicable, 0.1 mg of RNA was employed as the substrate. Similar results depicting the masking of enzyme activity were obtained with concentrated or diluted samples of seminal plasma or sperm cells which were not treated with the trichloroacetic acid.

Bovine pancreatic ribonuclease activity was not affected by either of the semen components. This may be explained by the fact that the active compound in semen degrades tRNA into non-dialysable fragments[5]. It can therefore be assumed that these fragments, being non-dialysable, are large enough to serve as a substrate for the exogenous ribonuclease. The data in these studies indicate that unless the hydrolytic substance in semen is differentially inactivated, it will be impossible to detect (except for diesterases) any exogenous or endogenous enzyme activity in semen, whereby RNA is synthesized, or utilized as a substrate or template. Initial studies have demonstrated that periodate oxidation destroys the hydrolytic substance in very dilute semen without affecting endogenous nucleic acid polymerase activity. Experiments are now being conducted to determine the proper conditions to inactivate this compound in undiluted semen by periodate oxidation. When this is accomplished, it will be possible to determine whether mature spermatozoa possess the enzyme complement necessary to synthesize nucleic acids and proteins.

This research was supported by a grant from the New York State Research Foundation.

BERTRUM SHEID
SUSAN M. WILSON

Department of Pharmacology,
State University of New York,
Downstate Medical Center,
Brooklyn, New York 11203

Received March 5; revised April 2, 1971.

[1] Mann, T., *Biochemistry of Semen and of the Male Reproductive Tract* (Methuen, London, 1964).
[2] Hakim, A. A., *Enzymologia*, **21**, 81 (1959).
[3] Smellie, R. M. S., *Prog. Nucleic Acid Res.*, **1**, 27 (1963).
[4] Sheid, B., and Wilson, S. M., *Fed. Proc.*, **29**, 3883 (1970).
[5] Sheid, B., and Wilson, S. M., *Biochim. Biophys. Acta*, **224**, 382 (1970).
[6] Sheid, B., and Wilson, S. M., *Life Sci.* (in the press).
[7] Libonati, M., and Floridi, A., *Europ. J. Biochem.*, **8**, 81 (1969).
[8] Holley, R. W., Apgar, J., Doctor, B. P., Farrow, J., Marini, M. A., and Merrill, S. H., *J. Biol. Chem.*, **236**, 200 (1961).
[9] Bresnick, E., *Molec. Pharmacol.*, **2**, 406 (1966).
[10] Grunberg-Manago, M., *Prog. Nucleic Acid Res.*, **1**, 93 (1963).

Possible Role for Prostaglandin F$_{2\alpha}$ in Parturition in Sheep

PROSTAGLANDINS F$_{2\alpha}$ (PGF$_{2\alpha}$), E$_1$ (PGE$_1$) and E$_2$ (PGE$_2$) have potent oxytocic properties that have led to their successful use in induction of labour and abortion in women[1]. They have been found in tissues and biological fluids of women in spontaneous labour but it is not clear whether the release of prostaglandins has an important physiological role in the control of labour or whether it is a consequence of labour. Karim and Devlin[2] found PGE$_1$, PGE$_2$, PGF$_{1\alpha}$ and PGF$_{2\alpha}$ in amniotic fluid of women in labour although only PGE$_1$ was present in samples obtained before labour. The source of prostaglandins in amniotic fluid may be the decidua, which shows the same pattern of prostaglandins as amniotic fluid—PGE$_1$ before labour and PGE$_1$, PGE$_2$, PGF$_{1\alpha}$ and PGF$_{2\alpha}$ during labour[2]. The decidua may also be the source of PGF$_{2\alpha}$ found in peripheral venous blood[3].

In sheep, the mechanism of onset of labour seems to be controlled chiefly by corticosteroids secreted by the adrenals of the foetal lamb[4]. The length of pregnancy can be manipulated in this species by experimental procedures such as foetal hypophysectomy or foetal infusions of either adrenocorticotrophic hormone or corticosteroids that depress or augment foetal adrenal function. We have investigated the role of PGF$_{2\alpha}$ in premature parturition induced by infusion of a glucocorticoid into the foetal lamb.

Samples of maternal cotyledons, foetal cotyledons and myometrium were obtained by killing ewes with an overdose of phenobarbitone and gallamine and then rapidly excising the tissues. The control ewes were normal pregnant animals of known gestation length. In one experimental group, the foetuses had received an intra-carotid infusion of dexamethasone (1 mg/24 h) either for 24 h or until labour contractions were observed on continuous intra-amniotic pressure recordings (at 46–52 h). In another experimental group the foetuses were infused with dexamethasone for 52–57 h but the onset of labour was prevented by giving the ewe progesterone in oil 100 mg/12 h. Before and during corticosteroid-induced labour large samples of plasma (100 ml.) were obtained by plasmaphoresis from chronically implanted catheters in the maternal jugular (JV) and uterine veins (UV). The fresh samples were immediately frozen on dry ice and stored at −10° until analysed.

Weighed portions of tissue were homogenized in 4 volumes of ethanol at 4° C. The supernatant of the homogenates and the plasma samples were extracted with ethyl acetate at *p*H 3.0 after the addition of 200 nCi of tritiated PGF$_{2\alpha}$. After methylation, the PGE and PGF compounds were separated by thin layer chromatography (TLC) using system M1 of Gréen and Samuelsson[5]. Authentic standards of methylated PGF$_{2\alpha}$ and PGE$_1$ were run beside the unknowns and were identified by spraying with 1% of iodine in methanol. R_F values (relative to the solvent front) of the methyl derivatives of PGF, PGE and PGA were respectively 0.42, 0.605 and 0.87. Areas of the TLC plates corresponding to the standards were removed and the extracts were further identified and purified by paper chromatography at room temperature using a mobile phase of ethylene dichloride/heptane 1 : 1 and a stationary phase of 70% glacial acetic acid. The area

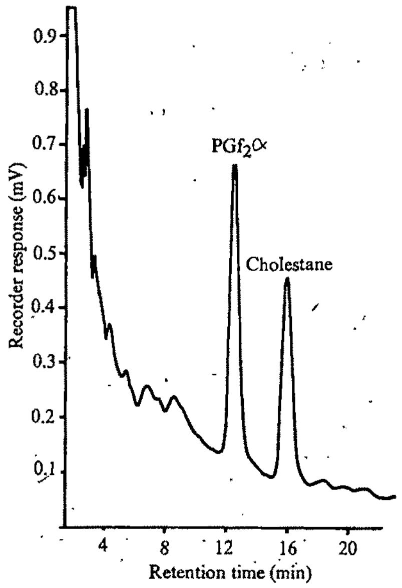


Fig. 1 Gas chromatographic assay of $PGF_{2\alpha}$ in 39 g of maternal cotyledons from sheep No. 0/102. Overall recovery during purification was 36%. One-twentieth of the trimethylsilyl ether derivative of the final extract was injected. Cholestane was added as an internal standard. The $PGF_{2\alpha}$ peak corresponds to 190 ng of authentic material. Column conditions: 1.8 m × 4 mm glass column, 2.03% SE 30 on 80–100 mesh 'Diaport S'. The column temperature was 225° C; injection port temperature was 260° C. Nitrogen flow rate was 25 ml./min.

corresponding to $PGF_{2\alpha}$ was identified by radiochromatogram scanning. The R_F values of PGF, PGE and PGB methyl ethers were respectively 0.52, 0.64 and 0.73. The trimethylsilyl ether derivatives were quantitated by gas chromatography (GLC) using conditions described in Fig. 1 and were also applied to a column packed with 3.8% 'Hi-Eff 3A' on 80–100 mesh 'Diaport S' at a column temperature of 218° C. Cholestane was used as an internal standard for PGF assays. Retention times relative to cholestane for authentic $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were respectively 0.78 and 0.84 on the SE 30 column and 0.45 and 0.45 on the succinate column. PGE compounds eluted from TLC plates were converted to PGB compounds by treatment with NaOH and were then subjected to paper chromatography, acetylation and GLC. The internal standard used for GLC of PGB compounds was 20β-acetoxyprogesterone and the relative retention time of authentic PGB_1 and PGB_2 on an SE 30 column was 0.72. Peaks with the same relative retention time as authentic materials on both SE 30 and succinate columns were measured and the amounts of prostaglandin in the samples were determined from a standard curve. The lower limits of detection of both PGF and PGE compounds were 100 ng/20 g tissue sample and 200 ng/100 ml. plasma sample. Procedural losses were estimated from the recovery of radioactivity after silylation.

$PGF_{1\alpha}$, PGE_1 and PGE_2 were not detected in any of the tissue or plasma samples. $PGF_{2\alpha}$ was not found in any of the JV samples or in UV samples taken before labour but was present in UV plasma during labour in nine of ten ewes (Table 1).

The concentration of $PGF_{2\alpha}$ in maternal cotyledons was raised above the levels found in two control animals after dexamethasone had been infused into the foetus for 24 h, but the concentration in myometrium was unchanged (Table 2). In labour, a further increase in concentration in maternal cotyledons was found and, in addition, the concentration in the myometrium was increased. Similarly increased concentrations of $PGF_{2\alpha}$ were also found in maternal cotyledons and myometrium of ewes that would have been in labour had they not received progesterone. The concentration of $PGF_{2\alpha}$ in foetal cotyledons was relatively low in all samples.

The results show that an increase in concentration of $PGF_{2\alpha}$ in maternal cotyledons precedes the onset of induced, premature labour by at least 24 h. Furthermore, an increase in concentration in both maternal cotyledons and in myometrium occurs not only in labour but also when uterine activity is suppressed by large doses of progesterone, indicating that the appearance of $PGF_{2\alpha}$ is not a consequence of labour. These observations are consistent with a causal relationship between $PGF_{2\alpha}$ and the onset of labour in sheep.

Table 1 Concentration of $PGF_{2\alpha}$ in Uterine Venous Blood before and during Premature Labour induced by Infusion of Dexamethasone into Foetal Lambs

Sheep No.	Length of gestation (days)	$PGF_{2\alpha}$ concentration (ng/m.l) Before labour	In labour
0/6	110	—*	31
0/39	111	—	11
0/36	117	—	5.4
9/104	120	—	—
9/82	126	—	7
9/107	127	—	37
0/86	131	—	35
0/64	138	—	2.4
0/61	140	—	29
0/85	140	—	3.5

* Not detected (<2 ng/ml.).

Table 2 Concentration of $PGF_{2\alpha}$ (ng/g wet tissue) in Maternal Cotyledons, Foetal Cotyledons and Myometrium before and during Premature Parturition induced by Infusion of Dexamethasone into Foetal Lambs

Sheep No.	Gestational age (days)	Maternal cotyledon	Myometrium	Foetal cotyledon
			Untreated	
0/92	126	82	70	47
0/93	140	164	88	122
			After 24 h dexamethasone	
0/108	128	382	99	101
0/102	130	315	97	86
			After about 48 h dexamethasone (in labour)	
0/100	128	543	490	77
0/101	128	470	154	
0/113	130	633	257	
0/50	140	875	417	100
0/62	140	—*	515	114
			After about 48 h dexamethasone (labour suppressed by progesterone)	
A254	126	329	122	110
0/107	127	723	236	33
0/103	134	275	306	

* Sample lost during purification.

The plasma concentration of progesterone decreases sharply before parturition at term[6] and also before premature parturition induced by administration of corticosteroids or ACTH to the foetus[4]. Although it is likely that withdrawal of the inhibitory effects or progesterone on uterine contractility plays a part in the physiological mechanisms regulating labour in sheep, there is doubt that it is the major mechanism since relatively large doses of progesterone fail to prevent labour[4,7]. Progesterone withdrawal may have a facilitatory role in parturition, secondary to a mechanism that is dependent on prostaglandin release. The recent demonstration that the uterine luteolytic agent is likely to be $PGF_{2\alpha}$ (refs. 8, 9) raises the possibility that $PGF_{2\alpha}$ could serve a dual role in relation to parturition, being luteolytic in species in which luteolysis immediately precedes parturition and being oxytocic in species in which pregnancy is maintained by placental hormones.

We thank the trustees of the Wellcome Trust for financial support, and Dr J. E. Pike, of Upjohn, Kalamazoo, for prostaglandins.

G. C. LIGGINS
SUSAN GRIEVES

Postgraduate School of Obstetrics and
Gynaecology,
University of Auckland

Received March 15; revised April 30, 1971.

[1] Sperof, L., and Ramwell, P. W., *Amer. J. Obstet. Gynec.*, **107**, 1111 (1970).
[2] Karim, S. M. M., and Devlin, J. J., *Obstet. Gynaec. Brit. Comm.*, **74**, 230 (1967).
[3] Karim, S. M. M., *Brit. Med. J.*, **4**, 618 (1968).
[4] Liggins, G. C., in *Foetal Autonomy*, Ciba Symp. (edit. by Wolstenholme, G. E. W., and O'Connor, M), 218 (Churchill, London, 1969).
[5] Gréen, K., and Samuelsson, B., *J. Lipid Res.*, **5**, 117 (1964).
[6] Bassett, J. M., Oxborrow, T. J., Smith, I. D., and Thorburn, G. D., *J. Endocrinol.*, **45**, 449 (1969).
[7] Bengtsson, L. Ph., and Schofield, B. M., *J. Reprod. Fertil.*, **5**, 423 (1963).
[8] McCracken, J. A., Glew, M. E., and Scaramuzzi, R. J., *J. Clin. Endocrinol.*, **30**, 544 (1970).
[9] Poyser, N. L., Horton, E. W., Thompson, C. J., and Los, M., *J. Endocrinol.*, **48**, xliii (1970).

Purity of Synthetic Peptide Preparations

WE strongly endorse Vane's comments[1] on the pitfalls associated with the use of synthetic peptides in biological studies. It is particularly misleading to express results in terms of weight without evidence of homogeneity, because apart from error peptides (which may not be inert), account is seldom taken of salt and solvent content.

The impact of these problems in the field of radioimmunoassay was discussed at some length[2,3] at the recent workshop meeting in Edinburgh. In the case of one commercial synthetic angiotensin already widely used in research, evidence has been recorded of varying stability between batches[4] and apparent variations in the peptide content of different batches[5]. Furthermore, extending Vane's comments on the chemical purity of these peptides, it may not be generally appreciated that rearrangement of the aspartyl residue in angiotensin can occur in solution even at neutral *p*H; the biological activity of the β aspartyl isomer in the rat pressor assay is some 150% that of the α form[6]. The activity of successive batches of peptide could thus differ according to the degree of contamination by the β isomer. If, however, as suggested[1], manufacturers provided comprehensive analytical data for each batch of synthetic peptide, the user would be aware of this possibility.

When precise quantification is needed, the practice of describing results in terms of apparent peptide content is misleading and could be avoided by assay in terms of a suitably characterized standard preparation. Such a preparation of angiotensin II (asp^1 ileu^5 angiotensin II) has been chemically characterized and its stability studied; this preparation (coded 70/302) and also a human renin preparation (coded 68/356) are now available from this division to research workers for the purpose of standardization.

In due course a preparation of human type angiotensin I[7] will also be provided. It is hoped that use of these will make possible valid quantitative comparison of results in the renin/angiotensin field.

We would emphasize that these general comments apply to many of the synthetic peptide hormones used quantitatively in research which include oxytocin, vasopressins, angiotensins I and II, bradykinin, corticotrophins, human calcitonin M and secretin.

D. R. BANGHAM
D. H. CALAM
J. A. PARSONS
C. J. ROBINSON

Division of Biological Standards,
National Institute for Medical Research,
London NW7

Received May 14, 1971.

[1] Vane, J. R., *Nature*, **230**, 382 (1971).
[2] Rudinger, J., *Radioimmunoassay Methods* (edit. by Kirkham, K. E., and Hunter, W. M.), 379 (Churchill Livingstone, Edinburgh, 1971).
[3] Bangham, D. R., and Cotes, P. M., *Radioimmunoassay Methods* (edit. by Kirkham, K. E., and Hunter, W. M.), 345 (Churchill Livingstone, Edinburgh, 1971).
[4] Freedlender, A., *Radioimmunoassay Methods* (edit. by Kirkham, K. E., and Hunter, W. M.), 387 (Churchill Livingstone, Edinburgh, 1971).
[5] Nielsen, M. D., *Radioimmunoassay Methods* (edit. by Kirkham, K. E., and Hunter, W. M.), 388 (Churchill Livingstone, Edinburgh, 1971).
[6] Riniker, B., and Schwyzer, R., *Helv. Chim. Acta*, **47**, 2357 (1964).
[7] Hollemans, H. J. G., *Clin. Chim. Acta*, **27**, 99 (1970).

Role of Reduced TPN in the Response to Interstitial Cell Stimulating Hormone

DISCUSSING the mechanism of action of interstitial cell stimulating hormone (ICSH), also called luteinizing hormone, on the ovary, Flint and Denton[1] concluded that the "primary steroidogenic response" of rat lutein tissue to ICSH involves no change in the cytoplasmic concentration of TPNH (NADPH). This argument was based on two observations: (i) glucose added *in vitro* to luteal tissue increased TPNH/TPN$^+$ but not steroid synthesis and (ii) ICSH *in vivo* increased steroid synthesis but not TPNH/TPN$^+$. Steroid synthesis was observed to increase by 0.19 μmol/g/h after injection of ICSH. The interpretation proposed reveals a fundamental misunderstanding of the significance of steady state values of TPNH/TPN$^+$ which this letter attempts to clarify.

In steady state conditions the concentration of luteal TPNH (or the ratio TPNH/TPN$^+$) at any one time is the outcome of a balance between production and destruction of TPNH (effectively between the relative rates of oxidation and reduction of the pyridine nucleotide). Clearly, constancy of steady state value of TPNH (or TPNH/TPN$^+$) does not necessarily mean that the rate of reduction of TPN$^+$ remains constant; the only valid conclusion is that the balance between oxidation and reduction has not changed. If the rate of reduction of TPN$^+$ increased two-fold, steady state values of TPNH/TPN$^+$ would not alter, if at the same time, oxidation of TPNH also increased two-fold.

Steroid-forming organs do not maintain high levels of stored biosynthetic intermediates beyond cholesterol, so that trophic stimulation depletes such stores of cholesterol (refs. 3–5 and unpublished observations). The synthesis of 1 mol of gestagen (progesterone and 20α-dihydroprogesterone) requires at least 2 ml. of TPNH to cleave the side chain of cholesterol[2]. It follows that the administration of ICSH has resulted in the formation of at least 2 × 0.19 μmol TPNH/g/h above levels in control tissue (no ICSH). The fact that steady state levels of TPNH/TPN$^+$ are unchanged should not obscure this. At least three possible explanations for increased formation of TPNH without change in TPNH/TPN$^+$ following administration of ICSH can be suggested. (i) ICSH increases the rate of production of TPNH to the level required to accelerate steroid production by 0.19 μmol/g/h; the additional TPNH is oxidized in the process of producing the steroids at an accelerated rate; the ratio TPNH/TPN$^+$ remains unaltered. (ii) ICSH specifically decreases oxidation of TPNH by some means not connected with steroidogenesis and this decrease equals the increase in the rate of utilization of TPNH for accelerated steroidogenesis. The equality of these two rates may reflect some controlled coupling between the two processes concerned. (iii) ICSH stimulates steroid synthesis by a mechanism not involving increased production of TPNH. Steroid synthesis could in turn generate reduction of TPN$^+$ "as it goes", by some unknown mechanism; this coupling between steroid synthesis and the reduction of TPN$^+$ would preserve steady state levels of TPNH. If the experiments reported were not carried out in steady state conditions, the data are more difficult to interpret and thus less informative.

The same considerations apply to interpretation of steady state concentrations of hormones in plasma which do not necessarily reflect the rate of production of the hormones concerned; a certain concentration can be maintained by low production with slow metabolic clearance or by high production with rapid metabolic clearance[6].

The first experimental observation of Flint and Denton[1] tells us that glucose increases TPNH/TPN$^+$ but not steroid synthesis. As they point out, the TPNH required for side chain cleavage of cholesterol must act within mitochondria. Ratios of TPNH/TPN$^+$ for the whole cell tell us nothing about mitochondrial TPNH. There is no reason to believe that enhanced glucose-6-PO$_4$ dehydrogenase activity will automatically generate intramitochondrial TPNH; *prima facie* this possibility seems quite remote. The central dilemma of the Haynes hypothesis to account for the action of ACTH on adrenal steroid synthesis[7] is the difficulty of explaining how high levels of intramitochondrial TPNH can result from increase in cytoplasmic TPNH/TPN$^+$. Simpson and Estabrook[8] suggested that malate may serve to "shuttle" reducing equivalents from cytoplasm to mitochondria. If this is so there are two possible consequences.

(i) If ICSH acts by providing TPNH for side chain cleavage of cholesterol, total cell levels of TPNH need not change after administration of ICSH, because intramitochondrial TPNH would increase at the expense of cytoplasmic TPNH. The additional reducing equivalents would be used within the mitochondria as fast as they were transported there from the cytoplasm.

(ii) Increasing cytoplasmic TPNH/TPN$^+$ by addition of glucose or by enhanced activity of glucose-6-phosphate dehydrogenase, will not *per se* increase steroidogenesis, unless the production or transport of malate is also increased to convey the extra reducing equivalents into mitochondria. The two observations of Flint and Denton can therefore be readily explained, even if ICSH stimulates steroid synthesis only by increasing reduction of TPN$^+$.

The misunderstanding revealed in the proposed interpretation of these data arises from the idea that if stimulation of steroid synthesis results from increase in TPNH/TPN$^+$, the cell establishes this stimulation by first developing a head of reductive energy followed by increased output of steroids. It could equally well be that steroid synthesis is poised ready for any increase in TPNH; reduced pyridine nucleotide would be formed more rapidly but would not accumulate. One important result of a systems approach to the control of steroid synthesis has been the demonstration of rapid return of steroidogenesis to control levels when trophic stimulation is withdrawn[9]. The sort of lag expected from the "running down" of accumulated high levels of TPNH is not observed.

Even if the cell sets up a high ratio of TPNH/TPN$^+$ which then drives steroid synthesis at an accelerated rate, it seems likely that the high levels of TPNH would soon be consumed. High levels of TPNH may not persist for 1 h after injection of ICSH.

The importance of increased production of TPNH in explaining the mechanism of action of ICSH will only become clear when we know more about the intracellular compartmentalization of pyridine nucleotides and when we can monitor continuously the redox state of TPN. TPNH may or may not be the fundamental mediator or the action of ICSH, but negative data on steady state values of TPNH/TPN$^+$ at a single time interval do not facilitate evaluation of this concept.

PETER F. HALL

Department of Biochemistry,
University of Melbourne,
Parkville,
Victoria

Received March 15; revised June 9, 1971.

[1] Flint, A. P. F., and Denton, R. M., *Nature*, **228**, 376 (1970).
[2] Shimizu, K., Gut, M., and Dorfman, R. I., *J. Biol. Chem.*, **237**, 699 (1962).
[3] Long, C. N. H., *Rec. Prog. Hormone Res.*, **1**, 99 (1947).
[4] Hall, P. F., *Biochemistry*, **2**, 1232, (1963).
[5] Bell, E. T., Makerji, S., and Loraine, J. A., *J. Endocrinol.*, **28**, 321 (1964).
[6] Eik-Nes, K. B., and Hall, P. F., *Vitamins and Hormones*, **23**, 153 (1965).
[7] Haynes, R. C., *J. Biol. Chem.*, **233**, 1220 (1958).
[8] Simpson, E. R., and Estabrook, R. W., *Archiv. Biochem. Biophys.*, **129**, 384 (1969).
[9] Urquhart, J., and Li, C. C., *Amer. J. Physiol.*, **214**, 73 (1968).

Chromosomes of Cancer Cells

THE reported[1] alterations in the malignant potential of a virus transformed mouse fibroblast cell line and its relationship to variations in the chromosome number seems to have a parallel in the human cervix. Our studies of the chromosome patterns seen in pre-invasive and invasive carcinomas of the uterine cervix suggest that these patterns may be an indicator of the invasive potential of the pre-invasive lesion[2]. We have proposed a theory in which we contend that the tetraploid and hypertetraploid pre-invasive lesions are less likely to develop into invasive carcinoma and that a potentially invasive lesion will have a peri-diploid component in its cytogenetic profile[3].

In human cervical cancer the differences in chromosome number may be related to the nuclear size of the component cells. Small, mixed and large cell lesions occur in both types of neoplasm[2]. It seems from our data that large cell pre-invasive lesions are cytogenetically distinct from large cell invasive carcinomas, the latter having a mean count of seventy-one compared with seventy-nine for pre-invasive cancer.

In both pre-invasive and invasive carcinoma of the cervix there is a wide distribution of chromosome number which cannot be described fully by the median[4]. We devised a statistical model to describe the total chromosome number distribution within individual lesions[3]. The use of the mean and variance to describe the component populations of the individual lesions shows a change in pattern which seems to occur between the pre-invasive lesions and invasive lesions. Pre-invasive lesions with chromosome counts in the tetraploid, quintaploid and hexaploid ranges were not represented in the invasive cancers and we suggested that a peri-diploid or peri-triploid dominance was required before invasion could take place[3]. Few examples of progression from pre-invasive to invasive cancer have been observed in human material, but an example of progression to micro-invasion with the appearance of a peri-diploid chromosome complement has been reported[5]. Present studies in this laboratory also seem to indicate that the sensitivity of invasive carcinoma of the cervix to radiotherapy may be related to variations in the chromosome constitution.

Overall, the comparison of the patterns in pre-invasive and invasive cancer of the cervix suggests that hypertetraploidy is associated with a low degree of malignancy. It seems possible, therefore, that these previously extraordinary variations in chromosome constitution are related to the progression of the individual neoplasm, but only when the entire chromosome complement is considered. The changes in the chromosome pattern which have been reported in a virus transformed cell line seem to have a clinical counterpart, particularly when the total cytogenetic data for cervical neoplasia are considered. It is also of interest that a virus (Herpes type II) has been implicated as an oncogenic agent in the human cervix[6].

J. A. KIRKLAND
M. A. STANLEY

Department of Obstetrics and Gynaecology,
Queen Elizabeth Hospital,
Woodville,
South Australia

Received March 16, 1971.

[1] Pollack, R., Wolman, S., and Vogel, A., *Nature*, **228**, 938 (1970).
[2] Kirkland, J. A., Stanley, M. A., and Cellier, K. M., *Cancer*, **20**, 1934 (1967).
[3] Cellier, K. M., Kirkland, J. A., and Stanley, M. A., *J. Nat. Cancer Inst.*, **44**, 1221 (1970).
[4] Kirkland, J. A., *Obstet. Gynecol. Survey*, **24**, 784 (1969).
[5] Stanley, M. A., and Kirkland, J. A., *Acta Cytol.*, **13**, 76 (1969).
[6] Rawls, W. E., Tompkins, W. A. F., Figueroa, M. E., and Melnick, J. L., *Science*, **161**, 1255 (1968).

Collection of Lymph from Kidneys Homotransplanted in Man: Cell Transformation *in vivo*

ALTHOUGH there have been careful studies[1-3] of the successive steps of lymphocyte sensitization by histocompatibility antigens *in vitro*, it has not yet been demonstrated directly that the same cell transformation takes place *in vivo* after a human organ allograft.

The practical problem is to devise a method of collecting a representative sample of lymph from such a transplanted organ. The lymph formed in the merino sheep kidney is drained entirely by a small number of hilar lymphatic ducts, so that cannulating one of these vessels and tying off the others allows the collection of the entire renal lymph[4]. In most other animals, including man, this is not however possible, because the renal lymphatic ducts are dispersed and not concentrated in the hilum. We have, nevertheless, succeeded in cannulating one of the renal lymphatic ducts of kidneys homotransplanted in dogs and in man. This communication concerns our attempts in man.

One way to make visible the human lymphatic ducts is to inject 1 ml. of Evans blue into the renal cortex. Three minutes later, some lymph ducts are seen on the exterior aspects of renal vessels. A ligature of vascular silk can be tied around this duct to serve as a label. Lymphatic ducts are extremely difficult to see in the ischaemic kidney, but we find they become clearly visible 10 or 15 min after transplantation. A polyethylene cannula (interior diameter 0.35 mm) filled with a saline solution of heparin can then be introduced into the duct and fixed tightly with a vascular nylon ligature. The catheter is led out through the abdominal incision.

In four recipients of kidney allografts, renal lymph collection was possible for 7, 5, 10 and 8 days after transplantation respectively. Lymphocyte outflow was measured daily. A differential count of normal lymphocytes and other cells, if any were present, was performed. Cells were studied by light and electron microscope. Tritiated thymidine incorporation was measured daily in the lymph and every second day in the blood with a scintillation counter, using 1 µCi/10^6 lymphocytes. Cellular radioautographies were performed daily.

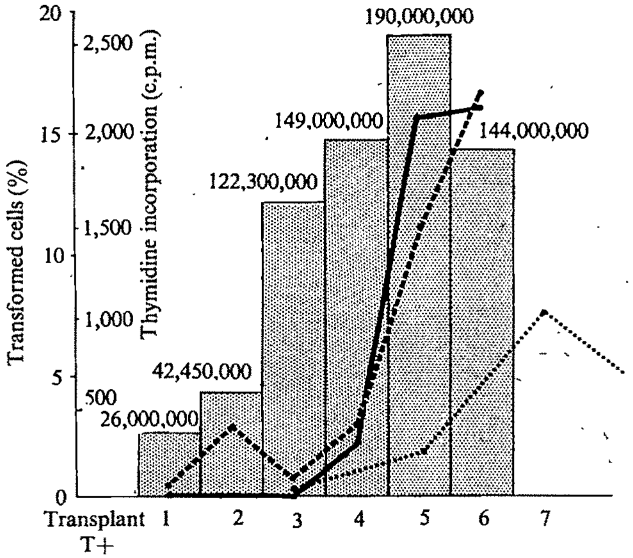


Fig. 1 Lymphocyte flow, percentage of lymph cell transformation and tritiated thymidine incorporation in lymph and in blood, following lymphatic duct drainage of an allografted kidney in man (case 1). Shaded sections, lymphocyte flow (cells/24 h); ● - - - ●, thymidine incorporation in lymph; ●—●, percentage of transformed cells; ● · · · ●, thymidine incorporation in blood.

The results in case 1 are shown in Fig. 1. This 29 yr old patient was given a kidney transplant taken from a cadaver 146 min after death. Renal ischaemia resulted in a 6 day oliguric renal failure followed by a progressive recovery. The patient received 300 mg of azathioprine, 5 ml. of antilymphocyte serum and 24 mg of betamethasone phosphate per day. The collected lymph contained 96–99% of small lymphocytes during the first 5 days. The total lymphocyte flow was successively 26 million, 42 million, 122 million, 149 million, 190 million and 144 million cells per 24 h. On days 5 and 6 there was a sudden appearance of transformed cells identical to the immunoblasts observed in mixed lymphocyte cultures and reaching 16% of the total cells. At the same time, the tritiated thymidine incorporation measurement rose from 91 c.p.m. on day 3 to 2,258 c.p.m. on day 6. Radioautographies showed a similar elevation of labelled cells. Tritiated thymidine incorporation into blood leucocytes also increased, but more slowly than in the lymph; after a peak on day 8 it decreased regularly. Coagulation stopped the lymph flow on day 7 and the catheter was removed. Case 2, in which the transplanted kidney was also taken from a cadaver, gave results similar to those observed in case 1, but the lymph flow ceased on day 5.

In cases 3 and 4 the kidney was taken from a living related donor. In case 3 (transplantation between siblings) a marked *in vitro* cell transformation (23% transformed cells) had been found in the mixed lymphocyte culture of donor and recipient cells before transplantation; the *in vivo* examination of the renal graft lymph showed essentially similar results to those found in case 1. In case 4 (parent to child transplantation) the mixed lymphocyte culture had shown no stimulation (1.6% transformed cells) and no cell transformation was found *in vivo* in the allograft lymph; neither was there any increase in thymidine incorporation. The clinical course in the latter case was remarkably uneventful.

These *in vivo* findings reinforce the suggestion that mixed lymphocyte culture may be considered "a model for allograft rejection"[5]. The similarity between the cell transformation taking place in the recipient of a human renal allograft and the cell transformation found in the *in vitro* mixed lymphocyte culture is striking. This has practical as well as theoretical importance because it suggests the immunological reaction may be studied and followed for several days in patients receiving a kidney allograft by using a daily collection of the graft lymph.

This may provide a new tool for recognizing and measuring the recipient sensitization, even in cases where the clinical signs of "rejection" are lacking or ambiguous. It also opens a new field for the *in vivo* collection and study of sensitized cells in human organ transplantation.

J. HAMBURGER
A. DIMITRIU
L. BANKIR
M. DEBRAY-SACHS
J. AUVERT

Hôpital Necker,
Paris XV^e

Received October 22, 1970.

[1] Bach, F. H., Bock, H., Graupner, K., Day, E., and Klosterman, H., *Proc. US Nat. Acad. Sci.*, **62**, 377 (1969).
[2] Bain, B., *Clin. Exp. Immunol.*, **6**, 255 (1970).
[3] Hirschhorn, K., in *Human Transplantation* (edit. by Rapaport, F. T., and Dausset, J.), 406 (Grune and Stratton, New York, 1968).
[4] Pedersen, N. C., and Morris, B., *J. Exp. Med.*, **131**, 936 (1970).
[5] Hävry, P., and Defendi, V., *Science*, **168**, 133 (1970).

Effect of Thalidomide on Human Embryonic Tissues

WE wish to report results which can be used in an attempt to define the level of action of thalidomide on human embryonic tissues *in vitro*. Because it is difficult to obtain human embryonic tissues in quantities sufficient for biochemical measurements, these results are based on the morphology and histology of cultured human tissues.

The starting point for these studies was the observation[1] that the mesonephros of 2–3 day old chick embryos undergo chondrogenesis if cultured on nutrient agar, but not if it is wrapped in chorioallantoic membrane (compare ref. 2). The chondrogenic potential of the mesonephros adjacent to the appendages was greater than that of the intervening region[2]. Thus chondrogenesis seemed to be related to girdle or appendage cartilage. Indeed, chondrogenesis is enhanced in 2–3 day limb buds cultured in association with the adjacent mesonephros, but not in buds from older embryos[3].

An investigation of the effects of thalidomide on mesonephros–limb bud chondrogenesis in cultured chick tissues has shown that thalidomide inhibited mesonephric chondrogenesis, whereas 'Doriden' (a non-teratogenic analogue of thalidomide) had no effect. The inhibitory effect was clearly on the mesonephric tissue, not the limb bud tissue[3].

Like most teratogenic agents, thalidomide has its greatest effect during a restricted period of development, with the human limb this occurs during the second month of pregnancy[4].

We obtained human embryos from the Boije Clinic, Helsinki, and dissected out somites from the posterior limb region; presumptive girdle tissue from the same region; ventral spinal cord and notochord; limb mesenchyme, and the nephric tissue complex (mesonephros, metanephric bud and metanephric mesenchyme). These were cultured on nutrient agar (1% agar containing Simm's balanced salt solution (SBSS), foetal calf serum (FCS) and Coon's modification of Ham's F12 (F12X)), in the ratios 2 : 2 : 1. Foetal calf serum and F12X were obtained from Grand Island Biological Co. The liquid feeding medium used was SBSS : FCS : F12X, (2 : 2 : 1) with no supplement, with thalidomide (obtained from W. S. Merrell before court litigation began), or with 'Doriden' (Ciba).

Thalidomide and 'Doriden' were used in a concentration of 0.8 mg/100 ml. The nutrient medium was too dense to allow spectrophotometric measurements of the thalidomide solution to check the concentration after the culture period. The half life of thalidomide *in vivo*, or at *p*H 7.4 at 37° C is reported to be 2–3 h (ref. 5 and personal communication from Schumacher *et al.*). The maximum aqueous solubility of thalidomide is 20–30 µg/ml.

After feeding with the liquid nutrient medium, tissues were covered with mineral oil ('Klearol'), and cultured at 37° C in 5% CO_2 : 95% air, in a humidified incubator. Cultures were kept for 3 days, when cartilage was visible in the controls, and then fixed in acetic acid : absolute ethanol (1 : 3).

The cultures were: (*a*) mesonephros; (*b*) mesonephros plus adjacent limb bud; (*c*) limb buds; (*d*) metanephric mesenchyme; (*e*) metanephric bud; (*f*) metanephric bud recombined with metanephric mesenchyme; (*g*) embryonic somites, and (*h*) embryonic somites plus a section of the ventral spinal cord or of the notochord. Although many cells died in older cultures, there was enough viable tissue in 3 day cultures to ascertain tissue differentiation.

Metanephric mesenchyme from 6 week old embryos formed tubules only if cultured in association with the ureteric bud, which corroborates Grobstein's findings[6] with mouse embryos. Embryonic somites from 6–7 week old embryos formed small amounts of cartilage if cultured alone, but significantly more in association with the embryonic spinal cord or notochord. These results agree with findings using embryonic chick tissues[7].

Cultures of mesonephros from 6–8 week embryos consistently formed small amounts of cartilage matrix, as shown by staining with Alcian blue, which is diagnostic for glycosaminoglycans. Six experiments were performed with embryos between 6 and 8 weeks old, as judged by size, or the stage of development of tissue fragments found in the debris. The mesonephros was dissected free of adhering tissues, and one side was cut into 1 mm³ pieces and cultured in the presence of thalidomide. The other half was similarly dissected, and cultured either in plain nutrient medium, or with 'Doriden'.

Table 1 Qualitative Scoring for Cartilage Matrix in Cultures of Human Mesonephros

Case No.	Age (weeks)	Treatment	Matrix scoring Range	Matrix scoring Cumulative sum
056–5	6+	Thal.	2–3	7
056–7	6+	'Doriden'	3–5	14
056–6	6+	Thal.	2–3	6
056–8	6+	'Doriden'	3–4	11
060–3	7+	Thal.	1–2	4
060–4	7+	'Doriden'	2–3	8
073–1	8+	Thal.	1–3	5
073–2	8+	'Doriden'	1–2	4

The range represents the minimum and maximum values for the three parameters used (amount, density and maturation of matrix). The cumulative sum represents the total for all three parameters. Scoring was from 1 (minimum) to 5 (maximum). The tissues were dissected from 6–8 week old human embryos and cultured on F12X nutrient agar for 3 days. Thalidomide and 'Doriden' were at a concentration of 0.8 mg/100 ml. of medium.

The results are shown in Table 1 and Fig. 1. Cartilage matrix was scored on histological slides, and the following assay procedures were used. The tissue sections stained with Alcian blue were examined independently and "blind" by four scientists and scored on a scale from 1 to 5 with respect to the relative amount of cartilage matrix (that is Alcian blue positive material), the density and the maturation of the matrix. The lowest values represent the least chondrogenic differentiation, and the cumulative sum represents the total of the three parameters (amount, density, maturation) indicating the degree of chondrogenesis. Table 1 shows that chondrogenic inhibition by thalidomide was most noticeable on the younger embryos (6–7 weeks old). The older the mesonephros, the less cartilage formed and the difference between the experi-

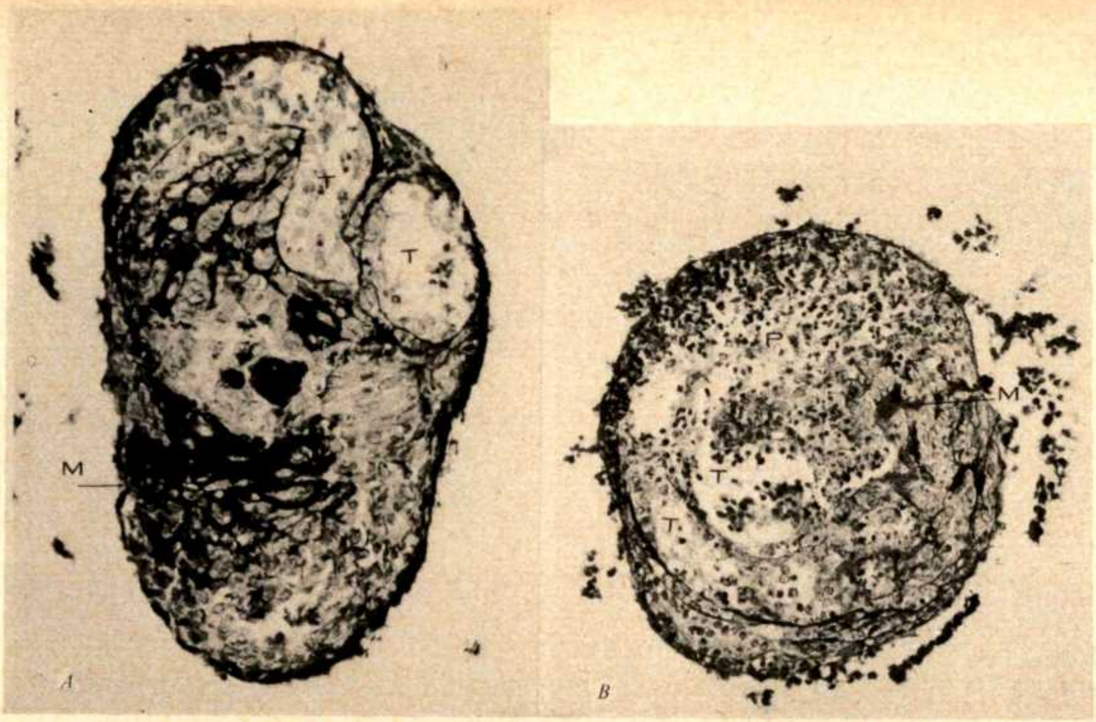


Fig. 1 *A*, Cultured human mesonephros from a 6 week old human embryo treated with 'Doriden'. The matrix (M) appears black in the photograph and tubules (T) are evident. (Alcian blue × 200.) *B*, Contralateral mesonephros from the same embryo, treated with thalidomide. The matrix is much less prominent, and the tubules are still evident. Pycnosis (P) is visible. (Alcian blue × 100.)

mentals (thalidomide) and the controls ('Doriden') was negligible.

Thus tissue associated with the human mesonephros at an early stage of development (6–7 weeks of age) seems to form cartilage in tissue culture. As the embryo ages, the amount of cartilage diminishes. The scoring for cartilage matrix in 6 week old tissues cultured in 'Doriden' ranged from 3 to 5 for the three parameters, with a cumulative sum of 14 (Table 1). For 8 week old tissues the corresponding values were 1–2 and 4, indicating significantly less matrix. These results agree well with the results obtained with chick mesonephros[3]. In addition, the tissues treated with thalidomide showed a significant decrease in matrix formation in 6 week old tissues, a similar decrease in 7 week old tissues, but no difference in 8 week old tissues. This again is similar to chick mesonephros[3].

When human limb buds from these embryos were grown in culture, cell death was more rampant, but nevertheless cartilage formed and there was no noticeable effect of thalidomide. None of the other chondrogenic tissues were inhibited.

These results suggest that a major site of action of thalidomide on limb-related chondrogenesis in human embryos is tissue associated with the embryonic mesonephros at a certain stage of development.

This work was supported by awards from the Lalor Foundation and the Paulo Foundation.

James W. Lash
Lauri Saxén

Department of Anatomy,
School of Medicine,
University of Pennsylvania,
Philadelphia, Pennsylvania 19104, *and*
III Department of Pathology,
University of Helsinki,
*Helsinki SF*00290

Received March 16; revised May 12, 1971.

[1] Lash, J. W., *Dev. Biol.*, **6**, 219 (1963).
[2] Strudel, G., and Pinot, M., *Dev. Biol.*, **11**, 284 (1965).
[3] Lash, J. W., *Amer. J. Obstet. Gynec.*, **90**, 1193 (1964).
[4] Nowack, E., *Humangenetik*, **1**, 516 (1965).
[5] Schumacher, H., Blake, D. A., and Gillette, J. R., *J. Pharmacol. Exp. Ther.*, **160**, 201 (1968).
[6] Grobstein, C., *J. Exp. Zool.*, **130**, 319 (1955).
[7] Lash, J. W., *J. Exp. Zool.*, **165**, 47 (1967).

Rabbit Blastocoele Perfusion Technique

Trophoblast cells must play a significant role in the movement of solute and water into the blastocoele[1]. The epithelial trophoblast (Fig. 1*A*), exposed on one side to the uterine environment and on the other to the blastocoele fluid, appears structurally and functionally similar to other solute and water transporting organs with epithelial cells which separate the blood stream from body cavities[2]. There is evidence for active ionic transport across the rabbit trophoblast[2–5], perhaps in connexion with the accumulation of blastocoele fluid[2,4].

The active transport components of the trophoblast membrane could be studied by techniques which have been applied successfully to various epithelial membranes, if the composition of the blastocoele fluid could be controlled. We have therefore investigated this problem. My first approach was to form a flat sheet of cells out of the blastocyst which could be placed between two fluid chambers, similar to the method used to study frog skin epithelium. My second method was to perfuse the blastocoele cavity using glass pipettes. Perfusion methods have been developed for various tissues, such as the squid giant axon[6], marine algae[7], and isolated renal tubules[8]. After many attempts at both approaches, the latter proved to be the better solution. I now describe a successful method and report electrical parameters of the 6 day rabbit blastocyst under continuous-flow perfusion.

Blastocysts were flushed from the uteri of naturally mated New Zealand rabbits. One of them (2–3 mm, average diameter) was secured in a special environmental chamber[4] and glass perfusion pipettes (40–60 μm tip diameter) were inserted into

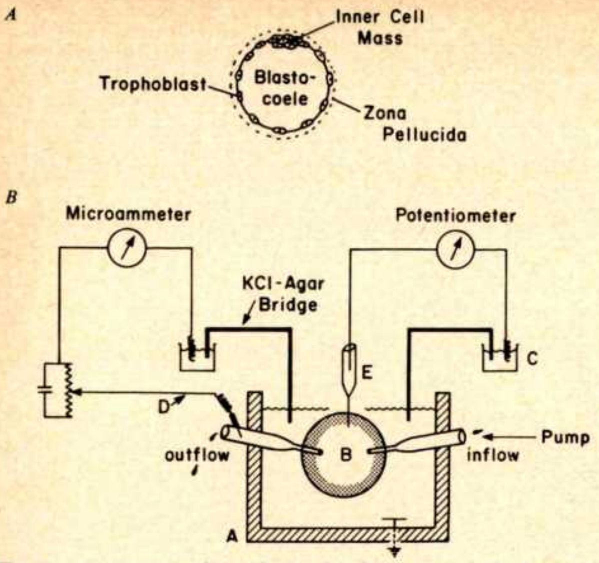


Fig. 1 *A*, Cross section of a 6 day blastocyst. *B*, Assembly for internal perfusion and electrical measurements of the blastocyst. A, Chamber used to secure embryo and maintain constant temperature, humidity, and gas phase (5% CO_2 in air); B, blastocyst; C, Ag-AgCl-KCl half cell; D, Ag-AgCl wire used for applying outside EMF; E, microelectrode.

opposite sides of the blastocyst. All manipulations were carried out using micromanipulators under a dissecting microscope.

The two chief difficulties in the perfusion of the blastocoele are: (1) how to penetrate the zona pellucida and trophoblast with pipettes which are both large enough in tip diameter to allow relatively low resistance to perfusion flow, and small enough not to cause the embryo to collapse when penetrated; (2) how to prevent leaks at the junction between the trophoblast and the pipette.

The solution was first to penetrate the tough inert zona pellucida and the trophoblast layer with a micropipette (tip diameter <1 µm), and then advance the micropipette into the blastocoele cavity until the shank of the micropipette was of similar diameter to that of the inflow pipette to be used. The micropipette was then withdrawn, leaving a small wound in the trophoblast wall and a small opening in the zona. The inflow pipette, filled with medium, connected to the inflow reservoir and clamped, was then inserted through the opening in the zona and the wound in the trophoblast without collapse of the blastocyst and with very little loss of blastocoele fluid. Using the same procedure, the outflow pipette, filled and connected to the outflow reservoir, was inserted on the opposite side. Any fluid loss from the blastocoele could now be replaced by opening the input side of the perfusion system and pumping in fluid while maintaining the output side closed. Once the perfusion pipettes were in place, a microelectrode was inserted into the blastocoele cavity for electrical measurements. The trophoblast epithelium was then allowed to seal around the three inserted pipettes (Fig. 2).

Surprisingly, the trophoblast epithelium adhered naturally to the glass pipettes forming a tight seal. By regulating the inflow perfusion rate by adjusting the pumping speed and the outflow rate by regulating the height of the outflow reservoir in relation to the chamber, the blastocyst could be maintained at its original volume without leakage around the trophoblast-pipette junction. By regulating inflow and outflow rates in this manner, the blastocoele contents could all be removed so that the cavity collapsed, and then replaced almost at will. Leaks around the pipettes could be detected microscopically and electrically. If perfusion pressure were increased, the blastocoele cavity expanded, causing the junction between the trophoblast and the pipette to be broken and the potential to decrease rapidly to near zero. Finally, to assess the possible leakage of the perfused blastocyst, ^{14}C-sucrose was added to the inflow reservoir and the outside solution was tested for the appearance of radioactivity. In sixteen collections in four preparations no activity was detected in the bathing solution, showing that a sensitive perfusion control system eliminated leakage problems. The perfusion procedure was started by quickly replacing the contents of the blastocoele with perfusion solution, after which a constant perfusion rate was maintained. Flow rates averaged between 2 and 3 µl./min. In a blastocyst of 2.5 mm average diameter there would be a complete exchange of solution about every 3–4 min.

Table 1 Electrical Properties of the Perfused Rabbit Blastocyst

Blastocyst	Time (min)	E_t* (mV)	I_{sc} (µA/cm²)	R (Ω cm²)
1	210	−6.2	5.2	1,204
2	70	−4.4	2.9	1,509
3	90	−4.8	2.8	1,783
4	150	−4.4	3.8	1,237
5	70	−5.7	3.0	2,218
6	110	−4.3	3.6	1,824
7	90	−6.6	6.5	1,099
8	180	−8.0		
9	180	−9.7		
10	180	−5.7		
11	90	−8.2		
12	130	−4.1		
Mean ± s.e.		−6.0 ± 0.52	4.0 ± 0.52	1,553 ± 154

* Blastocoele cavity negative with respect to external medium.

E_t and I_{sc} were recorded at 10 min intervals throughout each experiment.

The potential difference (E_t) across the trophoblast epithelium, short-circuit current (I_{sc}) and resistance (R) are given along with the time during which E_t and I_{sc} remained relatively constant. The perfusion and bathing medium were Eagle's containing 20% calf serum.

When experience with the technique permitted a high frequency of successful perfusion, the electrical properties of the epithelial trophoblast were studied. The potential difference (E_t) and short-circuit current (I_{sc}) across the trophoblast wall were measured as described by Ussing and Zerahn[9] with the circuit shown in Fig. 1*B*. Eagle's medium containing 20% calf serum was used for the perfusion and outside bathing media. The trophoblast membrane behaved as a simple linear resistor, and so resistance measurements were computed from the ratio of open-circuit potential to short-circuit current (Fig. 3). The resistance of the bathing solution measured without the blastocyst in position was always less than 40 Ωcm.

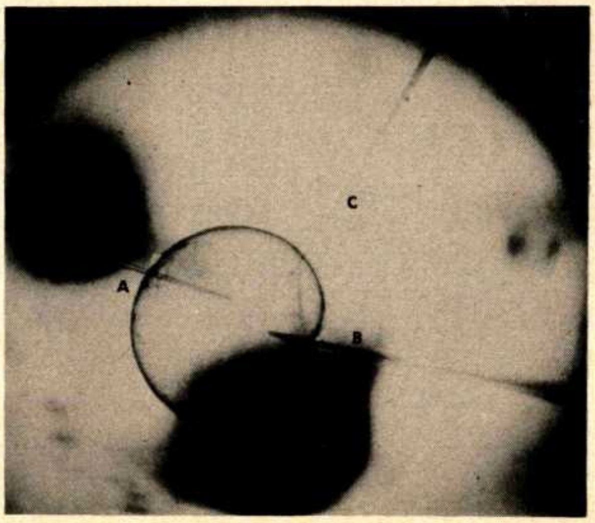


Fig. 2 Perfusion of a 6 day rabbit blastocyst during measurement of the electrical properties of the trophoblast wall. A, Inflow perfusion pipette; B, outflow pipette; C, microelectrode. The two dark spots in the photograph are caused by two gaskets at the bottom of environmental chamber.

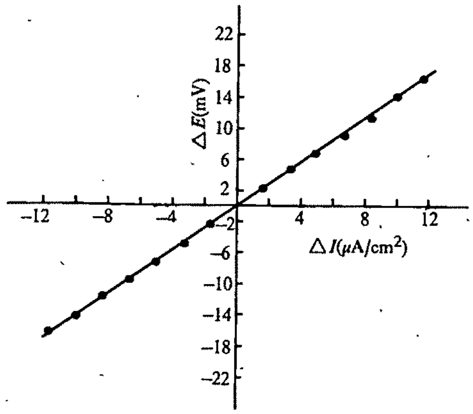


Fig. 3 A typical current-voltage graph for a perfused rabbit blastocyst. The origin of potential is −4.4 mV. Negative values of ΔE represent hyperpolarized potentials, produced by current passing inward across the trophoblast.

Table 1 shows the electrical properties of continuously perfused rabbit blastocysts. The average potential across the epithelial trophoblast (±s.e. of the mean), of twelve blastocysts under continuous perfusion with identical solutions on both sides, was −6 ±0.52 mV, whereas the value for twenty-three non-perfused embryos was −11.1±0.79 mV. The average specific membrane resistance of seven blastocysts measured under continuous perfusion was 1,553±154 Ωcm² and compares with 2,188±202 Ωcm² averaged from fifteen non-perfused embryos. In many cases, a non-perfused and a perfused blastocyst were obtained from the same rabbit. The average short-circuit current recorded in seven perfused blastocysts was 4±0.52 μAmp/cm² or about 0.15 μequiv./cm²/h. When both sides of an epithelial membrane are bathed with identical solutions, the short-circuit current represents the net active movement of ions across that membrane[9]. The time during which both E_t and I_{sc} remained relatively constant averaged 129 min lasting 70–90 min in five embryos and 110–210 min in seven others (Table 1). Some embryos may have maintained steady electrical properties longer than the times shown (180–210 min) for these experiments were arbitrarily terminated. Three blastocysts, which had their zonae pellucidae mechanically removed before perfusion, showed electrical properties similar to those with intact zonae. A technique has therefore been developed which will permit the study of transport phenomena across a mammalian embryonic membrane, using methods which are well documented for other epithelial membranes.

This research was supported by a grant from the National Institutes of Health, the Center for Population Research, and the National Institute for Child Health and Human Development.

MORTON H. CROSS

Laboratory of Reproductive Physiology,
School of Veterinary Medicine,
University of Pennsylvania,
Philadelphia,
Pennsylvania 19104

Received November 12, 1970; revised March 15, 1971.

[1] Lutwak-Mann, C., Bourswell, J. C., and Bennett, J. P., *J. Reprod. Fertil.*, **1**, 169 (1960).
[2] Gamow, E., and Daniel, J. C., *Wilhelm Roux Archiv.*, **164**, 261 (1970).
[3] Cross, M. H., and Brinster, R. L., *Exp. Cell Res.*, **58**, 125 (1969).
[4] Cross, M. H., and Brinster, R. L., *Exp. Cell Res.*, **62**, 303 (1970).
[5] Smith, M. W., *Experientia*, **26**, 736 (1970).
[6] Tasaki, T., *Amer. J. Physiol.*, **200**, 11 (1961).
[7] Gutknecht, J., *J. Gen. Physiol.*, **50**, 1821 (1967).
[8] Burg, M., Grantham, J., Abramow, M., and Orloff, J., *Amer. J. Physiol.*, **210**, 1293 (1966).
[9] Ussing, H., and Zerahn, K., *Acta Physiol. Scand.*, **23**, 110 (1951).

Limited Synergism between Rat Thymus and Mouse Bone Marrow

IN a number of experimental systems[1] it has been shown that thymus and bone marrow-derived cells can cooperate in the production of humoral antibodies. In some instances this synergism has been demonstrated when the interacting cells are antigenically similar but in other experiments allogeneic cells have been found to cooperate. We have now demonstrated cooperation between rat and mouse cells. A two-step experiment was performed, following the design of Miller[2]. Rat or mouse thymus cells were introduced with antigen into irradiated recipients. The donor cells were collected and injected into secondary host mice. The details of these experiments and their results are as follows.

Twenty adult RK mice, 10–12 weeks old, were given 900 r. of total body irradiation from a ^{60}Co source. Ten were injected with 2×10^7 mouse thymus cells plus 0.1 ml. of SRBC at 20% (group 1), and ten were injected with 2×10^7 rat thymus cells plus 0.1 ml. of SRBC at 20% (group 2).

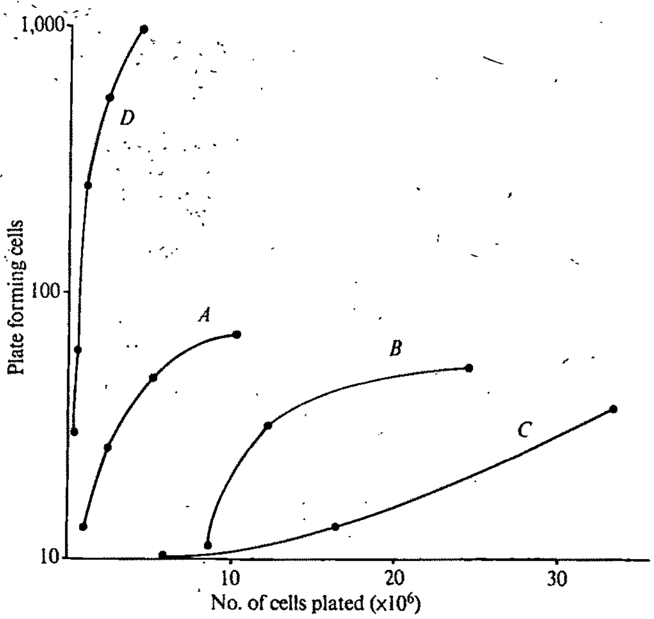


Fig. 1 Effect of mouse and rat thymus on the immunocompetence of mouse bone marrow cells (see text). Group *A* lethally irradiated mice repopulated with mouse thymus and mouse bone marrow cells; group *B*, lethally irradiated mice repopulated with rat thymus and mouse bone marrow cells; group *C*, lethally irradiated mice repopulated with mouse bone marrow cells; group *D*, unirradiated control mice given only SRBC.

Five days later another twelve RK mice, 10–12 weeks old, were given total body irradiation, and then three were injected with $1\cdot5\times10^6$ cells from group 1 pooled spleens plus 2×10^7 mice bone marrow cells and 0.1 ml. of SRBC at 20% (group *A*), three were injected with 1.5×10^6 cells from group 2 pooled spleens plus 2×10^7 mice bone marrow cells and 0.1 ml. of SRBC at 20% (group *B*), and three were injected with 2×10^7 mice bone marrow cells and 0.1 ml. of SRBC at 20% (group *C*). As controls three irradiated and three non-irradiated mice (group *D*) were immunized with 0.1 ml. of SRBC at 20%.

Six days later we counted plaque forming cells against SRBC—of pooled spleens of the different groups—by the Jerne technique[3]. All cellular suspensions were prepared in cold phosphate buffer saline, P 7.4, and injected intravenously within 3 h of the irradiation.

Fig. 1 shows that rat and mouse cells can cooperate, although not as efficiently as mouse–mouse combinations. The mechanism of cooperation is not understood but if it involves recognition of organ specific surface antigens it seems that these may be recognized across genetic barriers.

FERNANDO MORGADO
HUGO FOLCH

Department of Experimental Medicine,
Austral University of Chile,
Box 867, Valdivia

Received March 25; revised May 5, 1971.

[1] Claman, H. N., and Chaperon, E. A., *Transplant. Rev.*, **1**, 92 (1969).
[2] Miller, J. F. A. P., and Mitchell, G. F., *Transplant. Rev.*, **1**, 3 (1969).
[3] Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).

Cytotoxic Antibody for Epithelial Cells in Human Graft versus Host Disease

THE transplantation of immunocompetent cells into an immunologically suppressed individual frequently results in a syndrome believed to be due to an immune reaction of the grafted cells against the host. In man this graft versus host (GVH) reaction prominently involves the skin, gastro-intestinal tract and liver, and is characterized pathologically by lymphoid cell infiltrates, basal cell layer destruction and dyskeratosis[1]. The mechanism by which the GVH reaction damages epithelial structures has not been studied in man. Streilein and Billingham have studied a GVH-like reaction prominently involving the skin in hamsters and have made a number of observations pertinent to the mechanism of the GVH pathology[2,3]. One of their observations, the finding of a cytotoxic factor against hamster and rat epidermal cells in the serum of hamsters with GVH-like skin lesions stimulated us to investigate this in man. We have found in human patients with GVH an IgM antibody which is cytotoxic for epithelial but not lymphoid cells.

We studied sera from recipients of bone marrow transplants being cared for on the Leukemia Service of the National Cancer Institute. Of eight patients who underwent bone marrow transplants from their HL-A identical siblings, four developed clinical manifestations compatible with GVH, and in three of these the diagnosis was confirmed pathologically[4–6]. Sera were tested for cytotoxicity using a $^{51}\mathrm{Cr}$ cytotoxicity technique[7]. Target cells labelled with $^{51}\mathrm{Cr}$ and serum were incubated for 10 min before addition of rabbit complement, and then for 90 min at room temperature. A given serum was considered to be cytotoxic if it caused a minimum of 15% more $^{51}\mathrm{Cr}$ release from the target cells than normal human serum. The target cells used were monolayer cultures of HeLa and Hep-2 cells, both epithelial cell lines derived from a squamous cell carcinoma of the cervix and a squamous cell carcinoma of the larynx respectively. Lymphoid target cells were from lymphoid cell lines maintained in long term culture.

Reactivity of one patient's serum with epithelial and lymphoid cells at various intervals after transplantation is shown in Fig. 1. Pretransplant serum contained no cytotoxicity against either lymphoid or epithelial cell lines. During the third week after grafting, however, his serum contained cytotoxicity against the epithelial cells. No cytotoxic reactivity against lymphoid cells was present. The appearance of epithelial cytotoxicity coincided with the development of a rash, diarrhoea, jaundice and desquamation, all of which seemed compatible with GVH reaction. A skin biopsy on the twentieth day after the transplant as well as autopsy findings in skin, liver and intestine on the 32nd day were all compatible with GVH disease.

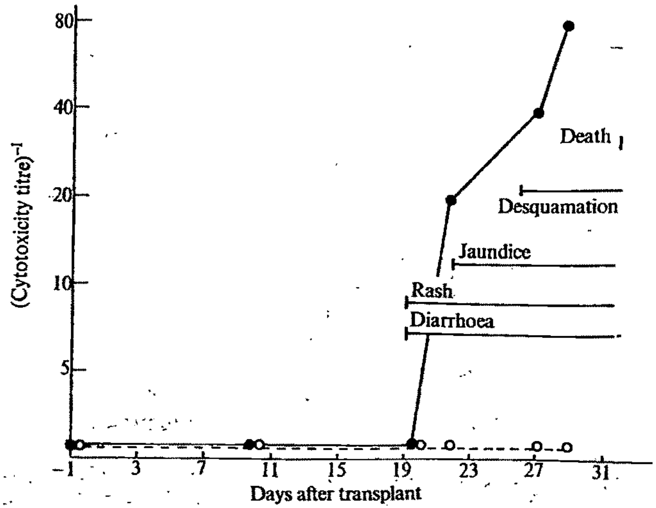


Fig. 1 Cytotoxic activity of serum from a patient with GVH following bone marrow transplantation. The solid line shows reactivity against epithelial (HeLa and Hep-2) cells; the broken line reactivity against lymphoid cells. The onset of each manifestation of GVH is also shown.

Two further patients had clinical evidence of GVH which was confirmed pathologically, and a third had mild symptoms compatible with GVH, although the diagnosis was not confirmed pathologically. In each of these patients, serum cytotoxicity against epithelial but not lymphoid cells appeared coincident with the development of rash and other manifestations of GVH. In contrast with sera from patients with GVH, sera from four transplant recipients in whom there was graft take but no GVH were not cytotoxic for epithelial cells. Similarly, sera from fifteen patients with acute lymphocytic leukaemia who did not receive marrow transplants, and sera from fifteen normal individuals were not cytotoxic for epithelial cells (Table 1).

Table 1 Antiepithelial Activity of Sera from Patients with GVH

Epithelial cell antibody (No. positive/No. tested)	Patient group studied
3/3	Clinical GVH, confirmed histologically
1/1	Probable GVH, not confirmed histologically
0/4	Graft take without evidence of GVH
0/15	ALL not grafted
0/15	Normal individuals

Sera were from non-affected transplant recipients, non-transplanted leukaemics, and normal individuals. ALL, acute lymphocytic leukaemia.

The cytotoxic factor was characterized with respect to molecular properties and specificity. The cytotoxicity of the sera for epithelial cells was complement dependent. Reactive sera from two of the patients were fractionated by gel filtration through 'Sephadex G-200'. The cytotoxic activity was found in the excluded protein peak which contained IgM but not IgG or IgA when screened by Ouchterlony agar diffusion using specific antisera. Treatment of cytotoxic sera with 0.1 M 2-mercaptoethanol destroyed the ability of the sera to lyse epithelial cells. These results provide evidence that the cytotoxic factor in these patients with GVH disease is an IgM antibody.

To investigate the possibility that the sera were reacting against an HL-A antigen, they were screened against a panel of lymphocytes from ninety individuals; no pattern of HL-A reactivity was observed. In addition, absorption of the sera with lymphocytes having a broad range of HL-A alloantigens did not remove the cytotoxicity for the epithelial cells. Absorption of the reactive sera with one epithelial cell line removed reactivity for the other. Thus it seems that the GVH antibody is not directed against HL-A antigens but has specificity for epithelial cells. A further indication of this specificity is the finding that the sera failed to react with tissue culture hepatocytes and fibroblasts.

The pathogenesis of the GVH reaction is unknown; but the observation that patients with this disease have an antibody directed to epithelial cells suggests several mechanisms for this reaction. The antibody we are detecting may be directed against minor histocompatibility antigens that are expressed only as epidermal specific antigens not detected by the usual HL-A matching techniques. Boyse *et al.* have presented data suggesting similar antigens in mice[8]. Alternately, it may be that damage initially produced in the skin by the GVH reaction exposes hidden epidermal antigens and these then provoke the formation of "autoantibodies" against epithelial cells. A third possibility is that impaired immune function in the grafted recipient predisposes to certain viral infections, and that alteration of epidermal cell surface antigens induced by virus, or cross-reactivity between viral and epidermal antigens accounts for the antibody detected in these patients. Further investigation of these and other possibilities may provide information about the importance of tissue specific non-HL-A antigens and antibodies in the processes of graft rejection and in the pathophysiology of the GVH reaction.

We thank Dr E. S. Henderson, Medicine Branch, and Dr R. G. Graw, Human Tumor Cell Biology Branch, National Cancer Institute, for the opportunity to study sera from their patients. Miss B. A. Plocinik performed cytotoxicity assays.

C. B. MERRITT
D. L. MANN
G. N. ROGENTINE, JUN.

Immunology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda,
Maryland 20014

Received December 31, 1970; revised March 15, 1971.

[1] DeVries, M. J., *Exp. Hematol.*, **20**, 112 (1970).
[2] Streilein, J. W., and Billingham, R. E., *J. Exp. Med.*, **132**, 163 (1970).
[3] Billingham, R. E., and Streilein, J. W., *Arch. Dermatol.*, **98**, 528 (1968).
[4] Graw, jun., R. G., Brown, J. A., Yankee, R. A., Leventhal, B. G., Whang-Peng, J., Rogentine, jun., G. N., and Henderson, E. S., *Blood* (in the press).
[5] Graw, jun., R. G., Brown, J. A., Yankee, R. A., Weiderlite, L. W., Leventhal, B. G., Whang-Peng, J., Rogentine, jun., G. N., and Henderson, E. S., *Exp. Hematol.*, **20**, 35 (1970).
[6] Graw, jun., R. G., Rogentine, jun., G. N., Yankee, R. A., Whang-Peng, J., Herzig, G. P., Halterman, R. H., Kruger, G., Berard, C., and Henderson, E. S., *Lancet*, **ii**, 1053 (1970).
[7] Rogentine, jun., G. N., and Plocinik, B. A., *Transplantation*, **5**, 1923 (1967).
[8] Boyse, E. A., Lance, E. M., Carswell, E. A., Cooper, S., and Old, L. J., *Nature*, **227**, 901 (1970).

Teratocytes as a Means of Resistance to Cellular Defence Reactions

I HAVE suggested that teratocytes, the giant cells which develop from the trophamnion of many parasitoid Hymenoptera, serve as a means of resistance to the defence reactions of insect hosts[1]. These cells are relatively small when they dissociate from the embryonic membrane, but as they become distributed in the blood of the host they expand to as much as 3,000 times their original volume. Their growth is achieved by the absorption of nutrients from the host's blood and I suggested that the consequent depletion of materials in the haemolymph would impede or inhibit haematopoiesis and prevent an effective haemocytic reaction to the young parasitoid.

Evidence supporting the hypothesis has been obtained in the course of experiments designed for another purpose. Eggs and larvae of the ichneumon wasp *Nemeritis canescens*, which does not produce teratocytes, were implanted in caterpillars of *Eucosma hohenwartiana* collected out of doors from heads of knapweed, *Centaurea nigra*. In healthy caterpillars the parasitoid was promptly killed by cellular defence reactions. Against all expectation, one larva of *Nemeritis* was found feeding and developing without encapsulation when it was 5 days old. The caterpillar containing this individual, however, was also infected by a braconid parasitoid, and its blood contained numerous teratocytes about 50 μm in diameter. In two caterpillars infected by wild parasitoids which had not produced teratocytes, the *Nemeritis* was encapsulated as it was in healthy hosts.

Three more examples of this phenomenon have recently been observed in caterpillars of *Chilo phragmitellus* collected from reeds at Wicken Fen. When eggs of *Nemeritis* were implanted in healthy caterpillars of this species, they were encapsulated, melanized and killed; only two hatched, and the larvae were then promptly destroyed in the same way. Three caterpillars in which eggs were implanted turned out to be already parasitized by gregarious braconids, probably *Apanteles ferrugineus*, which had produced many teratocytes. From these three hosts, and from them only, larvae of *Nemeritis* 5 days old were retrieved alive, feeding and apparently developing normally, without any trace of a haemocytic defence reaction.

These observations show that teratocytes are indeed a means of resistance to the cellular defence reactions of insects, and that the protection they afford is not restricted to the species of parasitoid that produces them but extends to other species. Since their action is not specific, attrition of the host is most likely to be the mechanism by which teratocytes have their protective effect.

I thank Mr J. A. Lade for help with the collection of caterpillars.

GEORGE SALT

Department of Zoology,
University of Cambridge

Received April 13, 1971.

[1] Salt, G., *Biol. Rev.*, **43**, 200 (1968).

Pure Che istry Lib
SCIENCE COLLEGE
92, Upper Circular Ro
CALCUTTA.

Cellular Basis for Immunologic Memory

AFTER exposure to an antigen resulting in a primary immune response, experimental animals and human subjects possess the ability to react specifically and in accelerated fashion to a second exposure to the same antigen. This immunological memory presumably involves specific cells, but it is not known whether it involves generation of an increased number of cells or increased synthetic (or some other) ability in individual cells. Nobody has yet identified a cell which was generated by a previous contact with a specific antigen. Lymphocytes present in the circulation of rats have been shown to carry memory[1], and recent work has established that many such cells are thymus-derived and important in many aspects of the immune response[2,3]. Because they are long lived and constantly re-circulating throughout the peripheral lymphoid organs, thymus-derived cells make ideal candidates for the carriage of memory. Indeed, memory can be diminished by killing cells in this sub-population[4].

Exposure to an antigen results in a burst of mitosis in the lymphoid organs, and at least initially many of the cells in mitosis are thymus-derived[5]. Moreover, these cells respond

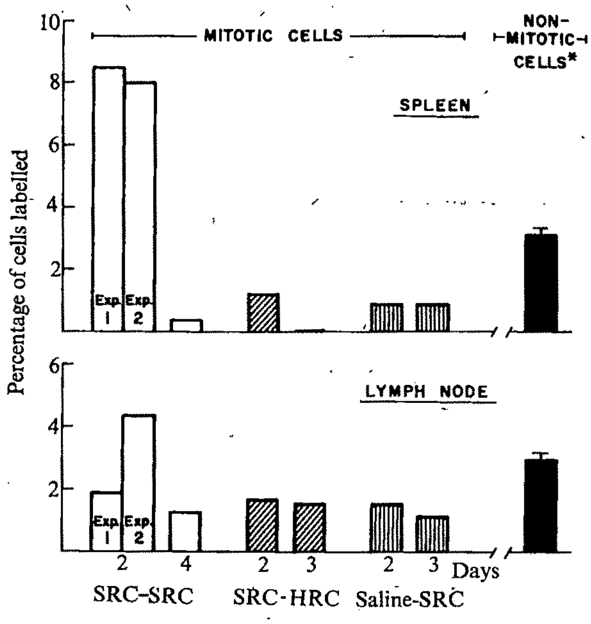


Fig. 1 Percentages of labelled mitotic and non-mitotic cells in spleen and mesenteric lymph nodes following secondary challenge. Results of two experiments are shown. The results are pooled from all the groups for they were all quite similar. The bar indicates the standard error obtained from meaning the seven groups.

with an accelerated burst of mitoses in the secondary response[3]. The peak of the mitotic response to sheep red cells (SRC) occurs on day 3 in the primary response and on day 2 in the secondary. The role of the mitosis is unknown, but it may involve the generation of specific cells some or all of which are destined to carry immunological memory. We have investigated this possibility by isotopically labelling cells synthesizing DNA in a primary response and observing the numbers of such labelled cells which responded mitotically to a second antigenic challenge. In doing this we took advantage of the work of Davies *et al.*[3,5], which clearly delineates the periods of peak mitotic activity of thymus-derived cells in mice following primary and secondary antigenic challenge. Thus we administered label for 4 days immediately after sensitization with SRC and re-stimulated with this antigen 3 weeks later. At this time we could expect continually dividing cell lines to have lost their label by dilution, while non-dividing cells would not. After a course of labelling with ^{3}H-thymidine similar to that described here, a population of heavily labelled lymphocytes is found in the spleen and lymph nodes of rats which diminishes in numbers very slowly with time[6–9]. By 21 days after cessation of labelling, when the initial rapid loss of label subsides, only a small population of heavily labelled cells persists[6–9].

Adult CBA mice received either 5×10^8 SRC or saline intraperitoneally, followed by injections of ^{3}H-thymidine every 6 h for 4 days (total dose = 7 μCi of 17.8 Ci/mmol specific activity of ^{3}H-thymidine/g body weight). Two groups of four mice, one immunized and one control, were given colcemid 3 h after the last thymidine injection. These animals were killed 1.5 h later and their spleens and mesenteric lymph nodes were removed. These tissues were divided roughly equally—half was used for tissue sectioning and the other processed by Ford's method[10] to give mitotic spreads. All other mice were challenged intraperitoneally with either 5×10^8 SRC or the same number of horse red cells (HRC) 3 weeks after primary immunization. On the second, third and fourth days after challenge, groups of three or four mice were killed and their spleens and lymph nodes processed in the same manner. All sections and spreads were dipped in diluted Kodak NTB3 nuclear track emulsion, exposed for 3.5 weeks, and developed in Kodak D19. Sections were stained with 0.1% toluidine blue and spreads with 2.0% aceto-orcein. The spreads were scored in a "blind" fashion by four persons, at least 1,500 non-mitotic nuclei and 300 mitoses being counted in each experimental group. Grain counts were performed in individual nuclei.

In an additional control experiment, two groups of six mice were immunized with 5×10^8 SRC intraperitoneally and one of these groups was given ^{3}H-thymidine as described above. All animals were bled weekly and then challenged after 3 weeks with 5×10^8 SRC and bled 5 days later. The immune response, assessed by haemagglutination titrations, was not altered by the injections of isotope.

Table 1 shows the distribution of label in the spleen cells of immunized and non-immunized mice killed immediately after the cessation of labelling (day 4). It can be seen that animals given SRC contain nearly twice as many labelled cells as control animals. In tissue sections, 4 days after the primary antigenic stimulus, many more labelled large lymphoid cells were present in the paracortical zones of mesenteric lymph nodes and the splenic white pulp than in animals given label but not antigen. There was also a diffuse increase in labelled reticulum cells in the marginal zone and red pulp of the spleen.

After specific challenge, that is, a secondary injection of SRC, very much greater numbers of labelled cells were found in mitosis in the spleens of animals at the peak of the mitotic response of thymus-derived cells[3] than were found in the spleens of animals originally given SRC and now challenged with HRC, or animals undergoing a primary immune response to SRC (Fig. 1). For the purpose of this communication a labelled nucleus is one which contained more than ten grains, although the average number of grains/labelled cell was less than seen immediately after the cessation of labelling. The numbers of labelled non-dividing cells in each of these groups did not differ significantly. Only in the group undergoing a secondary response to SRC was the proportion of labelled cells in mitosis greater than the proportion of labelled non-dividing cells. In spreads prepared from the mesenteric lymph nodes, a specific response of lower degree was observed in only one of the two experiments (Fig. 1). In histological sections of the spleen, labelled mitoses were limited to the white pulp. None was found in the germinal centres, and tingible bodies were also unlabelled. The labelled, non-dividing cells of the marginal zone and red pulp were equally numerous in specific animals and controls. In the lymph nodes, labelled mitotic cells were limited to the paracortical lymphoid tissue. By 4 days, when the mitotic activity of thymus-derived cells is over[3], labelled mitoses could no longer be found in either spreads or sections.

These results indicate that cells from the long lived popula-

Table 1 Labelling of Spleen Cells 4 Days after Induction of a Primary Response to SRC

	Non-mitotic cells (%)					Mitotic cells (%)				
mmunization	0–5 *	6–10	11–20	21–40	>40	0–5	6–10	11–20	21–40	>40
None	84.0	1.0	2.7	4.4	7.9	0	0	0	0	100
SRC	75.3	1.5	1.9	5.4	15.9	0	0	0	4.5	95.5

* Number of grains/cell.

tions, which are stimulated to synthesize DNA by contact with antigen, enter a resting phase until stimulated to re-enter division by secondary exposure to the same antigen. By definition these would be memory cells. They apparently correspond to Mitchell and Miller's "educated T lymphocytes"[2], for they were only observed at a time when the mitotic response of thymus-derived lymphocytes is maximal[3]. We are currently seeking direct experimental proof of their identity. Previous attempts to establish this point[11] have failed, perhaps because of the use of *Salmonella* antigens, which are poor stimulators of thymus-derived cells[12]. Our finding implies that cell replication may serve as a means of enlarging and/or altering the pool of thymus-derived cells capable of reacting with certain antigens. Replication may not, however, be essential to the helper function of these cells[13].

At the peak of the secondary response to SRC 30–40% of the cells in mitosis in the spleen are thymus-derived[3]. Thus we were initially surprised to find only 8–9% of the spleen cells responding mitotically to a second injection of antigen which showed evidence of having responded in the earlier primary response. It is important to remember that the work delineating the time and magnitude of the response of thymus-derived cells to antigen was carried out in irradiated, thymus-grafted mice. Thus it is possible that there may be some differences in normal mice. If there should be any significant differences, however, there would probably be an increase of thymus-derived cells responding in normal mice. Davies *et al.* have shown that increasing the number of reconstituting thymus grafts up to a point increases the number of peripheral thymus-derived cells[3]. This increase does not, however, alter the percentage of thymus-derived cells responding to antigen in lymph nodes nor does it change the timing of their response. It is possible that some thymus-derived memory cells lost their label through division after the period of labelling. We are investigating the possibility of recruitment of thymus-derived cells by other thymus-derived cells responding specifically. The small number (0–1%) of labelled cells found in control animals may be due, at least in part, to the emergence and subsequent response of a few thymus-derived cells which were undergoing division in the thymus at the time of thymidine administration.

The chief conclusion we should like to draw from this experiment is that one result of the DNA synthesis which follows primary antigenic stimulation is the generation of memory cells. The ability to identify these cells should be of value in characterizing them further.

R. K. G. is a recipient of a US Public Health Service Career Development Award. The work was supported by US Public Health Service grants from the National Cancer Institute, the National Institute of Allergy and Infectious Diseases and an American Cancer Society sub-grant. We thank Kazunari Kondo and Anne Shapiro for technical assistance.

Note added in proof: Since we submitted this manuscript, another report in which memory cells were identified by autoradiography, has appeared. Bosmann, C., and Feldman, J. D., *Clin. Exp. Immunol.*, 7, 565; 1970.

R. K. Gershon
J. Krüger
J. D. Naysmith
B. H. Waksman

Departments of Pathology and Microbiology,
Yale University School of Medicine,
New Haven,
Connecticut 06510

Received November 23, 1970; revised March 10, 1971.

[1] Gowans, J. L., and Uhr, J. W., *J. Exp. Med.*, **124**, 1017 (1966).
[2] Miller, J. F. A. P., and Mitchell, G. F., *Transplant. Rev.*, **1**, 3 (1969).
[3] Davies, A. J. S., *Transplant. Rev.*, **1**, 43 (1969).
[4] Raff, M. C., *Nature*, **226**, 1257 (1970).
[5] Davies, A. J. S., Leuchars, E., Wallis, V., and Koller, P. C., *Transplantation*, **4**, 438 (1966).
[6] Miller, J. J. III, *J. Immunol.*, **92**, 673 (1964).
[7] Miller, J. J. III, and Cole, L. J., *J. Exp. Med.*, **126**, 109 (1967).
[8] Miller, J. J. III, and Cole, L. J., *J. Immunol.*, **98**, 982 (1967).
[9] Bosman, C., and Feldman, J. D., *J. Exp. Med.*, **128**, 293 (1968).
[10] Ford, C. F., in *Tissue Grafting and Radiation* (edit. by Micklem, H. S., and Loutit, J. F.), 197 (Academic Press, New York, 1966).
[11] Miller, J. J. III, and Cole, L. J., *J. Immunol.*, **101**, 133 (1968).
[12] Carter, R. L., Davies, A. J. S., Leuchars, E., Wallis, V. J., and Gershon, R. K., in *Lymphatic Tissue and Germinal Centers in Immune Response*, 143 (Plenum Press, New York, 1969).
[13] Katz, D. H., Paul, W. E., Goidl, E. A., and Benacerraf, B., *Science*, **170**, 462 (1970).

Polychlorinated Biphenyls: Photolysis of 2,4,6,2′,4′,6′-Hexachlorobiphenyl

MANY aromatic organochlorine compounds are readily utilized or otherwise decomposed by a number of specific microorganisms. In the environment, however, these compounds are relatively stable and resistant to general microbial breakdown. For many of these compounds photochemical degradation, induced by the ultraviolet content of the Sun's light, may be a major pathway of decomposition.

Because a large number of pesticides contain a chloroaromatic moiety as a primary structural feature, considerable attention has been paid to the photolysis of these compounds[1–7]. Photochemical decomposition of chlorobiphenyls may be a significant process in the environment because they absorb in that region of the ultraviolet (ref. 8 and our unpublished demonstrations) which is available in natural conditions (compare ref. 1) from sunlight.

Commercially available polychlorinated biphenyls (PCB) for example 'Aroclor', consist of a complex mixture of chlorinated biphenyls which can be resolved into more than fifty peaks by gas chromatography on 'Scot columns' (private communication from D. J. Sissons and G. M. Telling). As it is difficult to correlate data from such a complex mixture we have synthesized a series of isomeric di, tri, tetra, penta, hexa, octa and decachlorobiphenyls to facilitate fundamental studies on analytical, biological and chemical aspects of PCB (manuscript in preparation). We have also shown that all these compounds give strong molecular ions on electron

Table 1 GLC and Mass Spectral Data for Hexachlorobiphenyl Photolysis Products

	Retention time* (min)	m/e of M^+	Chlorine† content
TLC band I			
Peak 1	5.6	—‡	0
Peak 2	8.2	—‡	0
Peak 3	9.9	222	2
Peak 4	13.5	256	3
Peak 5	14.1	290 (256)	4, (3^{tr})
Peak 6	19.0	324 (290)	5, (4^{tr})
Peak 7	29.7	358	6
Peak 8	56.9	304	2
TLC band II			
Peak 1	19.6	290	4
Peak 2	26.8	324	5
Peak 3	35.2§	358	6

* Conditions: instrument, Hewlett–Packard 5750; column, 6% QF-1+4% SE30 on 'Chromosorb' (AW) 60–80 mesh 5′, 1/4″ outside diameter, glass; injection port temperature, 220° C; column temperature, 175° C; flame ionization detector temperature and collection port temperature, 240° C; helium flow rate, 40 ml./min.

† From isotope peak distribution; tr, trace.

‡ Hydrocarbon peaks, M^+ not identified.

§ Corresponding to starting material.

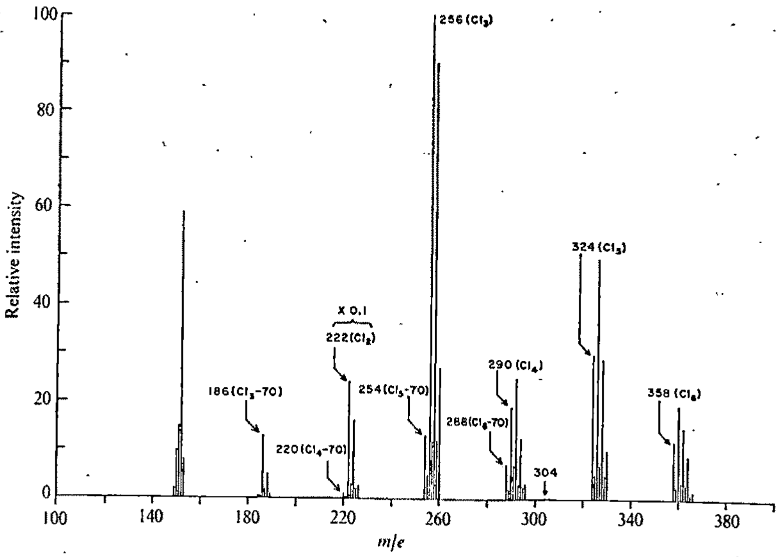


Fig. 1 70 eV mass spectrum (DuPont/CEC 21–110B instrument). The temperature of the sample, controlled independently from the source temperature[10], was 40° C. The intensity of ions corresponding to the components ($Cl_6 = C_{12}H_4Cl_6$, $Cl_5 = C_{12}H_5Cl_5$, and so on) in the mixture varies with sample temperature. Each isomer shows an M^+-70 ion as the most important fragment. These ions are indicated as Cl_6-70, Cl_5-70, and so on.

impact, followed by less intense ions resulting from successive loss of Cl and/or HCl (manuscript in preparation). As the general formula for PCB is $C_{12}H_{10-x}Cl_x$ the molecular ions of individual isomers are thus readily recognized in a mass spectrum of a mixture of these compounds (compare ref. 9).

We now wish to report the photochemical behaviour of 2,4,6,2′,4′,6′-hexachlorobiphenyl. A sample of 25 mg of this compound in 2.0 ml. of hexane was irradiated at λ_{max} 3100 Å for 100 min. Thin-layer chromatography, using 'Merck Silica Gel F-254' and hexane, revealed the presence of two major bands; band I (R_F 0.40) and band II (R_F 0.65, corresponding to starting material). The mass spectrum of band I (Fig. 1) indicated molecular ions corresponding to di, tri, tetra, penta and hexachlorobiphenyls. Gas liquid chromatography (GLC) of bands I and II revealed eight and three major peaks respectively. Retention times and mass spectra data for all peak components are reported in Table 1. When irradiation was carried out in methanol, similar results were obtained, but in addition a third and more polar band appeared, containing oxygenated polychlorinated compounds with structures which have not been investigated further.

Two conclusions can be drawn from the results of this study: (i) during photolysis, chlorine is removed rather readily from hexachlorobiphenyl yielding compounds of lower chlorine content; (ii) photochemical production of new compounds formed by loss of chlorine, rearrangement or condensation may lead to further complications in residue analysis with the possible appearance of chlorobiphenyls ("peaks") in environmental samples that are not present in commercial PCB mixtures.

S. SAFE
O. HUTZINGER

Atlantic Regional Laboratory,
National Research Council of Canada,
Halifax, Nova Scotia

Received March 22, 1971.

[1] Crosby, D. G., and Li, M.-Y., in *Degradation of Herbicides* (edit. by Kearney, P. C., and Kaufman, D. D.), 321 (Dekker, New York, 1969).
[2] Crosby, D. G., *Ann. NY Acad. Sci.*, **160**, 82 (1969).
[3] Crosby, D. G., and Leitis, E., *J. Agric. Food Chem.*, **17**, 1033 1036 (1969).
[4] Crosby, D. G., and Tang, C. S., *J. Agric. Food Chem.*, **17**, 1041 (1969).
[5] Mosier, A. R., Guenzi, W. D., and Miller, L. L., *Science*, **164**, 1083 (1969).
[6] Plimmer, J., Klingebiel, U. I., and Hummer, B. E., *Science*, **167**, 67 (1970).
[7] Miller, L. L., and Narang, R. S., *Science*, **169**, 368 (1970).
[8] Friedel, R., and Orchin, M., *UV Spectra of Aromatic Compounds* (Wiley, New York, 1951).
[9] Hutzinger, O., Jamieson, W. D., and Zitko, V., *Nature*, **226**, 664 (1970).
[10] Jamieson, W. D., and Mason, F. G., *Rev. Sci. Instrum.*, **41**, 778 (1970).

Selection for Resistance to Carbamate and Organophosphorus Insecticides in *Anopheles albimanus*

THE development of resistance in anopheline mosquitoes to DDT, HCH, and cyclodienes, and the consequent set back in malaria eradication programmes has lent urgency to the search for alternative insecticides to replace the chlorinated hydrocarbons. During the past few years, more than 1,300 new insecticides, chiefly organophosphates and carbamates, have been tested under an international collaborative programme of the World Health Organization[1]. Testing consists of seven sequential stages and only insecticides that satisfy the criteria in all stages are recommended for use on a large scale.

One of the insecticides that satisfied the criteria of stage VI (operational evaluation) and has been found suitable for stage VII (epidemiological evaluation) is the carbamate, propoxur[2]. It is intrinsically more toxic to adults of many mosquito species than dieldrin, malathion, fenthion and fenitrothion, and as a residual spray has been found to be effective for 2 to 4 months against *Anopheles gambiae* and *A. funestus* in Africa, *A. stephensi* and *A. dthali* in Iran, and *A. albimanus* in El Salvador[2].

No case of resistance to carbamate or organophosphorus insecticides has yet been reported although tolerance to malathion has been suspected in *A. albimanus* in Guatemala and Nicaragua[3,4] and subsequently demonstrated in El Salvador[16]. In this communication we report the development of high degrees of carbamate and organophosphorus resistance in *A. albimanus* resulting from intensive selection pressure in the field, and subsequently in the laboratory.

Earlier attempts to induce carbamate resistance in *A. albimanus* by laboratory selection have been unsuccessful, although in *Culex pipiens fatigans* a 30× level of resistance to propoxur developed in larvae within twenty-five generations of selection pressure[5,6]. A strain of *A. albimanus* from Panama selected with *m*-isopropylphenyl methylcarbamate for fifty generations developed only a 2.8× increase in the LC_{50} level[7]. Another strain from Haiti, containing dieldrin-resistant individuals, when subjected to larval selection pressure by propoxur for nineteen generations and to adult pressure during the

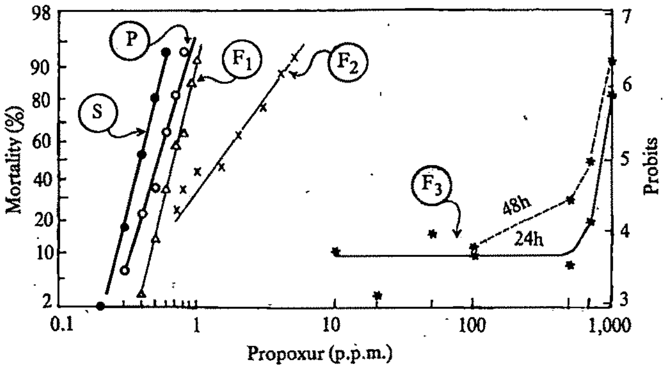


Fig. 1 Dose/mortality relationships of larvae of susceptible (S), El Salvador (P) and propoxur-selected (F_1, F_2, and F_3) populations of *Anopheles albimanus*.

subsequent fourteen generations, exhibited only a 3 × increase in larval LC_{50} and 3.5 × increase in adult LC_{50} (ref. 4).

We recently studied populations of this mosquito from El Salvador, where it is resistant to both DDT and dieldrin[3,8,9] and has also been exposed to parathion, methyl parathion, malathion, trichlorfon, thiometon, 'Azodrin' (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate), and DDT. In El Salvador and elsewhere in Central America, *A. albimanus* is often found breeding in or near cotton fields[10] which are heavily treated with these insecticides. As many as thirty applications of insecticides during the 6 month cotton growing season are not unusual (unpublished information from various sources including the El Salvador Department of Agriculture).

Eggs were obtained from adults collected on June 16, 1970, at Hacienda Melara, Department of La Libertad, El Salvador (given by Dr S. G. Breeland) and a vigorous colony was established in our laboratory. The larvae were fed on a mixture of Purina laboratory chow and yeast and the colony was maintained in controlled conditions of 26°–27° C and 60% relative humidity. The adults were fed on rabbits at the initial stages of this work and later on white mice. Larval and adult susceptibility tests were carried out as previously described[11]. In the adult tests glass-fibre filter paper was substituted for cellulose paper as the latter is known to reduce variability with carbamates[12]. The larval LC_{50} values obtained (Table 1) indicate increases in tolerance to propoxur, carbaryl, malathion, parathion and methyl parathion in the range of 1.1 × to 3.4 × compared with a mixed susceptible strain of Haiti/Panama origin.

When we subjected the El Salvador strain to larval selection pressure by propoxur at the 90–95% level of mortality, the larval LC_{50} rose only gradually at first, that is, from the parental 0.53 p.p.m. to 0.68 p.p.m. in the F_1 generation, and to 1.5 p.p.m. in the F_2 (Fig. 1). A change in slope of the dosage-mortality line of the F_2 generation from 3.3 in P_1 to 1.4 was more indicative of impending resistance. Selection for a third generation resulted in a marked increase in resistance to propoxur. Concentrations of 10–500 p.p.m. produced 24 h mortalities of from 3 to 15%; 1,000 p.p.m. resulted in only 81% mortality. If, at the end of the 24 h exposure, the larvae were held in clean water for an additional 24 h, only slight evidence of delayed mortality was observed. In these conditions, concentrations of 100, 500, 700 and 1,000 p.p.m. produced 11, 28, 48 and 91% mortality, respectively (Fig. 1).

Table 1 Response to Various Insecticides in Susceptible, Parental, and Propoxur-selected Strains of *Anopheles albimanus*

	24 h LC_{50} (p.p.m.)				
Compound	Haiti/ Panama susceptible	El Salvador P_1	RR*	Propoxur-selected F_{3+1}	RR*
Propoxur	0.39	0.53	1.4	> 100.0	> 100.0
Carbaryl	0.89	1.02	1.1	66.6	74.8
Malathion	0.085	0.25	2.9	1.7	20.0
Parathion	0.0031	0.0098	3.2	0.11	35.5
Methyl parathion	0.0065	0.022	3.4	0.24	36.9
Fenthion	0.023	0.026	1.1	0.027	1.2
Fenitrothion	0.025	0.045	1.8	0.24	9.6
Dichlorvos	0.11	0.11	1.0	0.21	1.9
DDT	0.01	0.046	4.6	0.20	20.0
Dieldrin	0.003 †	1.37	456.7	1.30	433.3

$$\text{* Resistance ratio} = \frac{LC_{50}\ P_1 \text{ or } F_{3+1} \text{ strain}}{LC_{50} \text{ susceptible strain}}$$

† LC_{50} of susceptible Gorgas strain.

To characterize the cross-resistance pattern of the strain, susceptibility tests were carried out on an unselected generation derived from the F_3, referred to as F_{3+1}. There was no detectable difference in susceptibility of F_3 and F_{3+1} larvae to propoxur. The tests indicated that resistance extends also to the carbamate carbaryl at a very high level (74.8 ×), and further it involves several organophosphorus insecticides, namely parathion (35.5 ×), methyl parathion (36.9 ×), malathion (20 ×), and fenitrothion (9.6 ×) (see Table 1). A lower level of resistance to paraoxon (approximately 5 ×) was found, and little or no resistance to dichlorvos and fenthion. The above levels of resistance are interesting because they appear to be approximately proportional to the pre-selection levels found in the parental field strain. Laboratory selection by propoxur therefore had the effect of magnifying the low levels of organophosphorus resistance which existed in the field population. Selection also increased the level of DDT resistance from the initial 4.6 × to 20 ×, but had no effect on dieldrin resistance.

Until biochemical tests are completed we cannot know the precise mechanisms responsible for this resistance. Evidence for physiological resistance, however, is readily apparent in the results so far obtained with synergists. The effects of piperonyl butoxide, triphenyl phosphate (TPP), S,S,S,-tributyl phosphorotrithioate (TBPT), and 1,1-*bis*-(*p*-chlorophenyl) ethanol (DMC) were studied by pretreating the resistant larvae with a sublethal dose of synergist 4 h before treatment with the insecticide (Table 2). Synergists were used at 10 p.p.m. except for TBPT which was used at 20 p.p.m. Piperonyl butoxide caused no increase in mortality with 100 p.p.m. propoxur, but with 500 and 700 p.p.m. it produced a marked increase. The absence of synergism at 100 p.p.m. of propoxur indicates that at this dosage a sufficient amount of detoxication enzyme remained uninhibited and was able to cope with the insecticide. At doses of 700 p.p.m. and above, however, the detoxication capacity of larvae was rapidly depleted. In contrast, carbaryl synergism occurs at lower dosages of insecticide. Synergism of carbamates by piperonyl butoxide suggests that microsomal mixed function oxidases are involved in resistance[13]. Further evidence of this is the effect of piperonyl butoxide on the toxicity of the phosphorothioates parathion, methyl parathion, fenitrothion, and fenthion. As expected, a strong degree of antagonism towards these was observed, indicating the inhibition of oxidative enzymes involved in the P=S to P=O activation step. With the phosphates dichlorvos and paraoxon no detectable effect was noted (Table 2). Because of the high degree of resistance to parathion (35.5 ×), some synergism of paraoxon might also be expected. That none was observed indicates that resistance involves an attack on the thionate before its conversion to the phosphate and/or non-oxidative degradation of the phosphate. In this respect, the low level of synergism obtained on paraoxon by TBPT, an inhibitor of desethylation[14], may be significant. The synergism of malathion by TPP would also suggest the presence of carboxyesterase detoxication enzymes[15].

A limited number of tests on adults revealed that resistance to propoxur is considerably lower in this stage than in larvae. The LC_{50} value of the parental adults (0.47 µg/cm²) rose only 4.3 × in the F_{3+1} generation (2.04 µg/cm²). It is therefore likely that selection for reduced cuticular penetration may also account for a portion of the observed resistance in larvae.

We expect that studies in progress in this laboratory will provide the necessary qualitative and quantitative information on the nature of the resistance factors involved. We believe, however, that the information so far obtained on resistance to propoxur and cross-resistance to fenitrothion is of major importance in mosquito control and in malaria eradication strategies in particular, since both compounds are considered as front line substitutes against DDT and dieldrin resistant anophelines.

Table 2 Effect of Synergists on the Toxicity of Various Compounds to Propoxur-selected *Anopheles albimanus*

Insecticide and dose (p.p.m.)		Synergist and dose (p.p.m.)		Percentage mortality	
				Insecticide alone	Insecticide + synergist
Propoxur	100	pb*	10	9	2
Propoxur	500	pb	10	7	90
Propoxur	700	pb	10	19	97
Carbaryl	30	pb	10	13	80
Carbaryl	50	pb	10	25	100
Malathion	2	TPP†	10	56	98
Parathion	0.175	pb	10	94	23
Methyl parathion	0.4	pb	10	93	2
Fenitrothion	0.3	pb	10	76	5
Fenthion	0.05	pb	10	95	0
Dichlorvos	0.2	pb	10	40	48
Paraoxon	0.2	pb	10	0	5
Paraoxon	1	pb	10	80	70
Paraoxon	0.2	TBPT‡	20	0	10
Paraoxon	1	TBPT	20	80	95
DDT	1	DMC§	10	76	87

* Piperonyl butoxide.
† Triphenyl phosphate.
‡ Tributyl phosphorotrithioate.
§ 1,1-*bis*-(*p*-chlorophenyl) ethanol.

This research was supported in part by a Public Health Service research grant from the Center for Disease Control, Atlanta, Georgia.

V. ARIARATNAM
G. P. GEORGHIOU

Department of Entomology,
University of California,
Riverside,
California 92502

Received March 8, 1971.

[1] Wright, J. W., *Cah. ORSTOM, Sér. Entomol. Méd. Parasitol.*, 7, 101 (1969).
[2] Wright, J. W., Fritz, R. F., Hocking, K. S., Babione, R., Gratz, N. G., Pal, R., Stiles, A. R., and Vandekar, M., *Bull. Wld. Hlth. Org.*, **40**, 67 (1969).
[3] WHO Expert Committee on Malaria, Thirteenth Report, *Wld. Hlth. Org. Techn. Rep. Ser.*, **357**, 59 (1967).
[4] Georghiou, G. P., *Wld. Rev. Pest Control*, **8**, 86 (1969).
[5] Georghiou, G. P., *Nature*, **207**, 883 (1965).
[6] Georghiou, G. P., Metcalf, R. L., and Gidden, F. E., *Bull. Wld. Hlth. Org.*, **35**, 691 (1966).
[7] Georghiou, G. P., and Metcalf, R. L., *Science*, **140**, 301 (1963).
[8] Brown, A. W. A., *Bull. Entomol. Soc. Amer.*, **14**, 3 (1968).
[9] Bruce-Chwatt, L. J., *Misc. Publ. Entomol. Soc. Amer.*, 7, 7 (1970).
[10] Busvine, J. R., and Pal, R., *Bull. Wld. Hlth. Org.*, **40**, 731 (1969).
[11] Georghiou, G. P., and Metcalf, R. L., *Mosquito News*, **21**, 328 (1961).
[12] Georghiou, G. P., and Gidden, F. E., *Mosquito News*, **25**, 204 (1965).
[13] Shrivastava, S. P., Georghiou, G. P., Metcalf, R. L., and Fukuto, T. R., *Bull. Wld. Hlth. Org.*, **42**, 931 (1970).
[14] Lewis, J. B., *Nature*, **224**, 917 (1969).
[15] Plapp, jun., F. W., and Eddy, G. W., *Science*, **134**, 2043 (1961).
[16] Breeland, S. G., Kliewer, J. W., Austin, J. R., and Miller, C. W., *Bull. Wld. Hlth. Org.*, **43**, 627 (1970).

Acceptability of Daily *l*-Tetramisole by Pound Dogs infected with *Dirofilaria immitis*

THIENPONT *et al.*[1] reported on the anti-helminthic potency of a new synthetic drug named tetramisole which, when given in a single dose, was effective against many of the intestinal and pulmonary nematodes of fourteen mammalian species. In the absence of any information on the host's reaction to tetramisole given for 30 to 40 days, an experiment was designed with the primary objective of testing the acceptance of daily doses of the drug by dogs from pounds. A secondary objective was to detect any therapeutic activity against adult *Dirofilaria immitis*.

Three pound dogs, weighing 40–50 pounds and aged 1–2 yr, were used. Each had a *D. immitis* microfilaraemia, with pretreatment concentrations of (*A*) 83, (*B*) 105 and (*C*) 5 microfilariae/20 mm^3 of blood respectively. Each was given one 50 mg tablet of tetramisole, directly or in a meatball, daily from Monday to Friday for 3 weeks, after which the dose was increased to one 100 mg tablet for the next 3 weeks. Four days after the last 100 mg dose, each dog was given a single dose at the rate of 9 mg per pound body weight.

The dogs were killed by intravenous injection of barbiturates 16 days after the last administration of drug and body cavities and viscera examined for worms. The great vessel leading to the heart was clamped off and the heart and lungs removed as a block. All chambers of the heart, the pulmonary artery, as well as all the dissectible branches of the arterial tree were opened in the search for adult heartworms. A gross examination for pathological changes was performed on all organs and principal tissues.

There were no unusual problems of drug acceptability with any of the three tetramisole regimens used. Dead worms were found in all dogs, often in the second order pulmonary arteries. All worms removed were placed in tissue culture fluid so that any movement could be observed, but none was seen. The microfilariae concentrations just before necropsy were: (*A*) 29; (*B*) 0; and (*C*) 0/20 m^3 of blood.

In the conditions of this limited experiment, there is clear evidence that tetramisole can be given to pound dogs at low concentrations for extended periods. Indications against the therapeutic use of tetramisole as a microfilaricide and as an adulticide were not observed clinically. More study is needed to determine the minimum effective concentration of tetramisole to destroy both microfilariae and adults of *D. immitis*.

G. S. TULLOCH
R. A. ANDERSON

Veterinary Sciences Division,
USAF School of Aerospace Medicine,
Brooks Air Force Base, Texas 78235

Received August 29; revised October 1, 1970.

[1] Thienpont, D., Vanparijs, O. F. J., Raeymaekers, A. H. M., Vandenberk, J., Demoen, P. J. A., Allewijn, F. T. N., Marsboom, R. P. H., Niemegeers, C. J. E., Schellekens, K. H. L., and Janssen, P. A. J., *Nature*, **209**, 1084 (1966).

Rational Design of Degradable Insecticides

I HAVE used a sterically based hypothesis of the mode of action of aryl insecticides, including DDT[1], to predict possible structures of new compounds, the synthesis[2] of which has been directed at finding insecticides with low mammalian toxicity and which, because of their inherent chemical instability, would readily degrade. Insecticidal activity has been studied

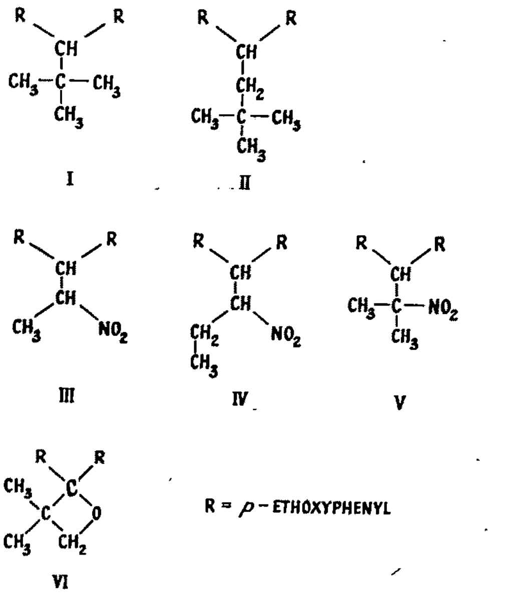


Fig. 1 Three series of non-halogenated insecticides designed from a theoretical model. In the nitroalkane and oxetane series the compounds containing two or at least one *p*-ethoxyphenyl-substituent exhibit a higher insecticidal activity than their *p*-chlorophenyl-analogues.

in susceptible and resistant housefly and investigations have been made of acute mammalian toxicity and chemical *in vitro* degradation.

The model consists essentially of a size limited "molecular wedge" which relates the physiological properties of the insecticides to their ability to keep the channel of Na^+ ions open[3] during an action potential pulse in a nerve membrane. The broad base of the "wedge" (for example, the *p*-chlorophenyl rings of DDT) was assumed to lock the whole structure by charge transfer complex formation into the protein matrix of the nerve membrane. This usually requires two phenyl rings substituted with electron donor groups. Chlorine atoms in active structures are not essential, as demonstrated by the activity of several series of new insecticides (Fig. 1 and Table 1) which do not contain halogens. These were tested for insecticidal activity in a DDT susceptible strain of housefly (WHO/IN/*Musca d.*/1) by the method described before.

An alternative to chlorine substitution in the phenyl rings comprising the base of the "wedge" was found in alkoxyphenyl derivatives. The *p*-ethoxy substituent, for example, has optimum electron donating properties within the model size limitation. Using this group the investigation of new structures centred on the design of the apex of the "wedge". In the original hypothesis, the chemical structure of the apex was only relevant with respect to size and chemical stability, and on this principle three types of compounds, branched hydrocarbons, nitroalkanes and dimethyl oxetanes, were selected and prepared (Fig. 1). The three series illustrate the importance of relating the size of the group at the apex to constructed steric three dimensional atomic models. We propose that the limit of the projected maximum van der Waals diameter (0.6 nm ±0.05) of this group determines the activity or inactivity of the compounds. In neopentane I (Fig. 1, Table 1) which is related to the less active dimethoxy analogue XV (Table 1)[4,5], the shift of the *t*-butyl group in its homologue, the neohexane II (Fig. 1, Table 1), does not alter its downward projection and the same insecticidal activity is retained.

The three nitroalkanes III, IV and V (Fig. 1) also demonstrate the importance of the design. The *p*-chlorophenyl analogues of the first two are insecticides, 'Prolan' and 'Bulan'[6], and the inactivity of 1-*o*-ethoxyphenyl-1-*p*-ethoxyphenyl-2-nitropropane[7], can be explained by its wrong dimensional fit into the described model, which suggested the high activity of the *bis*-*p*-ethoxyphenyl-compounds and the activity of the previously unknown nitroalkane isomer V (Fig. 1, Table 1). The best illustration of this design, however, is in the oxetane series VI–X (Table 1). The oxetane heterocyclic ring was selected on the basis of the construction of three dimensional atomic models and the projections of the groups. Fig. 2 shows the effect of the change of conformation of the methyl groups on the oxetanes from the 3,3-dimethyloxetane VII

Table 1 Structure-Activity Relationship of New Insecticides, their Synergism and Mammalian Toxicity

No.	Compound	LD_{50} *Musca d.* (μg/insect)	LD_{50} *Musca d.** + synergist (μg/insect)	Synergistic ratio	LD_{50} mouse (mg/kg)	$\frac{LD_{50}\text{ mouse}}{LD_{50}\textit{ Musca d.}}$ (mg/kg)
I	1,1-*bis*-(*p*-ethoxyphenyl)-2,2-dimethylpropane	1.92	0.32	5.5	5,200	54
II	1,1-*bis*-(*p*-ethoxyphenyl)-3,3-dimethylbutane	2.00	0.36	5.5		
III	1,1-*bis*-(*p*-ethoxyphenyl)-2-nitropropane	0.48	0.065	7.4	1,150	48
IV	1,1-*bis*-(*p*-ethoxyphenyl)-2-nitrobutane	0.55	0.061	9.0	1,160; 980†	42; 326†
V	1,1-*bis*-(*p*-ethoxyphenyl)-2-methyl-2-nitropropane	1.01	0.053	19.6		
VI	2,2-*bis*-(*p*-ethoxyphenyl)-3,3-dimethyl oxetane	0.52	0.010	52	1,200	46
VII	2,2-*bis*-(*p*-chlorophenyl)-3,3-dimethyl oxetane	1.27	0.66	1.9	3,500	55
VIII	2,2-*bis*-(*p*-chlorophenyl)-*trans*-3,4-dimethyl oxetane	>100	>100			
IX	2,2-*bis*-(*p*-methoxyphenyl)-3,3-dimethyl oxetane	5.3	0.25	21.0		
X	2,2-*bis*-(*p*-*n*-propoxyphenyl)-3,3-dimethyl oxetane	5.3	0.53	10.0		
XI	2-*p*-ethoxyphenyl-2-*p*-methoxyphenyl-3,3-dimethyl oxetane	1.43	0.086	17.9		
XII	2,2-*bis*-(*p*-ethoxyphenyl)-3,3,4-trimethyl oxetane	>100				
XIII	1-*p*-ethoxyphenyl-1-*p*-methoxyphenyl-2-nitropropane	0.25	0.014	17.9		
XIV	1,1-*bis*-(*p*-methoxyphenyl)-2-nitropropane	>20				
XV	1,1-*bis*-(*p*-methoxyphenyl)-2,2-dimethylpropane[4,5]	3.2	1.70	1.9		
XVI	1,1-*bis*-(*p*-chlorophenyl)-2-nitrobutane (Bulan)	1.69	1.78	0		
XVII	1,1-*bis*-(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT)	0.24	0.25	0	570	47

* 'Sesamex' 1% solution, 0.5 ul. added with each dose of insecticide.

† Toxicity test using 2 : 1 'Sesamex' to insecticide ratio.

(Table 1) to the *trans*-3,4-dimethyl- VIII, or 3,3,4-trimethyl oxetane, XII which alters the downward projection and the diameter of the end group. According to the theoretical model the projected maximum diameter of the last two derivatives is too large and the compounds are inactive. The dimensionally correct structures are highly active insecticides. The potentiated 1,1-*bis*-*p*-ethoxyphenyl derivative VI (Table 1) was twenty-five times as active as DDT against the housefly.

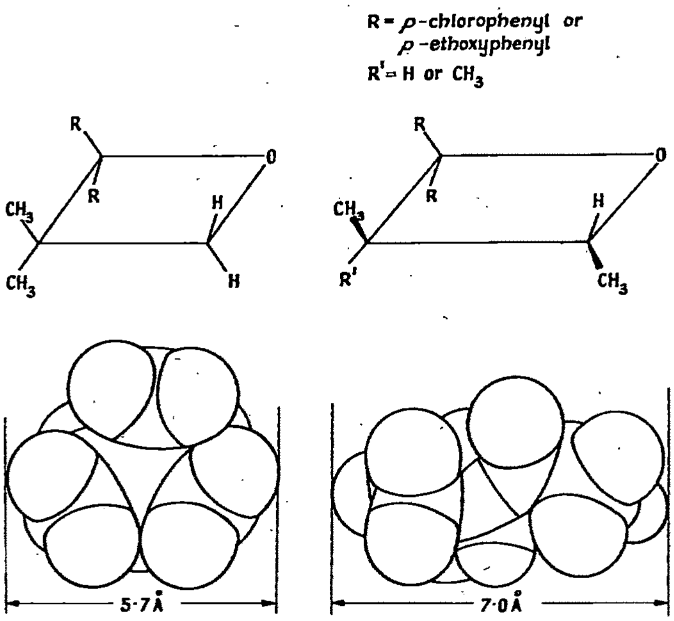


Fig. 2 The effect of conformation on activity of 2,2-*bis*-phenyl-dimethyl oxetane insecticides. The downward projection of steric atomic models with phenyl rings removed shows the change in diameter (in Ångstrom units) of the end groups for the insecticidally active 3,3-dimethyl oxetanes and inactive 3,3,4-trimethyl or *trans*-3,4-dimethyl oxetanes.

A major degradative mechanism of organic insecticides in insects and mammals is the action of microsomal mixed function oxidase[8,9]. The susceptibility of the new alkoxyphenyl structures to such attack was predicted from their chemical properties, and demonstrated by the strong potentiation of activity (Table 1), even in susceptible houseflies, by inhibitors of this enzyme system[10]. Potentiation was tested by applying 0.5 µl. of a 1% solution of 'Sesamex' (2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane) in acetone immediately after each dose of insecticide applied to the dorsum of the thorax of the housefly.

The ethoxyphenyl neopentane I (Fig. 3) is readily oxidized *in vitro* at the benzylic carbon and the neopentyl carbonium ion Ib formed from the labile 1-carbinol Ia rearranges spontaneously by the illustrated mechanism to a single product, the biologically inactive olefin Id. When R was phenyl or *p*-chlorophenyl, small amounts of olefin Ic were also isolated. Similar degradation should take place in the case of the related neohexane homologue II (Fig. 1). The major modes of biological degradation may parallel these *in vitro* transformations, and this is supported by the finding[8,11] that microsomal oxidation converts DDT by hydroxylation at the benzylic carbon to 1,1-*bis*-*p*-chlorophenyl-2,2,2-trichloroethanol. This metabolite, however, in contrast to carbinol Ia (Fig. 3), is stable.

In vitro degradation of all the insecticidal 2,2-dimethyl oxetanes VI, VII, IX, X, and XI (Table 1) was a major factor in their selection for synthesis, which takes place spontaneously (Fig. 3), yielding the inactive 1,1-diaryl-2,2-dimethylethylene and formaldehyde, and can be controlled by the addition or removal of free radical initiators[12]. When adsorbed on non-biological surfaces the oxetanes degrade rapidly. A sample of 1,1-*bis*-(*p*-chlorophenyl)-2,2-dimethyl ethylene cochromatographed with the 1,1-*bis*-(*p*-chlorophenyl)-3,3-dimethyl oxetane VII, on a deactivated preparative TLC silica gel plate using benzene as the eluent giving bands with Rfs 0.18 and 0.42 respectively. When extracted after 24 h exposure, both bands yielded only the 1,1-*bis*-(*p*-chlorophenyl) dimethyl ethylene. This rapid surface degradation of the oxetane insecticides contrasts with their stability in tests on solid samples or solutions of the compounds lasting several months, disproving the reported[13] inherent instability of some 3,3-diaryl oxetanes which could not be isolated but only detected spectrometrically in solution.

Acute mammalian toxicity was tested for several new compounds in each series (Table 1) using Swiss albino female mice of average weight 20 g. Compounds were administered intraperitoneally in olive oil and toxic effects were observed for 5 days. Five animals were used per concentration and the median lethal dose for each compound was calculated. The last column of Table 1 shows the ratios of mammalian to insecticidal toxicity in identical units (mg/kg; tested average 50 flies/gm). Even though the compounds were applied in different ways—some by topical application, others by intraperitoneal injection—a comparison of the values for the two species demonstrates an approximate fifty-fold safety margin in the acute toxicity in favour of the mammals, which is constant for different structures. The ratio of toxicities using the alkoxyphenyl nitrobutane IV (Table 1) with added

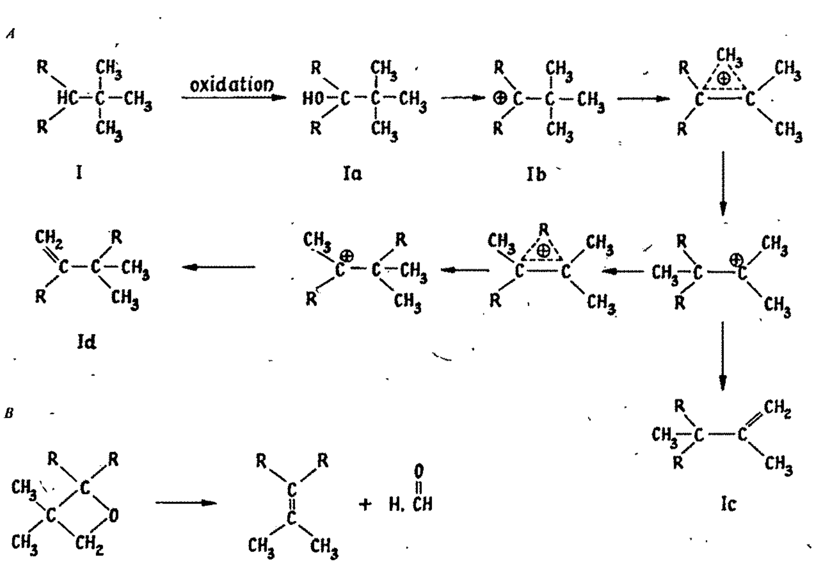


Fig. 3 Chemical degradation in two series of the new insecticides. *A*, The phenyl neopentyl structures are degraded to inactive olefins Ic and Id after hydroxylation to carbinol Ia. This rearrangement takes place at room temperature in the presence of even weak Lewis acids such as LiCl. *B*, The dimethyl oxetanes are degraded spontaneously when adsorbed on surfaces to formaldehyde and inactive 1,1-diaryl-2,2-dimethyl ethylenes. This degradation can be controlled by the addition of free radical initiators. All degradation products were identified by proton NMR, mass spectra and elemental analyses.

synergist demonstrates the selectivity of the synergist to insects and also the advantage of the potentiation in improving mammalian-insect toxicity ratio. For DDT XVII (Table 1) the ratio cannot be increased as its insecticidal activity cannot be potentiated. Although tests were carried out on only two species it is reasonable to conclude that for these compounds there is no intrinsic acute mammalian toxicity inherent in the chlorine substitution and that toxicity is potentiated by microsomal oxidation inhibitors predominantly in the insect.

Table 2 Mortality Data for DDT Resistant Strains of Housefly

Numbers from Table 1 of 1,1-*bis*-(*p*-ethoxy)- or (*p*-chlorophenyl)-compounds	Turramurra *	WHT (P) †	Housefly strain and LD_{50} values (μg/insect) F_3	F_{10}	F_{41}	F_{56}	DDT F_{37}
I -2,2-dimethylpropane	3.2	3.3	3.6	4.7	>200		
I+'Sesamex'	0.32 (0.1%)				4.2 (0.1%)		
III -2-nitropropane	0.80	0.32	0.86	2.74	—	0.33	
III+'Sesamex'	0.06 (0.1%)	0.09 (1.0%)	—	0.19 (0.5%)	—	0.04 (1.0%)	0.11 (0.1%)
IV 3-nitrobutane	0.86						
VII -3,3-dimethyl oxetane	2.38						
VII+'Sesamex'	0.66 (0.1%)						
XVII -2,2,2-trichloroethane (DDT)	>200	37.3	76.5	>200	>200		
DDT+'Sesamex'	>200 (1.0%)				>200 (1.0%)		

* Activity of compounds against a DDT resistant strain kept under DDT pressure and † against a field strain resistant to DDT, selected with compounds I, III and DDT for F number of generations. The selection with each compound was carried out on approximately 2,000 flies at 75% mortality level for each generation. Values in brackets are solution concentrations of 'Sesamex' synergist applied with each dose of the insecticide. All test values are for female flies. Mortality data were determined 48 h after topical application of insecticide and synergist in an acetone solution to two day old female flies fed on sugar only diet. Mortalities were compared with zero mortality in identically treated controls.

The decrease in the degree of oxidative degradation of *p*-chlorophenyl analogues such as DDT (revealed comparing the synergism of oxetanes VII and VI of Table 1) probably accounts for their persistence and that of their metabolites in the environment.

The most important factor which would account for the general low mammalian toxicity of this type of insecticide is the negative coefficient of insect mortality to temperature, which was also demonstrated for the new structures[14]. In warm blooded animals this effect would decrease the "locking" of the aryl structures into nerve membranes and diminish the nerve ion-flux disturbances attributed to this action.

The genetically developed enzyme DDT-dehydrochlorinase, which is a major factor in the build-up of resistance[15] to insecticides containing the 2,2,2-trichloroethane structural group, obviously cannot affect the nonhalogenated compounds listed in Table 1. The new insecticides had high activity against a field strain of the housefly resistant to DDT (Table 2). In a laboratory study using the 1,1-*bis*-(*p*-ethoxyphenyl)-2,2-dimethylpropane I and 2-nitropropane analogue III (Table 2) the parent generation (WHT-P) was a heterozygous field strain of the housefly with considerable resistance to DDT. For the three compounds tested in each generation approximately 2,000 flies were selected at a 75% level. A sample of female flies was then treated and the mortalities were counted 48 h later. The parent strain rapidly developed a complete resistance to DDT; resistance to the dimethylpropane I developed more slowly, while the nitropropane III gave inconsistent results and the initially developed resistance could not be maintained. Although the use of the 'Sesamex' synergist controlled the resistance to the new compounds, additional modes of resistance to DDT-type insecticides are now recognized[16] and further studies are required to determine their development by selection with the listed compounds.

Although positive results were obtained in the rational design of insecticides the question remains whether higher mammalian safety margins, non-halogenated, degradable structures and activity against resistant pests, outweigh the economic disadvantages of the compounds. Beneficial properties are sometimes coupled with decreased persistence and need for potentiation which may make the new compounds unacceptable under present agricultural and public-health practice of pest control.

Mammalian toxicity tests were carried out in part at the Department of Pharmacology, University of Melbourne, and the Commonwealth Serum Laboratories, Melbourne. I thank Dr E. Shipp for supervising the entomological tests and Mr R. Eibl for technical assistance.

G. Holan

Division of Applied Chemistry,
CSIRO,
P.O. Box 4331,
Melbourne, Victoria 3001

Received December 22, 1970; revised March 22, 1971.

[1] Holan, G., *Nature*, **221**, 1025 (1969).
[2] C.S.I.R.O., *Australian Patent Applications* Nos. 33896/68, 40743/68, 40745/68, PA 1945/70, PA 2367/70.
[3] Narahashi, T., and Haas, H. G., *J. Gen. Physiol.*, **51**, 177 (1968).
[4] Rogers, E. F., Brown, H. D., Rasmussen, I. M., and Heal, R. E. *J. Amer. Chem. Soc.*, **75**, 2991 (1953).
[5] Skerrett, E. J., and Woodcock, D., *J. Chem. Soc.*, 2718 (1950).
[6] Hass, H. B., Neher, M. B., and Blickenstaff, R. T., *Ind. Eng. Chem.*, **43**, 2875 (1951).
[7] Jacob, T. A., Bachman, G. B., and Hass, H. B., *J. Org. Chem.*, **16**, 1572 (1951).
[8] Agosin, M., Michaeli, D., Miskus, R., Nagasawa, S., and Hoskins, W. M., *J. Econ. Entomol.*, **54**, 340 (1961).
[9] Stritmatter, P., in *Biological Oxidations* (edit. by Singer, T. P.), 171 (Interscience, New York, 1968).
[10] Wilkinson, C. F., *World Review of Pest Control*, **7**, 155 (1968).
[11] Tsukamoto, M., *Botyu Kagaku*, **25**, 156 (1960).
[12] Margerum, J. D., Pitts, jun., J. N., Rutgers, J. G., and Searles, S., *J. Amer. Chem. Soc.*, **81**, 1549 (1959).
[13] Arnold, D. R., in *Adv. in Photochem.* (edit. by Noyes, jun., W. A., Hammond, G. S., and Pitts, jun., J. N.), **7**, 340 (Interscience, New York, 1968).
[14] Holan, G., *Bull. Wld. Health Org.* (in the press).
[15] Lipke, H., and Kearns, C. W., *Advan. Pest Control Res.*, **3**, 253 (1960).
[16] Plapp, jun., F. W., in *Biochemical Toxicology of Insecticides* (edit. by O'Brien, R. D., and Yamamoto, I.), 179 (Academic Press, New York, 1970).

Photomimetic Effect of Acetylcholine on Morphogenesis in *Trichoderma*

BIETH *et al.*[1] have demonstrated that synthetic photochromic agents can effect control of acetylcholinesterase activity *in vitro*, and suggest that photomorphogenetic processes are mediated by similar systems. Jaffe[2] has shown that acetylcholine mimics red light in some plant photomorphogenetic processes in which phytochrome is considered to be the photoacceptor. He also found increased efflux of acetylcholine from roots following red light treatment.

Various fungi have a photomorphogenetic pigment system which differs from the phytochrome system of higher plants insofar as the photoacceptor absorbs blue instead of red light[3,4].

Sporulation (conidiation) is induced at the growing edge of 1 day old cultures of the imperfect fungus *Trichoderma viride* Pers. ex Fries by minuscule quantities of blue light; the half maximum response is reached with 6.6×10^{-10} Einsteins/cm^2 at 447 nm[3]. Evidence with inhibitors suggests a requirement for nucleic acid synthesis for morphogenesis as sporulation can be selectively suppressed by some base analogues while only slightly affecting growth[5].

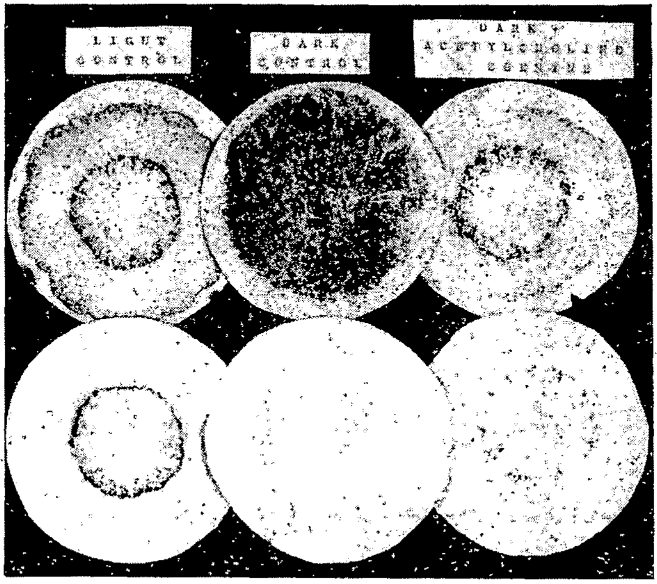


Fig. 1 Dark induction of sporulation (conidiation) in *Trichoderma* by acetylcholine and eserine. An agar block with mycelium of *Trichoderma viride* Pers. ex Fries was cultured for 34 h on one circle of Whatman No. 50 hardened filter paper, soaked with 2.3 ml. of double strength Difco potato dextrose broth, in sterile conditions at 24° C. The filter paper circles were then transferred to a second dish containing a 7 cm circle of Whatman No. 1 filter paper, soaked with 1.0 ml. of the above medium with or without the addition of 10^{-4} M acetylcholine (BDH) and 5×10^{-6} M eserine (Calbiochem) final concentrations. The light controls were then illuminated for 3 min with fluorescent lights. All other culturing procedures were performed with a red safelight. Bottom row: fixed in 80% ethanol 32 h after transferring; top row: fixed as above, dried and then stained with cotton blue to show mycelia as well as spores.

We wished to ascertain whether the blue light-induced morphogenetic response could be mediated through an acetylcholine type response, in a similar manner as that of the higher systems.

Agar blocks of plant fungal mycelium were planted on filter paper soaked with medium and cultured for 30–34 h. The filter papers were then transferred to Petri dishes containing fresh media with the experimental additives. Control cultures were illuminated for 3 min to induce sporulation. Detailed methods are described in ref. 5.

Preliminary experiments showed that acetylcholine alone could not induce sporulation in the dark under a wide range of concentrations (10^{-5} to 10^{-1} M). In the presence of the acetylcholinesterase inhibitor eserine (physostigmine) it was possible to induce sporulation, mimicing photoinduction in conditions which slightly inhibited growth (Fig. 1). Harsh physical and nutritional conditions such as desiccation or temporary starvation can induce a diffuse sporulation quite different from the distinct ring of spores induced by light or by acetylcholine. The acetylcholine antagonist curare suppressed light-induced sporulation, as would be expected from our preliminary findings, but it inhibited growth as well.

The ranges of effective acetylcholine and eserine concentrations inducing sporulation were found to be narrow (Fig. 2).

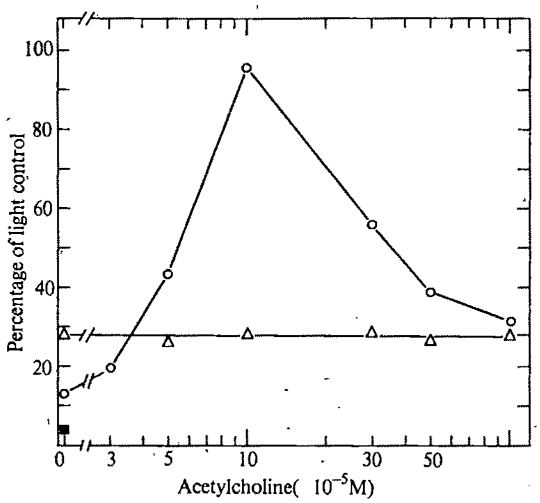


Fig. 2 Effect of acetylcholine and eserine concentrations on dark sporulation of *Trichoderma*. The experiments were performed as in Fig. 1. After ethanol fixation and drying, the spores were washed off in distilled water with a rubber policeman and counted microscopically in a Levy–Hausser corpuscle counting chamber. About 3×10^3 spores were contained in a light control spore ring. Results with 10^{-5} M eserine were similar to those with 3×10^{-6} M eserine shown above. ○, 5×10^{-6} M eserine; △, 3×10^{-6} eserine; ■, dark control.

Higher plants contain a blue light absorbing photomorphogenetic pigment system as well as the red light absorbing phytochrome. This blue light system has a similar action spectrum to that of *Trichoderma*. In some plants the direction of morphogenesis is sometimes controlled by an interplay of the two pigment systems[6]. If both systems act through acetylcholine some rethinking of the modes of action of each system will be necessary.

We thank Dr Israel Silman for useful discussion, and Dr M. J. Jaffe for access to an unpublished manuscript. We also thank Miss Tami Shihi for technical assistance. This research was supported in part by a grant from the US Department of Agriculture.

J. GRESSEL
LYNN STRAUSBAUCH
E. GALUN

Department of Plant Genetics,
Weizmann Institute of Science,
Rehovot

Received January 12, 1971.

---

[1] Bieth, J., Wassermann, N., Vratsanos, S. M., and Erlanger, B. F., *Proc. US Nat. Acad. Sci.*, 66, 850 (1970).

[2] Jaffe, M. J., *Plant Physiol.*, **46**, Suppl., 2 (1970); Jaffe, M. J., *Plant Physiol.*, **46**, 768 (1970).
[3] Gressel, J. B., and Hartmann, K. M., *Planta*, **79**, 271 (1968).
[4] Delbruck, M., *Bact. Rev.*, **33**, 133 (1969).
[5] Gressel, J., and Galun, E., *Develop. Biol.*, **15**, 575 (1967).
[6] Curry, G. M., in *Physiology of Plant Growth and Development* (edit. by Wilkins, M. B.), 243 (McGraw-Hill, Maidenhead, 1969).

Proof of Two Distinct Enhancement Effects by Blue Light on Oxygen Uptake in *Chlorella*

MANY authors have reported an enhancement of oxygen uptake in *Chlorella* by blue light[1-4]. The pigment responsible has not been definitely established, but may well be a flavin[5]. The mechanism of the enhanced oxygen uptake is also uncertain.

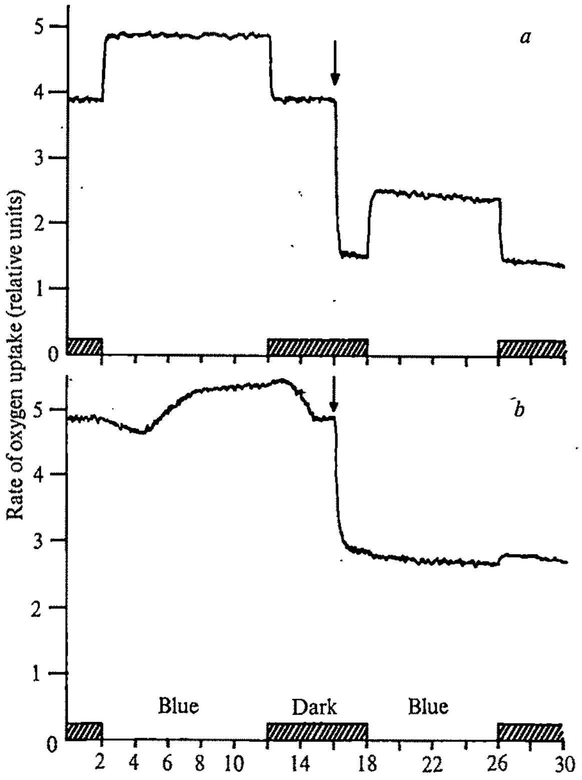


Fig. 1 The effect of blue light and cyanide poisoning on the respiration rate of two *Chlorella* strains. *a*, Colourless *Chlorella* mutant "G-27" in the presence of 3×10^{-5} M FMN (the curve has been corrected for uptake due to the auto-oxidation of FMN[9]; *b*, *C. pyrenoidosa* chick treated with 5×10^{-6} M DCMU. Cyanide (final concentration 2×10^{-4} M) was added to the preparations at the time indicated by the arrows. The enhancement was stopped in *b*, but not in *a* (pH=6.6 in all cases). Note the immediate onset of the enhancement in *a*, in contrast to *b*.

The enhanced respiratory activity has a respiratory quotient (RQ) of 1, and the same temperature dependence as dark respiration[5,6]; the cyanide sensitivities of the light stimulated oxygen uptake and of that part of glucose (dark) respiration which is inhibited by cyanide are identical[5]. It has been suggested[5,6] that the effect is due to a stimulation of normal respiratory pathways, possibly caused by an increased level of substrate (for example, a release of stored carbohydrate). Laudenbach and Pirson[7] investigated the effect of red and blue light on the carbohydrate content of the cells, and concluded that the effects of light on respiration are secondary and that a more direct effect might be a change in permeability of the plastid membrane.

On the other hand, Schmid and Schwarze[4] have reported oxygen uptake enhanced by blue light in a colourless *Chlorella* mutant, which they attribute to an FMN-dependent amino-acid oxidase. Crude enzyme extracts of this oxidase showed enhanced oxygen uptake in blue light, implying that blue light acts directly to increase oxygen uptake. With this amino-acid oxidase Schmid and Schwarze could account for the fact (reported in most of the above mentioned publications) that starvation of the cells for many hours before illumination gave an increased blue effect. They reasoned that in starvation the cells start hydrolysing their own protein, giving a higher concentration of free amino-acids available for direct oxidation. The authors assumed the enhancement effect observed in their mutant was the same as that reported by other groups, although one would not expect an RQ of 1 for protein oxidation. No inhibitor, temperature dependence or other studies were reported. We have found a second *C. pyrenoidosa* mutant ("G-27", described by Bendix and Allan[8]) with this FMN-dependent amino-acid oxidase activity[9].

Table 1 Distinguishing Features of the Two Types of Blue Light-stimulated Oxygen Uptake in *Chlorella*

Enhancement effect	Type I	Type II
Time of appearance	Delayed 6–10 min	Within 5 s
Cyanide sensitivity	97% inhibited at concentration of 2×10^{-4} M (ref. 5)	Unaffected by concentration of 2×10^{-2} M
Respiratory quotient	1	?
Pigment responsible	Possibly FMN	Probably FMN[9]
Probable mechanism	Stimulation of normal endogenous respiration and electron transport chain	FMN-dependent amino-acid oxidase
References cited	1–7	4, 9

We have now shown that this blue light phenomenon is different from that found in the normal (photosynthetic) *Chlorella* strain (Fig. 1). In the light of this we re-examined other reports of blue light enhancement effects in *Chlorella* and found that both effects had been observed, but not distinguished. We therefore wish to alert other investigators to this possible pitfall. Two easily checked, distinguishing features are the time course of the effects and their cyanide sensitivities. In DCMU-treated cells of the normal, photosynthetic strain, blue light causes an initial inhibition of oxygen uptake, which is followed 6–10 min later by an enhancement. This enhancement is virtually eliminated by 2×10^{-4} M cyanide (Fig. 1*b*), and is the same effect reported by Ried and further described by Pickett. The blue light-enhanced oxygen uptake in the "G-27" mutant, however, appears within 5 s (Fig. 1*a*) and is not inhibited even by 2×10^{-2} M cyanide.

For ease of identification we suggest the delayed, cyanide-sensitive effect be labelled "type I" and the immediate, cyanide-insensitive effect "type II". The properties of each are summarized in Table 1, together with the cited references allocated according to which effect they seem to describe. In the future authors should make clear which effect is being studied.

This research was supported by the National Research

Council of Canada. D. F. S. is the recipient of a Medical Research Council of Canada studentship.

DAVID F. SARGENT
C. P. S. TAYLOR

Biophysics Department,
University of Western Ontario,
Ontario

[1] Emerson, R., and Lewis, C. M., *Amer. J. Bot.*, **30**, 165 (1943).
[2] Ried, A., *Carnegie Inst. Wash. Yearbook*, **64**, 399 (1965).
[3] Kowallik, W., *Plant Physiol.*, **42**, 672 (1967).
[4] Schmid, G. H., and Schwarze, P., *Physiol. Chem.*, **350**, 1513 (1969).
[5] Pickett, J. M., *Carnegie Inst. Wash. Yearbook*, **66**, 197 (1967).
[6] Kowallik, W., and Gaffron, H., *Nature*, **215**, 1038 (1967).
[7] Laudenbach, B., and Pirson, A., *Arch. Mikrobiol.*, **67**, 226 (1969).
[8] Bendix, S., and Allan, M. B., *Arch. Mikrobiol.*, **41**, 115 (1963).
[9] Lee, D., Sargent, D. F., and Taylor, C. P. S., *Canad. J. Bot.*, **49**, 651 (1971).

Possible Ball Court at La Venta, Mexico

LA VENTA, one of the earliest Olmec sites, is located on an island in the Tonala River which forms the boundary between the Mexican states of Veracruz and Tabasco (18° 10′ N, 94° 5′ W). The site is believed to have been built and maintained during the period from 1000 BC to 600 BC or even later[1], primarily as a ceremonial or religious enclave[2]. For years, no ball court, usually an important feature of sacred Mesoamerican precincts, had been found at La Venta[3].

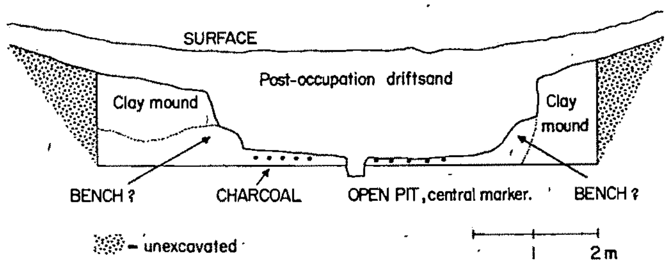


Fig. 1 Cross section through suspected ball court at La Venta, Mexico.

By 1963, at least 267 ball courts had been identified in Mesoamerica[4]. The Mesoamerican ball game is said to have originated in the La Venta culture and to have spread to the Maya and other Mesoamerican cultures[5]. An early stela found on the Veracruz Gulf Coast (the Matisse stela) depicts what is thought to be a ball player with hands upraised in front of his face[6]. Monument thirty-four at San Lorenzo, another Olmec site, also probably represents a ball player[7], while the helmeted colossal Olmec stone heads possibly represent champions of the ball game[8]. At San Lorenzo, ball player figurines have been found in the levels of the San Lorenzo phase which has been placed by radiocarbon dating to a range from 1150 to 900 BC (private communication from M. Coe). It has even been suggested that the ball court heroes were all politicians[9].

A structural complex recently discovered at La Venta, which may be a ball court, consists of two parallel mounds at the southern end of the "Stirling group", each about 14 m wide. That on the west is 55 m long and the other 39 m long[10], which suggests that the ball court was of the open ended type and thus stylistically consistent with other early courts.

A sample of charcoal from the uppermost clay construction layers of the court floor (Fig. 1) has been dated (UCLA-1630) at 2710 years (760 BC) using the newer half-life of 5,730 ± 30 yr. This radiocarbon date is consistent with various others obtained from the site[1,11–13].

The earliest court so far found in the lowland Maya area is probably that at Copan which is the earliest in a superimposed series of three courts and which possibly belongs to the Early Classic period (AD 300–600). This was a comparatively narrow open court, smoothly paved with low slanting ramps on either side and possibly with plaster markers[14,15].

UCLA–1630 seems to be the first radiocarbon date for a ball court, previous dating having been based on methods of varying degrees of accuracy, from ceramic association to assessment of architectural style and hieroglyphic inscriptions[4]. Another suspected ball court of perhaps similar antiquity is that at the Olmec site of San Lorenzo, constructed of earth and clay fill and built during the Palangana phase. This phase appears to have cross-ties to Mamon and to Drucker's La Venta material which places it into the 600–400 BC range (Coe, private communication).

The date of 760 BC would make the feature at La Venta the oldest ball court in Mesoamerica. The oldest definitely identified ball court seems to be that at Monte Alban and attributed to the Monte Alban Period II, which began at about the end of the third century BC, when Maya-like features, including stelae engraved with hieroglyphics, appeared at Monte Alban[6]. The detailed features of this court are unknown, but later Alban and Zapotec ball courts are noteworthy in being fully enclosed and in having two niches in opposite diagonal interior angles of the court instead of rings or other devices[6]. The dating of Monte Alban has been subject to numerous uncertainties[16], and most recently excavations at Lambityeco, near Monte Alban, by John Paddock are said to raise implications that Monte Alban had fallen several centuries earlier than had been believed, leaving a serious gap in dating sequences[17].

This study was supported by the National Science Foundation.

LILLIAN WORTHING WYSHAK
RAINER BERGER

Departments of Anthropology and History
and Institute of Geophysics,
University of California,
Los Angeles

JOHN A. GRAHAM
ROBERT F. HEIZER

Department of Anthropology,
University of California,
Berkeley

Received March 8; revised April 5, 1971.

[1] Berger, R., Graham, J. R., and Heizer, R. F., *Contrib. Univ. Calif. Archaeol. Res. Facility*, No. 3 (Berkeley, 1967).
[2] Heizer, R. F., *Dumbarton Oaks Conf. Olmecs* (Washington, 1968).
[3] Heizer, R. F., *33rd Intern. Cong. Americanists*, **2** (1959).
[4] Clune, jun., F. J., thesis, Univ. California (1963).
[5] Krickeberg, W., in *Precolumbian American Religions* (Holt, Rinehart and Winston, New York, 1968).
[6] Acosta, J. R., in *Handbook of Middle American Indians*, **3** (University of Texas Press, Austin, 1965).
[7] Coe, M. D., America's First Civilization (American Heritage Publishing Co., New York, 1968).
[8] Clewlow, C. W., Cowan, R. A., O'Connell, J. F., and Benneman, C., *Contrib. Univ. Calif. Archaeol. Res. Facility*, No. 4 (Berkeley, 1967).
[9] Coe, M. D., *Dumbarton Oaks Conf. Olmecs* (Washington, 1968).
[10] Heizer, R. F., Graham, J. A., and Napton, L. K., *Contrib. Univ. Calif. Archaeol. Res. Facility*, No. 5 (Berkeley, 1968).
[11] Berger, R., and Libby, W. F., *Radiocarbon*, **10**, 150 (1968)
[12] Berger, R., and Libby, W. F., *Radiocarbon*, **10**, 404 (1968).
[13] Berger, R., and Libby, W. F., *Radiocarbon*, **11**, 197 (1969).
[14] Pollock, H. E. D., *Handbook of Middle American Indians*, **2** (University of Texas Press, Austin, 1965).

[15] Stromsvik, G., *Contrib. Amer. Anthropol. Hist.*, No. 65 (Carnegie Institution of Washington, 1952).
[16] Paddock, J., in *Ancient Oaxaca* (Stanford University Press, Stanford, 1966).
[17] Sullivan, W., *New York Times*, March 16 (1970).

Do Intracellular Concentrations of Potassium or Sodium Regulate the Strength of the Heart Beat?

CERTAIN "inotropic" responses in which the strength of the heart beat increases are accompanied by a loss of potassium from the cells and a simultaneous gain of sodium[1]. The question of immediate interest is whether these changes of cellular ion content and of contractile force—if indeed they are always thus associated—reflect a mechanism which controls the heart twitch or whether they are incidental. We have set out to re-examine this question, much debated in the past but not yet entirely resolved[2].

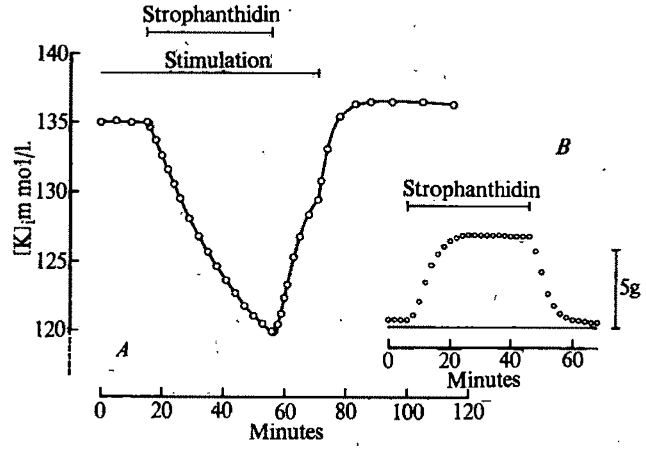


Fig. 1 Effects of 3-acetyl strophanthidin (5×10^{-7} M) on cellular potassium movements (*A*) and twitch tension (*B*). External medium: 0.5 mM $[\text{Ca}]_o$, 2 mM $[\text{K}]_o$, and isethionate substituted for chloride. *A*, Ventricle stimulated for 15 min immediately before start of the experiment, and thereafter for the time indicated by bar (rate 20 min^{-1}). Ordinate: Concentration in cell water. *B*, Peak twitch tension (plotted at 2 min intervals) developed by a ventricle stimulated (at 20 min^{-1}) throughout. (For details of technique, see ref. 14.)

Two parallel series of experiments were made to examine (i) the changes in the twitch tension developed by frog ventricles (*Rana pipiens*) during various inotropic responses, and (ii) the associated net movements of potassium between the heart and perfusion fluid (measured essentially as described in ref. 3, using the improved experimental procedure developed for the study of calcium fluxes[4]). We were thus able to confirm and extend some earlier results by showing that many, though not all, of the inotropic responses examined are accompanied by a net efflux of potassium ions from the heart cells, followed by a net influx during recovery from the inotropic effect. Potassium movements of this kind were quite marked during responses resulting from (i) changes in the stimulus rate ("staircase" phenomena), (ii) reduction of the external potassium concentration, $[\text{K}]_o$, and (iii) application of the cardiac steroid 3-acetyl strophanthidin (see magnitude of the potassium movements in Figs. 1, 3 and 4). Further, all these results were essentially unchanged when the normal perfusate containing 118 mM Cl was replaced by one in which chloride was substituted by larger anions such as isethionate or ethyl sulphate, which are known to penetrate the cell membrane only slowly[5,6]. While this excludes any appreciable contribution of anion movements, the possibility remains that the cation exchange, that is, cellular potassium for external sodium, is essential to the mechanism underlying the inotropic responses. Indeed, two hypotheses invoked to interpret the above and other related results are based on this idea, the earlier correlating strength of contraction with the level of potassium (refs. 1, 7, but compare 8), the later with that of sodium inside the heart cells[9]. In particular, it has been proposed[9,10], and widely accepted[11,12], that changes in cellular sodium concentration are linked with those of "activator" calcium and thus help to regulate contractile activity.

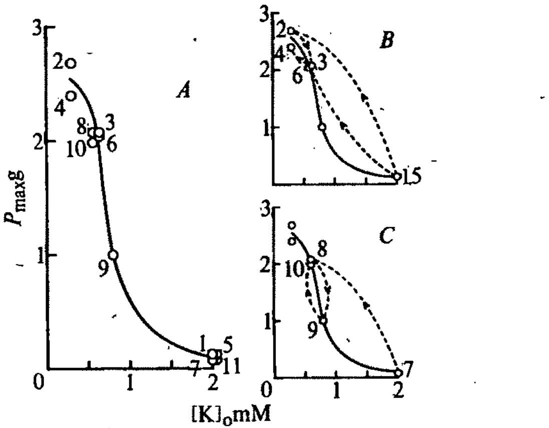


Fig. 2 Steady state twitch heights in media of different $[\text{K}]_o$. 0.5 mM $[\text{Ca}]_o$, isethionate substituted for chloride. Stimulus rate 20 min^{-1}. *A*, Dose-action curve, peak tension P_{max}, versus $[\text{K}]_o$, summarizing results of an experiment; numbers indicate sequence of changes in $[\text{K}]_o$. *B*, and *C*, Schematic representation of the different pathways (in the same experiment) leading from tension level in 2 mM $[\text{K}]_o$ to that in 0.6 mM $[\text{K}]_o$; cf. 1→2→3 and 5→6 in (B), 7→8 and 7→8→9→10 in (C). (Note also the two pathways to tension level in 0.3 mM $[\text{K}]_o$: 1→2 and 1→2→3→4 in (*B*).)

In analysing these ideas further, the subsequent experiments were made in Cl-free media, with isethionate as the substitute anion, to ensure that movements of sodium of essentially the same size, but opposite direction, accompanied the potassium movements which we determined. (Of course, the equivalence is not exact because of the movements of other ions, notably calcium. The change in the intracellular ion balance, however, as produced, for example, by the known calcium movements in similar conditions[13], is too small to affect the validity of the above assumption and of the conclusions to be derived later.)

Three sets of results emerged which are difficult to reconcile with the view that contraction is sensitive to changes in level of cell potassium or sodium. (1) During some of the inotropic responses, twitch tension and level of cellular potassium, $[\text{K}]_i$, altered with markedly differing time courses, the changes of tension being usually more rapid than those of $[\text{K}]_i$. Fig. 1 illustrates this difference by the effects obtained with a submaximal dose of acetyl strophanthidin (5×10^{-7} M; maximum twitch tension was obtained with concentrations greater than 10^{-6} M in otherwise similar experiments). Twitch tension rose to a new steady level within 15–20 min of the application of the drug (Fig. 1*B*), whereas the level of $[\text{K}]_i$ continued to decrease, though at a gradually declining rate, for much longer (> 40 min). The later part of Fig. 1 shows the recovery from the drug action: the tension declined to the original low level (Fig. 1*B*) and the tissue reabsorbed potassium after withdrawal of the drug, and subsequent cessation of stimulation (Fig. 1*A*).

Experiments in which the effects of low $[K]_0$ were examined in the same way as described for acetyl strophanthidin yielded closely similar results—a rapid increase of tension (within 10–20 min) to a steady high level, associated with a progressive loss of potassium (lasting >40 min).

(2) Steady twitch tensions recorded in a low $[K]_0$ medium were not related to intracellular potassium (or sodium) concentrations, which could be adjusted by pretreatment of the ventricles with various low potassium fluids. Fig. 2*A–C* illustrates the steady state tensions in an experiment in which a ventricle was exposed to fluids of various $[K]_0$ for 10–20 min (2 mM being the level in "normal" fluids). As is seen, twitch heights in a 0.6 mM $[K]_0$ medium were little different whether recorded after the concentration step from 2 to 0.6 mM $[K]_0$ or after the more complex sequences which included exposure of the ventricle to either 0.3 or 0.8 mM $[K]_0$. (Compare sequences 5→6 and 1→2→3, and sequences 7→8 and 7→8→9→10 in Fig. 2*B* and *C* respectively). By contrast, potassium release from the cells markedly depended on the sequence of concentration change, being, for example, 7.2 mmol/l. cell water (±0.7, s.e. of five determinations) after the change from 2 to 0.6 mM $[K]_0$, as against 16.3 mmol/l. (±1.2, s.e. of five determinations) after the change through the level of 0.3 mM $[K]_0$. (These results were obtained from ten experiments made in similar conditions to those of Fig. 2.)

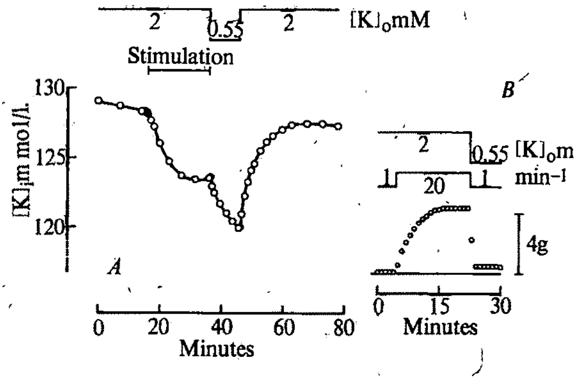


Fig. 3 "Ascending" and "descending" staircase in media of different $[K]_0$. 1.2 mM $[Ca]_0$, isethionate substituted for chloride. *A*, Cellular potassium movements associated with periods of quiescence and stimulation (rate 20 min^{-1}) in 2 mM $[K]_0$, and with a subsequent period of quiescence, first in 0.55 mM and then in 2 mM $[K]_0$. *B*, Peak tension of twitches (plotted at 1 min intervals) in response to a high stimulus rate (20 min^{-1}) in 2 mM $[K]_0$, and to a subsequent low rate (1 min^{-1}) in 0.55 mM $[K]_0$.

It should also be mentioned that pretreatment of heart cells with very low potassium fluids (<0.5 mM $[K]_0$) sometimes affected the subsequent twitch tension which, however, became weaker rather than stronger, possibly as the result of "fatigue" (compare tension levels 2 and 4 in 0.3 mM $[K]_0$ of Fig. 2, and also the results of Fig. 4*B*).

(3) Well defined conditions exist in which changes in twitch tension were associated with potassium and sodium movements in directions opposite to those predicted by any of the hypotheses outlined here. For example, the weakening of heart twitches during a period of reduced stimulus rate ("descending" staircase), which is usually associated with potassium uptake (and sodium loss), also occurred when heart cells were made to lose potassium (and gain sodium) by exposure to a low $[K]_0$ medium during this period. Indeed, the decline of twitch tension so obtained was quite similar to that of a normal "descending" staircase, except that the final steady level of tension was slightly higher in the low potassium fluid (Fig. 3*B*). The loss of potassium during this tension decline was considerable, being comparable with that associated with the build up of tension during the "ascending" staircase (compare cellular K loss during stimulation period in Fig. 3*A*) and was followed by a net gain of this ion by the cells during the final recovery period in normal $[K]_0$ medium.

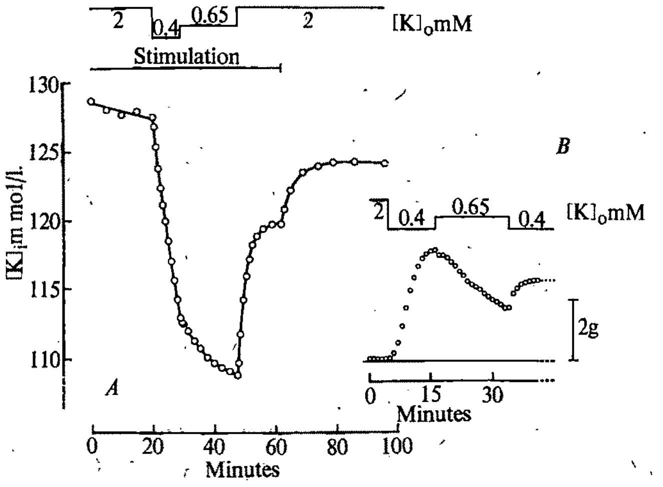


Fig. 4 Effects of several step changes of $[K]_0$ on cellular potassium movements (*A*) and twitch tension (*B*). External medium: 0.5 mM $[Ca]_0$, isethionate substituted for chloride. *A*, Ventricle stimulated as in Fig. 1. *B*, Peak tension of twitches (plotted at 1 min intervals) during continuous stimulation (rate 20 min^{-1}) in media of different $[K]_0$ as indicated. Final dotted line indicates that on return to 0.4 mM $[K]_0$ tension normally attained a plateau, though often of reduced height (compare discussion in connexion with Fig. 2).

Fig. 4 is another illustration of the concurrence of tension changes with ion movements in the "wrong" direction. Here the responses to a series of changes in $[K]_0$ were followed, first, to a step decrease, from 2 to 0.4 mM, which caused tension to rise, then to a smaller step up, from 0.4 to 0.65 mM, which caused tension to fall (Fig. 4*B*). The cellular potassium release (Fig. 4*A*) associated with the first period was in accordance with the hypotheses tested, but not its continuation during the second period, during which the direction of tension change reversed. The final sections of Fig. 4*A* and *B* show the essentially unimpaired responsiveness of the tissue to further tests, even after large and rapid losses of potassium. Thus, (*a*) potassium was taken up again after the re-establishment of normal $[K]_0$, and cessation of stimulation, respectively (Fig. 4*A*), and (*b*) the tension rose once more on again reducing $[K]_0$ (Fig. 4*B*).

In conclusion, although it is still conceivable that cellular potassium for sodium exchanges contribute in regulating the strength of the heart beat, this contribution is unlikely to be at all substantial. More probably, the principal factor common to many inotropic responses resides in the cell membrane, in as yet unknown processes which control the magnitude of cellular calcium fluxes.

This work was supported by a grant from the British Heart Foundation.

D. C. GADSBY
R. NIEDERGERKE
S. PAGE

Department of Biophysics,
University College, London

Received February 25, 1971.

[1] Hajdu, S., and Leonard, E., *Pharmacol. Rev.*, **11**, 173 (1959).
[2] Glynn, I. M., *Pharmacol. Rev.*, **16**, 381 (1964).
[3] Keenan, M. J., and Niedergerke, R., *J. Physiol.*, **188**, 235 (1967).
[4] Niedergerke, R., and Page S., *Pflügers Arch. Ges. Physiol.*, **306**, 354 (1969).
[5] Hutter, O. F., and Noble, D., *J. Physiol.*, **157**, 335 (1961).
[6] Dudel, J., and Rüdel, R., *Pflügers Arch. Ges. Physiol.*, **308**, 291 (1969).
[7] Müller, P., *Circulation Res.*, **17**, 46 (1965).
[8] Green, E. A., Loh, C. K., Lorber, V., and Tyberg, J. V., *Amer. J. Physiol.*, **219**, 1802 (1970.)
[9] Glitsch, H. G., Reuter, H., and Scholz, H., *J. Physiol.*, **209**, 25 (1970).
[10] Reiter, M., in *Calcium and Cellular Function* (edit. by Cuthbert, A. W.), 270 (Macmillan, London, 1970).
[11] *Gaddum's Pharmacology*, Sixth Ed. (revised by Burgen, A. S. V., and Mitchell, J. F.) 104 (Oxford University Press, London, 1968).
[12] Passmore, R., and Robson, J. S., *A Companion to Medical Studies*, 2 (Blackwell, Oxford and Edinburgh, 1970).
[13] Niedergerke, R., Page, S., and Talbot, M. S., *J. Physiol.*, **202**, 58 (1969).
[14] Chapman, R. A., and Niedergerke, R., *J. Physiol.*, **211**, 389 (1970).

Acquisition of Human Antigens by *Schistosoma mansoni* during Cultivation *in vitro*

Schistosoma mansoni may live in the portal circulation of man for many years. In the rhesus monkey, established adult worms persist even though their presence stimulates a powerful host immunity against reinfection with cercariae[1]. We have suggested that this concomitant immunity may be explained if the adult worms possess host antigens[2]; such an immunological disguise would allow these established worms to evade the efferent component of the host's immune response which kills new invading forms lacking the disguise. The existence of these host antigens at the surface of the worms has been demonstrated by transferring worms, grown to maturity in mice, directly into the portal circulation of rhesus monkeys immunized against mouse erythrocytes. Such mouse worms are rapidly destroyed by an antibody-mediated response directed against sites on the worms' surface; mouse worms survive well on transfer to normal monkeys[3].

These mouse antigens are not permanently expressed in the worm, and could no longer be detected using this system when mouse worms had been in the circulation of normal monkeys for 7 days. Nevertheless, there is good evidence that these antigens are not merely gross contaminants of host origin; they seem to be firmly bound to or incorporated into the surface membrane of the worm[3–5]. They do not seem to be mouse IgG immunoglobulin, neither are they Forsmann antigen; but they are antigenically identical to other components of the mouse erythrocyte membrane[4]. They are not present in cercariae but are partly expressed in 7 day schistosomula and fully expressed in 15 day schistosomula[5].

The origin and mode of acquisition of these antigens are unknown. We believe that they are truly of host origin and, by some means, they become incorporated into the surface structure of the schistosome. An alternative hypothesis, favoured by Capron and his colleagues[6], suggests that these antigens are synthesized by the schistosomes and mimic those of the host. It is postulated that the schistosome possesses a range of codes for these antigens and specific selection for a given mimicking antigen takes place when the worm enters a particular host.

We have recently studied the acquisition of host antigens by schistosomula during cultivation *in vitro*. Such a system is much more susceptible to detailed analysis than an *in vivo* system, and it may eventually be possible to select between the hypotheses outlined above. Because *S. mansoni* is primarily a parasite of man, and much is known of human erythrocyte antigens, we grew the schistosomes in human blood.

Schistosomula of a Puerto Rican strain of *S. mansoni*[7] were collected by allowing cercariae to penetrate specially prepared isolated mouse skin; they were then cultured *in vitro* for 15 days (J. A. C. and S. R. S., in preparation). The culture medium consisted of equal volumes of fresh human or monkey serum and Earle's balanced salt solution, lactalbumin hydrolysate at a final concentration of 0.25%, 1% washed human or monkey erythrocytes and antibiotics. The medium was gassed with 8% CO_2 in air to give a *p*H of 7.4. Incubation was at 37° C and the medium was replaced at intervals of 3–4 days, except that the original erythrocyte inoculum was retained. Two ml. of this medium, in a Leighton tube, supported the growth of 200–250 schistosomula. At 15 days, the schistosomula had shown satisfactory development according to the criteria of Clegg[8]; indeed they were more advanced than schistosomula grown *in vivo* in mice for the same period.

Meanwhile, rhesus monkeys were immunized with various types of washed human erythrocytes in Freund's complete adjuvant[3]; control monkeys were injected with adjuvant alone or not treated. Three weeks after primary immunization, the monkeys received a booster intraperitoneal injection of washed erythrocytes. One week later, the 15 day schistosomula were washed in Earle's solution containing 1% monkey serum and transferred to immunized and control monkeys by cannulation of the mesenteric veins[5]. Each monkey received a carefully counted number of schistosomula; the actual numbers transferred varied between 165 and 247 in the first experiment and 105 and 128 in the second. Four or 5 weeks after transfer, when any surviving worms would have reached maturity, the monkeys were killed and perfused and the adult worms were recovered[7].

Table 1 Acquisition of Human Host Antigens by Schistosomes *in vitro*

Monkey No.	Immunization	Erythrocytes in culture system	Worm recovery 5 weeks later (%)
601	FCA	Monkey	67
608	Nil	Monkey	79
602	FCA	AB−	73
607	Nil	AB−	45
603	Anti-AB−	Monkey	103
604	Anti-AB−	Monkey	96
605	Anti-AB−	AB−	3
606	Anti-AB−	AB−	5

FCA, immunization with Freund's complete adjuvant alone.

The human blood used in the first experiment was of type AB Rh−; control cultures were maintained in normal rhesus monkey blood. Table 1 summarizes the plan and the results of this experiment. Control monkeys accepted schistosomula grown in either human or monkey blood with equal readiness and survival rates were high. Anti-AB− monkeys accepted worms grown in monkey blood but almost completely destroyed those cultured in AB− blood. Evidently, these schistosomula had acquired human antigens during their cultivation *in vitro* and these served as targets for the immune response of the anti-human monkeys.

The second experiment was designed to determine whether blood group specific antigens were among those acquired by the schistosomula. Two bloods differing in respect of several major antigens were chosen: A, DE, N−, Fy(a+); and B, dē, N+, Fy(a−). Rhesus monkeys were immunized with one or other type of washed erythrocytes in Freund's complete adjuvant followed by a booster dose intraperitoneally; control monkeys were injected with Freund's complete adjuvant alone. Schistosomula were cultivated for 15 days in media based on these bloods and were then washed and transferred into rhesus monkeys according to the scheme shown in Table 2. Five weeks later, the surviving adult worms were recovered.

Table 2 Specific Blood Group Substances as Host Antigens

Group	Monkey No.	Immunization	Erythrocytes in culture system	Worm recovery 5 weeks later (%)
Control	618	FCA	A+	76
	619	FCA	A+	45
	624	FCA	B−	73
	625	FCA	B−	85
Homologous	614	Anti-A+	A+	3
	615	Anti-A+	A+	3
	622	Anti-B−	B−	1
	623	Anti-B−	B−	3
Heterologous	620	Anti-A+	B−	14
	621	Anti-A+	B−	23
	616	Anti-B−	A+	3
	617	Anti-B−	A+	33

FCA, immunization with Freunds complete adjuvant alone.
Students t test was carried out after arc sin transformation of the data. Control versus homologous: $P<0.001$; control versus heterologous: $P<0.01$; homologous versus heterologous: $P<0.05$

When the transfer was homologous, "A+ worms" into anti-A+ monkeys and "B− worms" into anti-B− monkeys, almost all the schistosomula were destroyed. With heterologous transfer, "A+" worms into anti-B− monkeys and vice versa survival rates were more variable. Certainly, significantly fewer worms survived than in the control monkeys, but more survived than in the homologous transfers. We interpret this result as meaning that the schistosomula acquired, *in vitro*, antigens which were common to the two erythrocyte types but also, to some degree, specific blood group antigens.

We may conclude that the schistosomula of *S. mansoni* can acquire human antigens and that these antigens can be acquired during cultivation *in vitro*. Thus the *in vitro* system appears to offer a valid approach to further studies. Human antigens common to A+ and B− erythrocytes are acquired by schistosomula in culture and there is some evidence that blood group specific antigens are expressed as well. If this last finding can be confirmed, we believe that it supports the view that host antigens are truly of host origin and are not synthesized by the worm. We doubt that the selective synthesis of human blood group specific antigens is within the capabilities of a trematode parasite, even one as well adapted as *S. mansoni*.

We thank Mr John Harris for determining the blood group specificities and Mr Kenneth Gammage for assistance.

J. A. Clegg
S. R. Smithers

National Institute for Medical Research,
London NW7 1AA

R. J. Terry

Brunel University,
London W3 9BX

Received May 13, 1971.

[1] Smithers, S. R., and Terry, R. J., *Trans. Roy. Soc. Trop. Med. Hyg.*, **61**, 517 (1967).
[2] Smithers, S. R., and Terry, R. J., *Ann. NY Acad. Sci.*, **160**, 826 (1969).
[3] Smithers, S. R., Terry, R. J., and Hockley, D. J., *Proc. Roy. Soc.*, B, **171**, 483 (1969).
[4] Clegg, J. A., Smithers, S. R., and Terry, R. J., *Parasitology*, **61**, 87 (1970).
[5] Clegg, J. A., Smithers, S. R., and Terry, R. J., *Intern. J. Parasitol.*, **1**, 43 (1970).
[6] Capron, A., Biguet, J., Vernes, A., and Afchain, D., *Path.-Biol.*, **16**, 121 (1968).
[7] Smithers, S. R., and Terry, R. J., *Parasitology*, **55**, 695 (1965).
[8] Clegg, J. A., *Exp. Parasitol.*, **16**, 133 (1965).

Biphasic and Reversible Adaptation of Goldfish to Alcohol

GOLDFISH are well suited to the study of the effects of alcohol on behaviour. The concentration of alcohol in their blood rapidly approximates the concentration of alcohol in the water in which they swim[1]. Thus the study of tolerance, for example, is not complicated by such variables as rate of alcohol absorption. We have studied certain responses of goldfish as a function of time in an alcohol solution, to learn whether tolerance to alcohol develops, and whether it persists after return to water.

Twenty-four goldfish (*Carassius auratus*), 3–5 cm long, were kept in 100 l. tanks containing tap water. At the start of each session, twelve were randomly assigned to an experimental group and twelve to a control group. The fish were transferred to smaller tanks containing 0.8% ethanol solution (experimental fish) or water (control fish), remaining in the respective solution, one fish per tank, for periods of 0.5 h to 42 h.

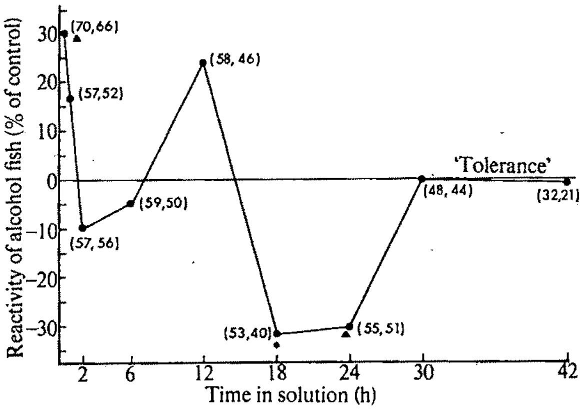


Fig. 1 Biphasic effects of alcohol on goldfish responding to an aversive stimulus after immersion in an 0.8% alcohol solution for varying periods. Each point represents the difference in the mean value of experimental fish from the mean value of concurrently run control fish, expressed as a percentage of the control value. Numbers in parenthesis represent, respectively, the experimental N and control N for each immersion period. Significant differences from control means are indicated by an asterisk ($P<0.05$) and triangle ($P<0.01$). The 12 h response was not significant ($P>0.10$).

We measured an escape reaction to an aversive stimulus. The experimental apparatus consisted of a trough 118 cm long, 3 cm wide, 4 cm deep. At one end was a "start box" 6 cm long, 3 cm wide, separated from the remainder by a trap door. A high intensity light faced the start box, opposite the trap door. For each trial a fish was placed in the start box for 30 s, the light was turned on for 3 s, and the trap door was lifted, allowing the fish to escape from the light. Response was measured as distance, in cm, the fish swam from the light in 5 s. The unresponsive fish or those that moved less than 5 cm were excluded from the analysis so that the numbers of fish in each group are unequal. Each fish was used only once so that they should not become "practised". Experimental and control fish were tested simultaneously, the trough containing the pretest-period fluid. Alcohol in water and blood was assayed by gas–liquid chromatography. Equilibration occurred within 60 min.

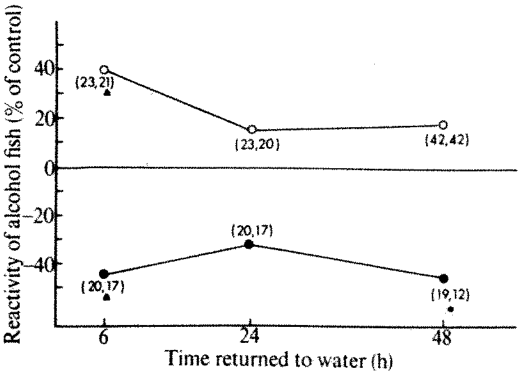


Fig. 2 Fish "tolerant" to alcohol after 42 h of immersion in an 0.8% alcohol solution returned to water for periods of 6, 24, and 48 h. Fish reimmersed in alcohol for 0.5 h continued to show an excitatory response (○). Fish immersed in alcohol for 24 h still showed a depressed response (●). Each point represents the difference in mean value of experimental fish from concurrently run control fish, expressed as a percentage of the control value. Significant differences from control means are indicated by an asterisk ($P<0.02$) and solid triangles ($P<0.01$). Numbers in parenthesis represent, respectively, the experimental N and control N.

Fish in alcohol for 0.5 h were significantly more reactive than control fish (Fig. 1). They also were more reactive after 1 h. Fish in alcohol for 2 and 6 h were a little less reactive. They were more reactive after 12 h. After 18 and 24 h, experimental fish were much less reactive. After this "depressed" phase, the groups responded similarly after 30 and 42 h of immersion.

These results indicate that during adaptation to an 0.8% alcohol solution, when the alcohol blood level rises from zero to 800 mg/100 ml. in 1 h, the goldfish pass through three phases of activity. They are hyper-reactive for about 1 h, then hypo-reactive after about 18 h, and lastly they become "tolerant" after about 30 h.

A second study was conducted to learn whether tolerance persists after return to water. Fish were kept in an 0.8% alcohol solution or water for 42 h as described. Experimental and control fish were then returned to separate water-filled tanks for 6, 24 and 48 h. After each interval, experimental fish were returned to individual tanks containing an 0.8% alcohol solution and controls were returned to individual tanks containing water. After 0·5 h, they were tested in the aversive stimulus trough. Another group of fish were immersed in alcohol or water for 24 h before testing. These periods were chosen because in the original experiment alcohol fish were maximally excited and maximally depressed at 0.5 h and 24 h, respectively. If tolerance persisted after return to water, reimmersion in alcohol could be expected to (1) abolish the excitatory phase at 0.5 h, (2) abolish the depressed phase at 24 h, or (3) do both. This did not occur. Tolerant fish, after return to water, still showed an excitatory phase after 0.5 h in alcohol and a depressed phase after 24 h in alcohol (Fig. 2).

As noted, fish occasionally failed to swim away from the light. The percentage of nonresponders in the experimental and control groups differed markedly. 9% of the alcohol group and 23% of the control group ($\chi^2=49.57$; $P<0.001$) failed to respond. The difference existed each time fish were tested (Table 1). To investigate this more deeply, we repeated the experiment with one change: two fish, instead of one, were placed in the small containers. Tested after 42 h of immersion (Table 1), the percentage of nonresponders was the same in both groups. A tank mate, in short, had the same effect as alcohol in reducing the number of nonresponders.

Table 1 Comparison of Nonresponders in Alcohol and Control Groups

Time in solution (h)	Alcohol group		Controls	
	N	Nonresponders (%)	N	Nonresponders (%)
0.5	75	7	75	12
1	62	8	62	16
2	62	8	62	10
6	61	3*	62	19
12	62	6*	62	26
18	60	12*	61	34
24	60	8	61	16
30	56	14	62	29
42	41	22*	47	55
42 (with two fish)	19	21	24	21

"Nonresponders" refers to fish that swam less than 5 cm from a start box in an aversive stimulus trough, the stimulus being a high intensity light. All fish were kept in single-fish tanks containing either alcohol or water, except in the final trial, when two fish were kept in each tank.

* Significant difference between alcohol and control fish ($P<0.01$).

It seems, therefore, that goldfish can become adapted to alcohol and that the initial response is biphasic. Tolerance of alcohol is rapidly extinguished if acclimated fish are returned to water. The excitatory phase may result from a rapidly rising blood alcohol level, but the depressed phase and subsequent tolerance apparently reflect duration of exposure, since blood alcohol concentration is constant after 1 h of immersion. Finally, "social isolation" of goldfish apparently reduces responsivity to an aversive stimulus, and alcohol cancels this effect.

This work was supported in part by the US Public Health Service and a Research Scientist development award from the National Institute of Mental Health (D. W. G.).

DONALD W. GOODWIN
CAROL P. DOWD
SAMUEL B. GUZE

Department of Psychiatry,
Washington University School of Medicine,
4940 *Audubon Avenue,*
St Louis, Missouri 63110

Received December 14, 1970; revised April 6, 1971.

[1] Ryback, R., Percarpio, B., and Vitale, J., *Nature*, **222**, 1068 (1969).

Larval Development and Metamorphosis of *Acanthaster planci* (Asteroidea)

LITTLE is known of the factors influencing survival during the planktonic phase of *Acanthaster planci* (Linnaeus), the crown of thorns starfish, apart from an observation of fish eating newly released eggs[1]. But it is not surprising that this phase has been suggested as the cause of the recent plague of the animal in the Pacific[2–4], for large females release many millions of eggs each spawning season[1] and any factor which slightly reduces the mortality rate during planktonic life and settlement greatly increases recruitment into the starfish populations.

In this study *A. planci* was reared in the laboratory from fertilization to juvenile starfish and a preliminary study has been made of the influence of temperature, salinity, food and substrate on survival and development. Previously, Henderson[5] and Mortensen[6] reared *A. planci* to late bipinnaria and brachiolaria stages, respectively.

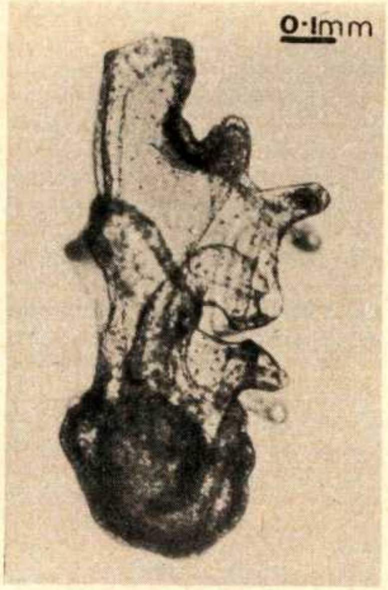


Fig. 1 Lateral view of an early brachiolaria of *Acanthaster planci*.

Mature starfish were collected from a cay 40 miles ENE of Cardwell, North Queensland (146° 51′ E, 18° 2′ S), on January 22, 1971; larvae were obtained by the method of Henderson[5]. On day 3 the larvae were transferred to 10 l. glass aquaria containing seawater, $33^{0}/_{00}$ salinity, with 50 IU penicillin G and 0.05 mg streptomycin sulphate/ml. A mixture of unicellular algae was added on days 3 and 9. On day 15 the larvae were transferred from the Fisheries Laboratory, Mourilyan, to James Cook University and placed in glass dishes, about 100 per dish containing 250 ml. of filtered seawater, in a shaker bath operating at forty-five shakes per minute. The larvae were transferred to fresh conditions every few days and unicellular algae added daily. Dishes were maintained at 24°–25° C until day 21, then some were raised to 28°–29° C by day 26.

Bipinnariae developed in the manner described before[5,6]. After day 23 some larvae developed two brachiolar arms on the pre-oral ciliated band; the pre-oral lobes enlarged, an adhesive disk developed and the posterior portion containing the starfish primordium became enlarged, irregular and brown (Fig. 1). The pre-oral lobe apex then developed dorsal and ventral projections, the latter becoming the third brachiolar arm (Fig. 2). Mortensen's brachiolaria[6] is similar, but the pre-oral arms are obscure and the primordium is poorly developed.

Late brachiolariae tested substrates with the brachiolar arms and settlement began on day 28. Some remained attached by the adhesive disk throughout metamorphosis; others either failed to attach or detached. Metamorphosis into a five-armed starfish, with absorption of larval structures, took two days. The early starfish were 0.3–0.5 mm diameter and each arm carried a red pigment spot, a primary podium and two pairs of tube feet on its oral surface (Fig. 3). Juvenile starfish, apparently still feeding on the unicellular algae, grew 0.1–0.2 mm in diameter in 10 days with subdivision of the arms and development of the mouth, spines and ossicles (Fig. 4).

Many larvae failed to reach brachiolaria or metamorphosis and regressed to a ciliated, globular form. Larvae completed development at 28°–29° C, while those remaining at 24°–25° C did not advance beyond early brachiolaria. The mean period from fertilization to starfish was 38 days with a range of 30 to 47 days. In other studies, *A. planci* larvae developed more rapidly, developing to late bipinnaria in 12 days at 27° ± 1° C[5] and to brachiolaria in 16 days[6]. Thus survival and developmental rate vary markedly within a small temperature range. Development may be more rapid in the field than in this study with a long period at 24°–25° C—Barrier Reef water temperatures are 27°–30° C during the breeding season.

A. planci bipinnariae tolerate abrupt salinity changes from $36^{0}/_{00}$ to $21^{0}/_{00}$[5]. Late brachiolariae and metamorphosing forms are much less tolerant and many ruptured when transferred from $33^{0}/_{00}$ to $35^{0}/_{00}$ salinity. The ruptures were invariably from the starfish primordium and earlier stages in

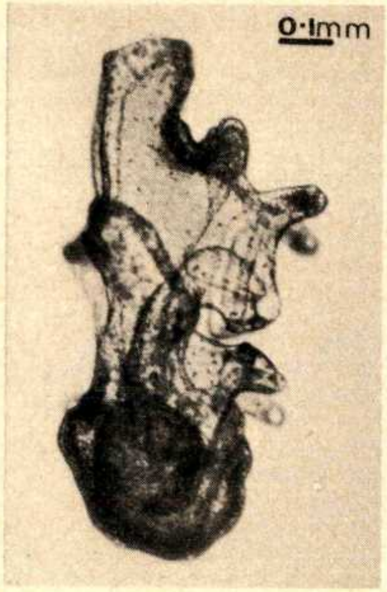


Fig. 2 Lateral view of a late brachiolaria of *Acanthaster planci* showing the three brachiolar arms (1) and adhesive disk (2) (preserved specimen).

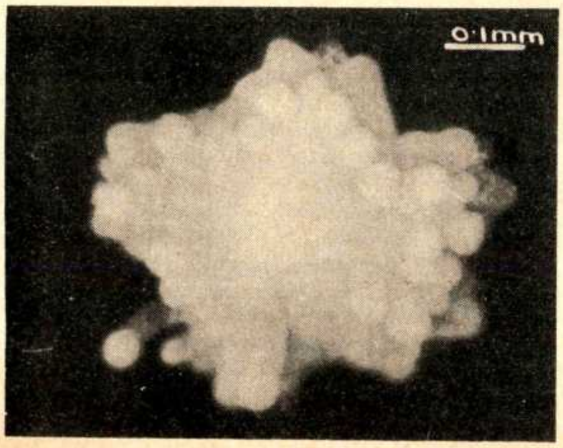


Fig. 3 Oral view of juvenile starfish of *Acanthaster planci* one day after metamorphosis (preserved specimen).

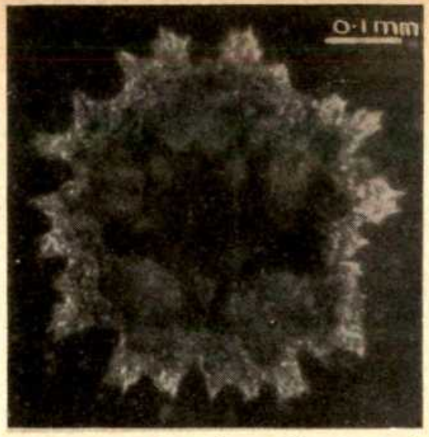


Fig. 4 Aboral view of juvenile starfish of *Acanthaster planci* 14 days after metamorphosis.

the cultures were unaffected. The metamorphosis of ruptured larvae was abnormal.

Larvae fed a mixture of unicellular algae, *Isochrysis galbana*, *Gymnodinium* sp., *Amphidinium* sp., *Cyclotella nana* and *Dunaliella primolecta*, had a predominance of the last alga in their stomachs and some were fed solely with *D. primolecta* from day 22. These larvae metamorphosed at the same time as those on the mixed diet.

Brachiolariae showed a degree of substrate selection at settlement, as observed in other echinoderm larvae[7]. Late brachiolariae kept for up to 14 days in clean, glass dishes without additional substrates failed to metamorphose and some regressed. Simultaneously larvae metamorphosed in other dishes in the presence of encrusting algae and algal detritus. With the exception of a larva which attached to an algal strand on dead coral, *Pocillopora damicornis*, larvae only settled on the bottoms of dishes and there was no significant difference between settlement rates in dishes to which the following substrates were added: macroscopic algae, *Cladophoropsis* sp.; serpulid tubes; fine, siliceous sand; lithothamnion; live *P. damicornis*; *A. planci* spines and tube feet and recently depredated *P. damicornis*.

The period of the *A. planci* life cycle between the earliest starfish, 0.3–0.5 mm diameter, and the smallest, field specimen, 11 mm diameter, collected from coral[1], is still to be studied. This important period includes the transition to adult feeding behaviour.

We thank Mr R. G. Pearson and Mr M. Lamont for their assistance and Professor C. Burdon-Jones for his advice in preparing the manuscript. The work was supported by the Government of Queensland and James Cook University of North Queensland.

J. A. HENDERSON

Department of Primary Industries,
Fisheries Branch,
Harbours and Marine Buildings,
Brisbane, Q 4000

J. S. LUCAS

School of Biological Sciences,
James Cook University of North Queensland,
Box 999 *PO,*
Townsville, Q 4810

Received April 26; revised June 7, 1971.

[1] Pearson, R. G., and Endean, R., *Fish. Notes Qd*, **3**, 27 (1969).
[2] Endean, R., *Report on Investigations made into Aspects of the Current Acanthaster planci (Crown of Thorns) Infestations of Certain Reefs of the Great Barrier Reef* (Fisheries Branch, Queensland Department of Primary Industries, Brisbane, 1969).
[3] Chesher, R. H., *Science*, **165**, 280 (1969).
[4] Vine, P. J., *Nature*, **228**, 341 (1970).
[5] Henderson, J. A., *Fish. Notes Qd*, **3**, 69 (1969).
[6] Mortensen, T. H., *K. Danske Vidensk. Selsk. Skr.*, **94**, 1 (1931).
[7] Thorson, G., *Biol. Rev.*, **25**, 1 (1950).

New Hypothesis of the Cause of Cytoplasmic Incompatibility in *Culex pipiens* L.

IF males of one strain of *C. pipiens* are crossed with females of a strain from a different geographical area, the number of offspring may be either normal or small, or there may be none at all[1]. These three results have been called compatible, partially compatible and incompatible, respectively. Reciprocal crosses may give the same or different results. For example, strain A males may be fully compatible with strain B females, but strain B males may be compatible, incompatible, or only partially compatible, with strain A females.

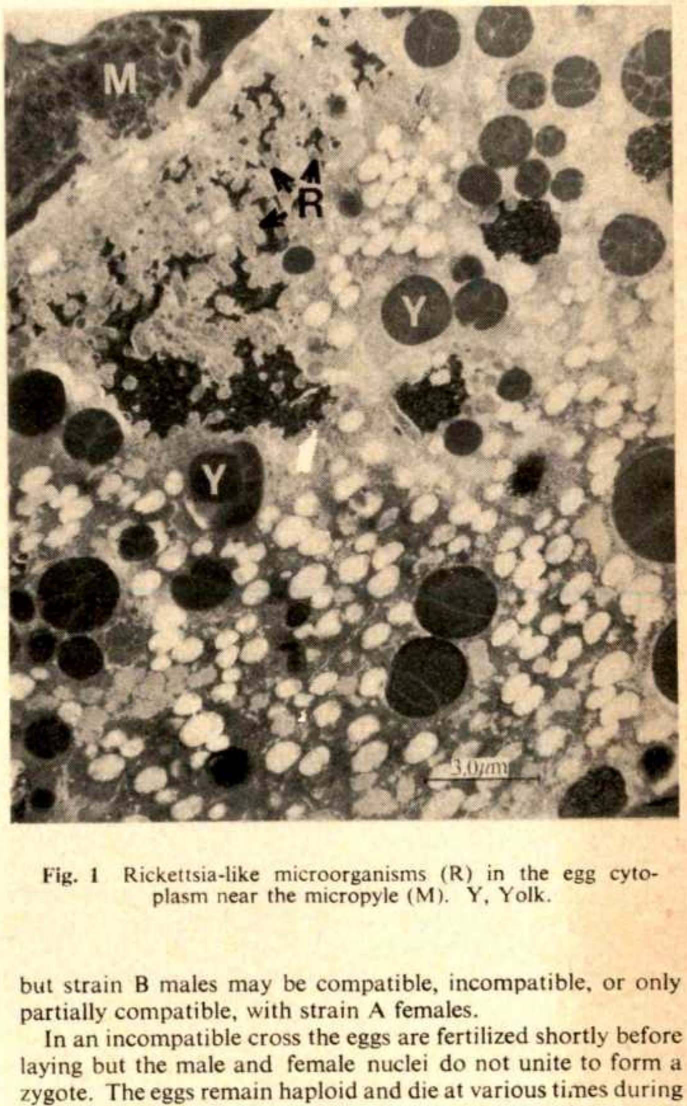


Fig. 1 Rickettsia-like microorganisms (R) in the egg cytoplasm near the micropyle (M). Y, Yolk.

In an incompatible cross the eggs are fertilized shortly before laying but the male and female nuclei do not unite to form a zygote. The eggs remain haploid and die at various times during embryogenesis[2]. Incompatibility is inherited strictly through the female, as shown by Laven's experiments on genome replacement[1]. For this reason the incompatibility is said to be cytoplasmic.

In a partially compatible cross many eggs die, sometimes before fertilization and sometimes after, so that embryonic death cannot be ascribed to haploidy[3]. It may be very difficult to distinguish between incompatible and partially compatible crosses. True hybrids may be produced in presumably incompatible crosses at a very low frequency, possibly once in 5 or 10,000 incompatible eggs (unpublished results of J. H. Y.).

Although a number of hypotheses have been put forward to explain the inheritance of cytoplasmic incompatibility, none of these offers an adequte explanation and none suggests how it might be caused[1,4,5]. We wish to propose a new theory based on a cytoplasmically inherited infectious agent. A rickettsia-like microorganism has been found in substantial numbers in the adults, eggs and embryos from normal and incompatible crosses of *C. pipiens*. These microorganisms are found in the follicles of the ovaries and in recently laid eggs, they are especially abundant near the microphyle (Fig. 1). They are approximately the size of mitochondria but of variable size. In sections prepared for electron microscopy they are seen to be elongate or spherical bodies surrounded by two membranes, and are often seen in association with accumulations of glycogen (Fig. 2). The sperms enter the egg through the micropyle and must pass near or through the mass of microorganisms before they can approach the female nucleus which lies midway down the length of the egg. It is possible that the microorganisms have a deleterious effect on presumably "incompatible" sperm so that they are unable to participate with the oocyte nucleus in the formation of a zygote. The microorganisms appear to be well adapted symbionts of their normal hosts, the particular strains of mosquitoes in which they are normally found, but may be deleterious to sperms of other strains.

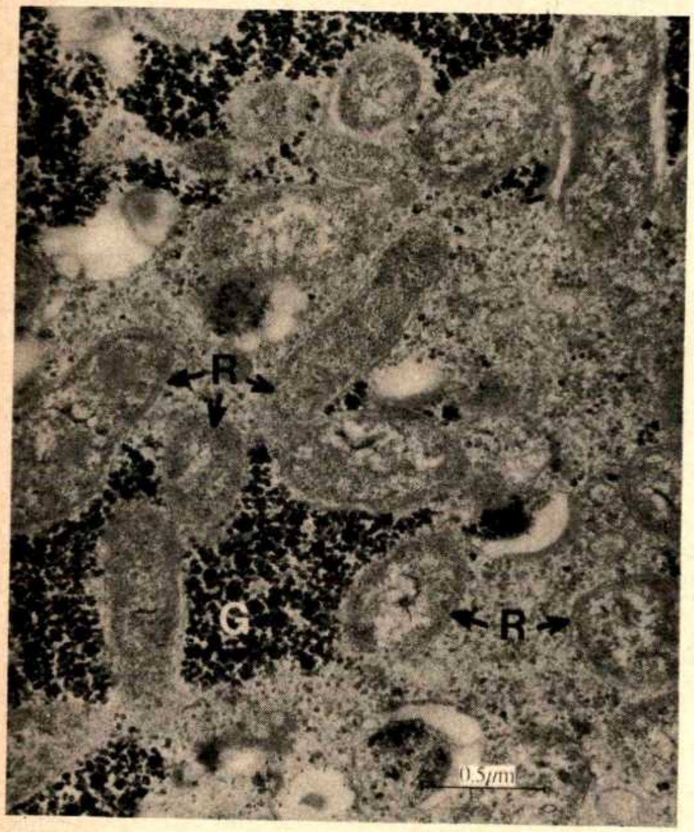


Fig. 2 Enlarged view of the microorganisms in the egg. G, Glycogen; R, rickettsia-like microorganism.

Hertig[6] described a rickettsia-like microorganism, *Wolbachia pipientis*, from *Culex pipiens* from Boston, Massachusetts. He also found them in specimens from Minnesota and China. Similar microorganisms have been found in other members of the *C. pipiens* complex, in *C.p. quinquefasciatus* Say in Colombia[7] and in the autogenous form of *C. pipiens* from England[7] and France[8]. Hertig failed to find them in other species of *Culex* in Massachusetts and Zulueta in other species of mosquitoes in England although he did find them in some species of forest culicines in Colombia. The microorganisms have been found in all the specimens of *C. pipiens* which have been examined.

The presence of coadapted combinations of *C. pipiens* and infectious agents may explain the spectrum of crossing types observed in *C. pipiens*. The microorganisms are consistently present in the reproductive organs of the mosquito throughout its life cycle and probably kill some follicles and sperm in each generation. If it is assumed that males and females of a strain of *C. pipiens* have the same strain of microorganisms but mosquitoes of different strains have somewhat different microorganisms, then in compatible crosses, the sperm of, for example, strain A which have survived the effect of the microorganisms in the developing testes, are not killed when they enter the eggs of females of strain A which contain the same strain of microorganisms. The sperm fuse with the oocyte nuclei and produce normal larvae. The same sperm from strain A when exposed to a different strain of microorganism, as, for example, in the eggs of strain B, are adversely affected and are prevented from forming normal diploid embryos. In these cases incompatibility is observed and the cross produces no offspring except for an occasional parthenogenetic female. In the reverse cross the effect on the sperm may not be lethal and in this case nonreciprocal incompatibility is the result. Partial compatibility is seen when the microorganisms of a strain of mosquito are not lethal to all sperms from another strain of mosquito. Some sperm survive passage through the mass of microorganisms and unite with the female nuclei to form diploid embryos although some of these succumb to infection later in embryogenesis. Hybrids which survive have gonads which are infected with the maternal strain of microorganism. The gametes they produce have survived the infection, so no effect of the infection is seen in F_2 matings.

The elucidation of the mechanism of cytoplasmic incompatibility in *C. pipiens* is of utmost importance because of its potential use in the control of medically important mosquitoes and because it may clarify our concept of speciation in the *C. pipiens* complex. Our proposed explanation for incompatibility provides a starting point for the solution of these two problems, control and speciation. Much work needs to be done to prove that the microorganisms do in fact cause incompatibility. Infectious agents are known to cause a number of unusual effects in other Dipterans such as sex-ratio distortion[9], CO_2 sensitivity[10] and male hybrid sterility[11]. Cytoplasmic incompatibility has also been found in the *Aedes scutellaris* group of mosquitoes[4] and a rickettsia-like microorganism has been reported from a related species, *A. aegypti*[12].

We have found a rickettsia-like microorganism, possibly *Wolbachia pipientis*, in eggs from incompatible and normal crosses of *Culex pipiens*. It is found in the cytoplasm of the eggs and other cells of the mosquito but not in mature sperms. The presence of the microorganisms in the maternal cytoplasm and their transovarial transmission suggests that they might be the cause of cytoplasmic incompatibility.

This research was supported by a Public Health Service research grant from the Center for Disease Control, Atlanta, Georgia, and by a Public Health Service training grant.

JANICE HARUMI YEN
A. RALPH BARR

School of Public Health,
University of California,
Los Angeles

Received May 24, 1971.

[1] Laven, H., *Z. Indukt. Abstamm. Vererbungslehre*, **88**, 478 (1957).
[2] Jost, E., *Wilhelm Roux' Archiv.*, **166**, 173 (1970).
[3] Barr, A. R., *Proc. Calif. Mosq. Control Assoc.*, **37**, 19 (1970).
[4] Smith-White, S., and Woodhill, A. R., *Proc. Linn. Soc. NSW*, **79**, 163 (1954).
[5] McClelland, G. A. H., *Genetics of Insect Vectors of Disease*, **277** (1967).
[6] Hertig, M., *Parasitology*, **28**, 453 (1936).
[7] Zulueta, J. de, *WHO/VB*/**125.65**, 69 (1965).
[8] Callot, J., *Ann. Parasitol. Human Comp.*, **25**, 354 (1950).
[9] Poulson, D. F., and Sakaguchi, B., *Science*, **133**, 1489 (1961).
[10] L'Heritier, P., *Adv. Virus Res.*, **5**, 195 (1958).
[11] Kernaghan, R. P., and Ehrman, L., *Chromosoma*, **29**, 291 (1970).
[12] Sellards, A. W., and Siler, J. F., *Amer. J. Trop. Med.*, **8**, 299 (1928).

BOOK REVIEWS

Old World Revisited

Old World Monkeys (Evolution, Systematics and Behaviour). Edited by J. R. Napier and P. H. Napier. Pp. xvi+660. (Academic Press: New York and London, 1970.) £9.50.

THIS volume contains nineteen contributions from a symposium in Burg-Wartenstein (July 1969), sponsored by the Wenner Gren Foundation. The primary intention was provision of a basis for revising the systematics of Old World monkeys (superfamily Cercopithecoidea), and that aim has been admirably fulfilled. The papers provide an excellent source of up to date information on a number of key topics. In particular, it is invaluable to find in one volume a review of the fossil history of the Old World monkeys (Simons), a concise survey of biochemical aspects of monkey evolution (Barnicot and Wade; Sarich), an account of the oft neglected area of Sundaland (Medway), a series of papers on the behaviour of forest-living monkey species (Lowe's Guenon–Bourlière *et al.*; Drill–Gartlan; Gray Langurs–Ripley), a fascinating account of cross-species modification of social behaviour in baboons (Kummer *et al.*), and an annotated classification (Thorington and Groves). Among the behavioural papers, Struhsaker's account of the relevance of *Cercopithecus* vocalisations for classification is particularly noteworthy. Academically, the overall product is a highly useful and stimulating book, for which tribute should be paid to the sponsors and the editors. This book should prove to be essential for anyone concerned with Old World monkeys and their evolution.

Interest in biochemical aspects of Primate evolution is growing rapidly, and outsiders will find the balanced account provided by Barnicot and Wade both readable and informative. Evolution operates through modifications in the structure of DNA, and such changes can be directly expressed in protein structure. Ideally, an analysis of amino-acid sequences in various proteins should provide highly reliable data on evolutionary change. Actual sequencing is rare, however, and most workers estimate amino-acid change largely with electrophoretic techniques (Barnicot and Wade) or through immunological cross-reaction (Sarich). In spite of the limitations of such techniques, Sarich claims to be able to provide an absolute evolutionary time scale, given calibration of *one* point from the fossil record. It is unfortunate that he should have selected an extremely questionable date (that of the divergence of Cercopithecoidea and Hominoidea) for calibration, and that he should have proceeded to a controversial dating for the divergence of apes and man, where the fossil record is comparatively good. As Thorington hints in his introduction, new techniques of this kind are best tested by comparison with existing classifications, rather than *vice versa.* Eventually, biochemical data will provide a precise tool for investigating evolutionary relationships; but there are still many difficulties of *interpretation* to be mastered. Nonetheless, it does emerge quite clearly from both biochemical contributions that the New World monkeys are probably not separately derived from prosimian ancestors; although the date of divergence is not clear, it seems obvious that there was a specific stock which gave rise to all the monkeys and the apes.

A review of the fossil history of the Old World monkeys has long been overdue, and Simons does much to clarify a somewhat confused situation. There is, however, one conspicuous omission. Simons states that *Apidium* should be classified within the Cercopithecidae, and he has also suggested that *Oreopithecus* is a close relative of *Apidium* (Simons, E. L., *Scientific American,* **211**, 50; 1964). Since this implies that *Oreopithecus* is an Old World monkey (contrary to Hürzeler's interpretation), adequate treatment of this fossil would have been welcome. Simons also gives the impression that available fossils are sufficient for zoogeographical interpretation, which is not the case. His apparent conflict with the views expressed in Napier's stimulating discussion of palaeoecology and evolution should be viewed in this light.

Typographically, this book does not do justice to its academic importance. The mixture of (sometimes inaccurate) typescript with neat printing is unpleasing, and it is disruptive to find the figures and tables grouped at the ends of the papers. There are many typing errors, one of which has led to the surprising statement in Ripley's paper that "The food of the grey langurs consists primarily of termite clay" (page 486). Even on the book jacket, there is a reference to "ethololologists" (?). Presumably, the justification for this layout could be that of economy; but such economy is not evident in the high price quoted.

R. D. MARTIN

Drugs in Confusion

Drug Dependence. Edited by Robert T. Harris, William M. McIsaac and Charles R. Schuster Jr. (Advances in Mental Science II.) Pp. xiv+342. (University of Texas: Austin and London, November 1970. Published for the Faculty for Advanced Studies of the Texas Research Institute for Mental Sciences.) 95*s*.

IN spite of the title, this is a miscellaneous collection of papers given at the "second annual symposium sponsored by the Texas Research Institute of Mental Science". The date of the meeting is nowhere mentioned but from internal evidence it seems to have been in the first half of 1968. There are twenty-four contributions of varying quality, arranged under the general headings "Biological Aspects" (eight papers); "Pharmacological Aspects" (three); "Behavioral Aspects" (five); "Therapeutic Programmes for Drug Dependence" (five) and "Social Aspects" (three). If the first group is redistributed (drug dependence is a biological phenomenon), the last sub-divided into "Penal Aspects" and "Social Aspects", and one or two of the most obviously misclassified papers helped into more appropriate company, we get pharmacology, nine; behaviour, seven; treatment, two; law and punishment, four, and society, two.

This kind of "balance" is fairly representative of collections of this kind, and it may well be due in part to the difficulty of collecting hard data in soft areas; although it should be pointed out that "hard" is not necessarily synonymous with "reliable" or even "objective", and is seldom coincidental with "relevant". But of course the hard scientists always have the best of that argument—to paraphrase the little girl (or, some say, the old lady), they can and do claim "you can't tell if it's relevant if you don't give me a grant". Another kind of imbalance is more worrying, however, and it is hard to say if it is exaggerated or lessened by mixing scientists from different disciplines together in this way. I mean the trespassing of scientists, sometimes eminent ones, into fields of which they are not landlords, nor even tenants, and where they describe the scene, enunciate laws, or give directives on the authority of their absentee reputation instead of the evidence before them. Is it my own prejudice that social scientists are less inclined to this kind of bad manners than basic ones? Probably; for example, the only piece of discussion (of which there must surely have been more than this) that the editors have thought worth including follows a paper by the assistant director for science of the United States Bureau of Narcotics and Dangerous Drugs; their purpose in doing so is perhaps to remind him that the high tension passing through the barbed wire does not respect the direction from which the cow approaches it.

On the other hand, the two "social" authors seem to me to grow in stature by putting their roots down firmly on their side of the fence. Helen Nowlis, cool and wise as ever, emphasizes that there are two separate drug problems—a medical problem and a people problem; that student drug use is a social problem that extends far beyond drugs; and that "to rely on presenting the effects of drugs on the body is not enough".

Dr Bueno, on the other hand, ends his paper "The Problem of Drug Addiction (*sic*) in Mexico", and indeed the book as a whole: "their lies are so clear to their hearts and senses that I fear they'd rather die than renounce them, if not treated with intelligence, so strongly do they persist in their doctrines and superstitions". One might be pardoned for thinking that he is referring to the current debaters about certain problems of drug dependence; in fact, he is quoting from an early seventeenth century *Manual for the Ministers to the Indians.* Perhaps we need a twentieth century "Manual for Professors ministering to Each Other".

C. R. B. JOYCE

Inside Plants

Introduction to the Fine Structure of Plant Cells. By Myron C. Ledbetter and Keith R. Porter. Pp. ix+188 (51 Plates). (Springer–Verlag: Berlin and New York, 1970.) 54DM; $14.80.

LEDBETTER and Porter have produced an outstanding collection of plant electron micrographs. Probably no other laboratory in the world could bring forward a group of such sustained high quality across such a wide range of material. Such atlases, which have now been emerging regularly from various laboratories for about seven years, conveniently chart the progress made in the techniques for the preparation of plant material. That plant techniques have now, in the best laboratories, caught up with animal preparative techniques, can be demonstrated by comparing one of the first atlases (Stephen Hurry's excellent *The Microstructure of Cells* of 1964) with this production.

The text, however, which is generous compared with most atlases and well referenced, has limitations. The highly critical approach, applied to the quality of the micrographs, has not been applied to the text. Several statements are either wrong or misleading, for example, "Apparently the S_3 layer has a higher content of matrix substances (pectins, lignins and *sporopollenins*) . . ." (page 61); "The cuticle . . . is in turn impregnated with . . . waxes and oils, which . . . render it resistant as well to insect pests and the invasion of microbial and viral diseases" (page 109); "The large size of mesophyll cells is due in part to their relatively large vacuole" (page 133); "The absence of starch from the plastids reflects only the inability of permanganate to fix it . . ." (page 161); ". . . the Ubisch bodies, which are absorbed onto the surfaces of the pollen grains" (page 168).

Other statements may be true, but have no scientific backing to my knowledge, for example ". . . the plasmodesmata provide for the intercellular movements of large and small molecules . . ." (page 49); ". . . the Casparian strip assures that entering ions must pass through the plasma membranes of the endodermal protoplasts . . ." (page 81); "Cutin . . . Produced by epidermal cells, it moves through their primary walls and floods out over their exposed surfaces . . ." (page 113).

Plate 6.3 is labelled "Collenchyma". It is, however, a filament cell, at best a very atypical form of this type of cell and not representative in a number of ways. The structure of companion cells is given in detail, but no mention is made of the fact that they are absent in all gymnosperms, in a few angiosperms as well, and in all angiosperms in early stages of development. Cutin chemistry is much better understood than is suggested on page 113. Lignin (page 109) is an infrequent component of the epidermal cells of higher plants.

In short, the micrographs of this work and their references may be used as the most up to date and (with regard to the micrographs) the most technically accomplished of their kind available in a collected form. But the text must be treated with great care.

B. E. JUNIPER

Comfort of Clothes

Clothing: Comfort and Function. By Lyman Fourt and Norman R. S. Hollies. Pp. ix+254. (Dekker: New York, November 1970.) $14.50; £6.90.

THIS book is concerned with the interactions of clothing with the thermal balance between man's metabolic production of heat and his exchange of heat with the environment. Readers interested in this subject will doubtless be familiar with the classic *Physiology of Heat Regulation and the Science of Clothing* by L. H. Newburgh (Saunders, Philadelphia and London, 1949). The present book discusses more recent work which has added to basic knowledge of the subject, and includes information derived from studies of the comfort properties of military clothing conducted in government research laboratories in the United States and elsewhere.

The book deals exclusively with the interaction of "normal" clothing as worn by the military and by civilians in everyday life with the transfer of sensible and evaporative heat between man and his environment. It does not deal with special clothing using auxiliary power for heating or cooling inside the clothing (such as space suits) nor with the mechanical properties of clothing (strength, durability and so on).

The first chapter discusses various quantities and units used in clothing comfort studies, and the second considers clothing as a quasiphysiological system, including discussion of the air content of fabrics and clothing systems and the effects of wind and body motion on the thermal balance. The third chapter consists of a discussion of clothing types for different parts of the body.

The next chapter, concerned with heat and moisture relations in clothing, describes test methods involving apparatus and human subjects. The latter aspect of such studies is treated in more detail in the fifth chapter. There is an account of various physical properties of clothing and clothing materials in relation to comfort, including among others air and water permeability, flammability, static electricity and radiant heat characteristics, in the sixth chapter. Next, natural and man-made fibres are compared with respect to the comfort properties of fabrics made from them, and in a short final chapter the authors discuss briefly current trends and developments involving new materials and finishes, new techniques and new concepts of clothing design.

This book is an extensive review of published works with 477 references. For this alone it is valuable and will be useful to people concerned with the comfort of clothing in spite of a few misprints and errors.

W. H. REES

The Seas are Changing

The Sea: Ideas and Observations on Progress in the Study of the Seas. Edited by Arthur E. Maxwell. Vol. 4: New Concepts of Sea Floor Evolution. Part 1: General Observations. Pp. xi+791. £15.25. Part 2: Regional Observations, Concepts. Pp. xii+664. £15.50. (Wiley-Interscience: New York and London, June 1971.)

THE third volume of *The Sea*, edited by Maurice Hill, was published in 1963 and dealt with the Earth beneath the sea. It provided, and still provides, an excellent introduction to the techniques, theory and fundamental results of marine geology and geophysics. However, within a few years of its publication the whole conception of the origin of the deep-sea floor had undergone a complete revolution, and those parts of the third volume that dealt with ocean crust structure and evolution began to look in need of refurbishing. Now we have the fourth volume, dedicated to the memory of Maurice Hill, and entitled *New Concepts of Sea Floor Evolution*, which takes the appropriate parts of the previous volume, and revises and expands them in the light of the new plate tectonics. The expansion is considerable. What was about 500 pages now occupies 1,455, split into three parts and bound as two books. The first book contains part I, "General Observations", which is essentially a revised and expanded version of the material in the third volume, introducing some new topics, such as petrology and seismology, and concentrating on techniques and global summaries of results. The second book is new material. Part II, "Regional Observations", consists of a number of regional syntheses covering different parts, but not all, of the deep ocean floor and continental margins. The articles generally combine results from several geological and geophysical techniques to arrive at statements about the structure and origin of the areas under discussion. Part III, "Concepts", contains two articles, by Vine and Hess, and by J. T. Wilson, which set the general and regional observations into the global framework of plate tectonics.

A volume like this must have two distinct aims, to interest active scientists with new syntheses and ideas, and to build up a fundamental framework, to act as an archival source for the future. The first aim is not well met here, chiefly because of publication delays. Each article is dated, and dates range from April 1968 to July 1969, with most in the range July 1968 to February 1969. Things have moved a bit since then, in particular with the arrival of results from the JOIDES drilling programme in the deep oceans, and with the realization of the importance of triple junction evolution in the geological history of plate margins. Thus only a few of the articles make an impact as original and new, though in this class must come the thoughtful article on the structure of the Pacific basin by Shor, Menard and Raitt (book 2), and that on the benthic boundary layer by Wimbush and Munk (book 1).

The second aim, though of less immediate interest, is particularly important in determining the life of a book, and this aim is much better satisfied. Many of the authors have taken a great deal of trouble in assembling references, in giving accounts of results published in widely scattered papers, and in drawing the results together to make a solid framework. This is particularly successful in the section on regional observations, where such papers as that on the Mediterranean by Ryan, Stanley, Hersey, Fahlquist and Allan, and that on Pacific boundary structure by Hayes and Ewing will be invaluable as sources of assembled basic information. The price of the two books, £30.75, will be a considerable deterrent to the private purchaser. My own advice would be to make sure that your library gets both books, while if you feel that you can afford only one, buy the second book, with parts II and III, rather than the first. On the other hand, if you get a chance to review a copy, do not hesitate, it is certainly time well spent.

J. R. CANN

Cambrian Collection

Cambrian of the New World. Edited by C. H. Holland. Pp. 456. (Wiley-Interscience: London and New York, April 1971.) £10.50.

THIS is the first volume of a series designed to describe the Lower Palaeozoic rocks of the world. As Professor Charles Holland puts it, the intention is to provide an up to date, yet reasonably circumscribed survey illuminated by the authors' own views but retaining some measure of common organization. If the series can maintain the standard of the first volume this aim should be achieved with success.

The book contains six contributions: an account of the Cambrian of the stable interior of the United States by Christina Lochman-Balk; descriptions of the Cambrian of the Great Basin to the west and of the Cambrian of the Appalachians and New England to the east by A. R. Palmer, together with three chapters which summarize the Cambrian of Canada and Alaska, by F. K. North, of the North American Arctic, by J. W. Cowie and of South America, by A. V. Borrello. They are all clearly presented accounts, written by authors of considerable experience. Judging from the bibliography the text seems to have been completed about two years ago, but there is much new and previously undocumented material, particularly on the Cambrian of the west of North America.

An attractive feature is the manner in which the authors hand on unpublished material to their successors and go out of their way to point out the possibilities for future work which might lead to definitive syntheses. It is to be hoped that this approach attracts stratigraphical palaeontologists to the rich fields awaiting them. As Palmer remarks, as many as seven different biostratigraphical entities can be recognized in the Early Cambrian sequences of the Great Basin.

It goes without saying that a central theme in this book is the record of transgressions and regressions of the Cambrian seas over the Americas. At some time or another in the Cambrian, a part of every state in the Union was covered by a marine incursion. There are admirable syntheses of the different assemblages of rock which resulted and discussions of such topics as the possible influence of the underlying geological structures on the extent of these seas and repeated references to the interplay of biological activity and the kind of rock that resulted. Such discussions bring out the necessity to disentangle contemporary faunas which differ because they inhabited different environments from groups of animals which evolved through the passage of time and which might be used to define subdivisions of the Cambrian.

The reader is left with a clear picture of Cambrian events as revealed by present knowledge and with a sense of where progress is being made in current research and what the future might hold in store as investigations continue. Professor Holland and his colleagues can be congratulated on a fascinating book which I can recommend to any geologist interested in the Lower Palaeozoic history of our planet.

J. SUTTON

Physics and God

Proceedings of the International Conference on Thermodynamics held in Cardiff, UK, April 1–4, 1970. Edited by Peter T. Landsberg. (International Union of Pure and Applied Chemistry, in conjunction with the International Union of Pure and Applied Physics, and the Institute of Physics and the Physical Society. *Pure and Applied Chemistry*, Vol. 22, Nos. 3–4, 1970.) Pp. ix+215–555. (Butterworth: London, December 1970.) £9.

LET me say at the outset that I do not think that, purely academically, I am properly qualified to evaluate this book. I propose to review it, all the same, for the sake of interest and amusement: because it seems to me, as a modest but persistent user of commonplace thermodynamic methods in experimental physics, that this conference, which I did not attend, must certainly have been those things—and more. It illustrates the power of a simple macroscopic principle such as thermodynamics to

Pure Chemistry Library

provide a magnificent variety of all things from the profoundly philosophic through the simply utilitarian to the distinctly risible. It is sometimes difficult to distinguish (if one wishes or ought to) between the first and last of these. Take, for example, the following exchange in the discussion reports concerning the direction of time.

D. Park—(Massachusetts, USA)—It seems that we get our sense of time direction very much more from the radiation of the Sun . . . than from anything the Universe is doing. Why on Earth should non-radiative living processes be bound up with the ultimate fate of radiation? This is not clear in Landsberg's statement, but Narlikar has his own answer. According to it, if suddenly the Universe started to contract, then it would seem to us that, as a results of distant events, the Sun would start re-absorbing radiation.

Landsberg—It would seem so to God, not to living things. God would say, ah, the Universe is contracting and everybody is getting younger while, I, God, am getting older.

Costa de Beauregard—No! Eternity is time-extended!

Landsberg—Mon Dieu (laughter)! I didn't really mean God.

What shall our undergraduates, struggling grimly uphill on their Carnot cycles, make of this? It was not, of course, a conference for undergraduates, but that is a legitimate question, and the answer is, I hope, that they should make much—in the sense that these are the sort of deep philosophical problems that stimulate people to study physics—at least they provided one of my own major motivations and I do not think, in spite of appearances, that things are so very much different now, fundamentally. It is a pity that the glamour of particle physics and expensive accelerators, and the hubris apparently inevitably engendered in some of their adherents, have distracted attention from the equally—or more—fundamental questions raised by less expensive disciplines. It is also anomalous (in a rational, not a political) sense in this age of relevance: a good deal of the work of this conference might well have application in industry and elsewhere now and in the foreseeable future.

Rather few reports of conference proceedings have more than a transient appeal, as a collection of papers of current interest in a special field. This is surely one of them, which should be widely used and be of lasting value. The section on foundations is perhaps difficult and esoteric and will be read mostly by thermodynamicists, but there is wide interest in, for example, phase transitions and surfaces. There are papers on the theory of fading memory, irreversibility and quantum mechanics, astrophysics and relativity. There are papers and discussions on the teaching of thermodynamics. It is impossible to get an adequate idea of the scope and balance of the book, however, without examining it, which I very strongly recommend.

It seems a pity to carp, but I must say that this book is so valuable that an index would have been well worthwhile. It might have enabled me to detect some reference to information theory, which has so far escaped me. One other omission is the thermodynamics of finite systems. This state of matter, intermediate between non-interacting single-particle behaviour and the infinite thermodynamic limit, is surely of fundamental interest. It concerns, for example, nuclear matter, polymers, macromolecules and many biological systems, and could lead to a radical reassessment of our understanding. Not much is known experimentally in this area, but there is an awakening interest in the properties of small assemblies of particles, and this may well be a useful addition in a future conference.

D. F. BREWER

Information Science

Introduction to Science-Information Work. By C. W. Hanson. Pp. 199. (Aslib: London, June 1971.) £3; members £2.40.

ALTHOUGH information science has been developing rapidly during the past twenty years, and education in the field has been offered in the past ten years, there are still no definitive textbooks. It is a pity, therefore, that Mr Hanson, after his long pioneering experience at the British Scientific Instrument Research Association and at Aslib, as head of research, should now present a book on the mixed objectives of "information, documentation and library work" and called "science-information work". Information science is largely recognized as distinct from librarianship; in Great Britain, the term covers both practical and theoretical aspects, so that the expropriation of the term in the United States for theoretical studies of the science of information is not sufficient justification for abandoning it in Britain. Furthermore, information science is now extending into non-scientific fields, so that it is not just science-information. It was Mr Hanson who suggested the title "information scientist" in 1957.

Even as an "introduction" the book is quite elementary in content, although what it covers is most lucidly, carefully, and thoroughly described. It is in five parts. The first part deals with the problems of communication and the flow of information, the growth of the literature and the media of communication. The second part describes the primary and secondary types of literature, and non-documentary sources of information—organizations and people—a much-needed reminder in these days of mechanized services. There is then a rather short adumbration of mechanization, information retrieval and selective dissemination of information, all in a very general manner and in only seven pages. The fourth part discusses the provision of information to enquirers and users, and the making of searches for such information. Finally, there is some account of library operations, including lending, reprography and microforms in relation to information services; mechanized methods of library housekeeping are also considered. A short appendix gives attention to aspects of writing, editing, printing, publishing, translating and public relations.

In general, the book is centred on conditions in the United Kingdom, and foreign literature and sources of information are given scant attention. There is much sound commonsense in the advice given, but there are some unexpected flaws. The production of abstracts bulletins within industrial firms and research associations is not described. Patents are dismissed as unimportant as sources of information, although they frequently describe research which may not achieve wider publication for several years. To suggest that *International Critical Tables* (1927) is the last publication of its kind is too insular and ignores many excellent foreign publications.

The book is specifically addressed to school leavers, undergraduates and even older unqualified people, although information science is rapidly becoming a fully graduate profession. It deals too much with routine matters and makes the work seem rather a pedestrian activity. This is a pity, because the principal attractions of information science are the wide variety of tasks, the breadth of knowledge to be covered by keeping up to date and in touch with current research, and the contacts with people, from the factory floor to management. Increasingly there are opportunities to develop advanced computer-based systems, and there is a growing field of research in information science itself. Useful though this book may be at basic routine practical levels, it lacks the enthusiasm and the intimations of advanced work and problems which will inspire new entrants to the profession. Information departments, handling both technical information and management information, may well become the essential nerve centres of industry in the future. In the absence of the much needed advanced textbooks, even an "introduction" should point out, boldly, the way ahead.

J. FARRADANE

CORRESPONDENCE

Chemical Societies

SIR,—Your recent editorial about the Society of Chemical Industry (SCI) has just been brought to my notice (*Nature*, **231**, 411; 1971) and, as I believe I was the only individual member to protest against the proposed merger of the SCI with the other chemical bodies, may I explain why I did so? Other protests came from members of one of the sections of the SCI (*Chemistry and Industry*, 1592, December 12, 1970).

I am a member of the Chemical Society (CS) and SCI, but not of the Royal Institute of Chemistry (RIC) because I do not satisfy the professional membership requirements.

The original proposals were for the amalgamation of the three bodies under a new unifying charter and, as long as this was the case, I was in favour and voted accordingly in the CS referendum. As long as a new charter was the ultimate aim, any divergence on the way towards it was not important, for it seemed certain that the lawyers advising the Privy Council would sort out anything which ought not to be allowed. Suddenly, however, in a document issued to members by the CS, what was to be the new CS became the "new" CS and it appeared obvious that amalgamation under a new charter was not contemplated within the foreseeable future. As none of the bodies have power in their charters to amalgamate, any other kind of amalgamation was, therefore, unlawful.

As far as the CS is concerned, I am now waiting to see the scheme which the Charity Commissioners must be drafting for the uniting of two charitable bodies (CS and the Faraday Society) with two which are not (RIC and the Society for Analytical Chemistry). Because there is no power to amalgamate in the charter of the CS, this scheme will show how it can all be done through new bye-laws alone, which themselves may be inconsistent with the charter (Council Members "appointed by" the RIC against "electing . . . other Members of Council" by the Fellows; Fellows of "any nationality" against "loving subjects"). It will also show how these new bye-laws are to be approved at a meeting which, somehow, took place on April 21, 1971, when a resolution was passed for the new bye-laws to come into force forthwith. It will then reconcile that with the supplementary charter which says that "no such resolution . . . shall become effective . . . until the expiration of one month" and will then reconcile the whole with the original charter, which declares "no resolution or Bye-law shall on any account . . . be made . . . in opposition to the general scope, true intent and meaning" of the charter or of the laws or statutes of the realm (including the Charities Act, 1960?) and that if any such "rule or Bye-law shall be made, the same shall be absolutely null and void to all intents, effects, constructions, and purposes whatsoever".

How, in 1971, can the powers of the CS be so wide under its charter that it can amalgamate with (to be taken over by?) the RIC, when, in 1876, the "limited powers of the Chemical Society under its charter" were such that it could not accommodate within its own corporate identity that body of professional chemists which eventually became the RIC? (See *The Chemical Society 1841–1941*, pp. 52 and 53.) In any explanation, I hope account will be taken of the fact that the only thing which seems to have changed since 1876 is the addition of a supplementary charter in 1920 and that took away the class of associateship under which professional chemists might possibly have been accommodated.

In your editorial of November 14, 1970 (*Nature*, **228**, 597; 1970), you mention the "enormous expense of re-negotiating a single" charter. Could not some of this expense have been avoided (anyway, for those bodies which are charities—the CS and SCI) by asking the Charity Commissioners to prepare a scheme under section 15 of the Charities Act? This provides machinery for amending, free of charge to the charity, the charters of those charities which are established by Royal Charter. The idea is partly to stop charities needlessly incurring legal costs when the Commissioners can do what is necessary without charge to the charity. Charity money should be spent on charitable objects and anything the commissioners do for free means that just so much more will be so spent.

Yours faithfully,

J. C. WILLIAMS

60A *High Street,*
Edgware
*Middlesex HA*8 7*EJ*

Smoking and Cancer

SIR,—It would be a pity if the somewhat personal nature of the exchanges between Dr John Higginson (*Nature*, **232**, 355; 1971) and Dr T. D. Sterling (*Nature*, **231**, 543; 1971), about the availability of unpublished data on smoking and lung cancer, were to obscure a real point of principle involved.

A scientific author basing his conclusions on the results of an experiment takes it for granted that he has to publish enough information for the experiment to be repeated, and its results confirmed or not, as the case may be. In biology and medicine, and in the social sciences, conclusions may sometimes be drawn from observations which cannot be repeated, but here also the data ought to be made publicly available, to allow other interpretations. No useful scientific purpose is served by publishing unverifiable hypotheses; and that is so even if they are unverifiable only because the information needed for verification is kept private.

Ideally everything relevant ought to be published, but for reasons of space that may be impossible. As Dr Sterling has pointed out, this has not been done with most of the smoking/lung cancer investigations, and perhaps in other medical fields as well, where published conclusions have been derived from data which are not generally available, except by favour of the authors. This is unsatisfactory, since it is in no way to "impugn the integrity and competence of the numerous scientists and committees in many countries who have reviewed exhaustively the extensive data published on this subject" (if I may quote Dr Higginson) to accept that there is at least a remote chance that some of them may have been mistaken, and that there might be advantages in allowing a fresh look from an unprejudiced, or at any rate a different, point of view.

Granted that much of the data may be in a form unsuitable for publication in scientific journals, and that it would clearly be quite wrong to conclude that a busy and important medical scientist "who fails to make his data available to any Tom, Dick or Harry is lacking in credibility" (to quote Dr Higginson again), would it not be possible to have an arrangement whereby the data are deposited in some central library or institute and publicly available without reference to the author, to be copied and quoted from just as though they had been published with the original paper?

The British Museum (Natural History) has for many years operated such a scheme for biological and ecological data, which are often both voluminous and undigestible, and which journal editors are rightly reluctant to print, substituting instead a note to indicate where they are to be found in full. This seems to work well enough, and allows an author to refer to unprinted results without any fear of being accused of keeping them to himself, away from public investigation and criticism.

Yours faithfully,

C. B. GOODHART

University Museum
of Zoology, Cambridge

Announcements

University News

Dr C. D. Papaliolios has been appointed professor of physics in **Harvard University** and **Dr David K. Cohen** has been appointed professor of education and social policy.

Professor F. M. Arscott, University of Surrey, has been appointed professor of pure mathematics in the **University of Reading,** in succession to **Professor R. Rado,** on whom the title of professor emeritus has been conferred.

Dr H. P. Rang, University of Oxford, has been appointed to the chair of pharmacology in the **University of Southampton.**

Dr W. M. Hutchison has been appointed to a personal professorship of parasitology in the **University of Strathclyde.**

Miscellaneous

The sixth annual **Karl G. Jansky lectureship** of the National Radio Astronomy Observatory, Charlottesville, Virginia, has been awarded to **Professor Charles H. Townes,** University of California.

The **Horace Le Marquand and Dudley Bigg research fellowship** has been awarded by the council of the Royal Society to **Dr G. R. Shellam,** Commonwealth Serum Laboratories, to enable him to study the immune response to tumour specific antigens at the Tumour Immunology Unit, University College London.

Special merit promotions have been awarded to thirty scientists and technologists employed in government and other public establishments: *Civil Service:* **D. Küchemann,** Royal Aircraft Establishment; **P. S. Bulson,** Military Vehicles Engineering Establishment; **J. F. Gittins,** Services Electronics Research Laboratory; **T. P. McLean,** Royal Radar Establishment; **R. J. Murgatroyd,** Meteorological Office; **D. W. Robinson,** National Physical Laboratory; **J. W. C. Gates,** National Physical Laboratory; **E. L. Goldsmith,** Royal Aircraft Establishment; **J. K. Lancaster,** Royal Aircraft Establishment; **K. R. May,** Microbiological Research Establishment; **R. L. Moss,** Warren Spring Laboratory; **J. B. Mullin,** Royal Radar Establishment; **M. E. Peover,** National Physical Laboratory; **P. B. J. Crisp,** Quantity Surveying Department. *Agricultural Research Council:* **F. Brown,** Animal Virus Research Institute; **C. G. Butler,** Rothamsted Experimental Station; **L. E. A. Rowson,** Unit of Reproductive Physiology; **A. Feinstein,** Institute of Animal Physiology; **J. W. B. King,** Animal Breeding Research Organization; **D. L. Lee,** Houghton Poultry Research Station; **W. G. Siller,** Poultry Research Centre. *Natural Environment Research Council:* **J. C. Swallow,** National Institute of Oceanography; **A. Watson,** Nature Conservancy. *Science Research Council:* **B. E. J. Pagel,** Royal Greenwich Observatory; **A. H. Gabriel,** Astrophysics Research Unit; **N. M. King,** Rutherford High Energy Laboratory; **P. F. Smith,** Rutherford High Energy Laboratory. *UK Atomic Energy Authority:* **R. Bullough,** Atomic Energy Research Establishment; **A. Gibson,** Culham Laboratory; **R. S. Nelson,** Atomic Energy Research Establishment.

Errata

IN the article "Gamma Rays (250 keV–2.3 MeV) from NP 0532" by L. E. Orwig, E. L. Chupp and D. J. Forrest (*Nature*, **231**, 171; 1971), the following corrections should be made. The end of paragraph 4 should read ". . . to account for the drift of the pulsar period as a result of the slowdown of the pulsar, the Earth's rotation, the Earth's orbital motion and the motion of the balloon". Paragraph 5, line 6, should read "$\gtrsim \pm 0.5$ ms", and line 4 should read "Another source of error . . .". Paragraph 6, line 3, should read "15 h 50 m 30 s UT . . .", and line 7 should read "15 h 50 m (29.9956 ± 0.0003) s . . .".

THE headline for the article "Formation of New Connexions in Adult Rat Brains after Partial Deafferentation" by P. D. Wall and M. D. Egger (*Nature*, **232**, 542; 1971) should read "Mapping of the receptive fields of cells in the thalamus and cortex after destruction of a dorsal column nucleus in the rat reveals evidence of functional reorganization in the central nervous system." The penultimate line of the legend to Fig. 1 should read ". . . the axes show distances".

PROFESSOR D. GWYNNE EVANS was mistakenly referred to as Professor D. Gwynne in University News in *Nature*, **232**, 508 (1971). Professor Evans is the director designate of the Lister Institute, as well as its professor of bacteriology and immunology.

British Diary

Wednesday, September 1

133rd Annual Meeting (eight days) British Association for the Advancement of Science, at Swansea.

Multivariable Control System Design and Applications (4th UKAC control convention, three days) Institution of Electrical Engineers, at the University of Manchester.

Thursday, September 2

518th Meeting (two days) Biochemical Society, at the University of Sussex, Falmer, Brighton, Sussex.

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight	
		U.S.A.	Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature + Nature Physical Science	**£24**	**$83**	**$90**
Nature + Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, August 27 1971

NATURE Volume 233 SEPTEMBER 3 1971

Pure Chemistry Library
SCIENCE COLLEGE
[illegible] Circular Road
[illegible]

Making Water Run Uphill

THE Annual Meeting of the British Association this year will not be the last or even the last but one, and indeed, if the programme arranged for Swansea is anything to go by, the occasion could mark a change in the association's intellectual fortunes. For a great many years, people have complained with some justice that the association's most spectacular public performance had become too dry and too academic to command the interest of younger professional scientists or even to enliven public awareness of what the association is for. Adversity seems to have brought out the best in the old organization, which for the past few years has steadily improved the sheer mechanics of presenting a kind of review of what is happening in professional science to an audience of interested bystanders and professionals. At the same time, the organization has taken, admittedly a little late in the day, some steps to put its constitution in order. That there should be talk of electing the members of the association's governing body, which has previously perpetuated itself from decade to decade, is a sign of how far the reforming spirit has gone. Yet it remains the sad truth that the sheet anchor of the association in the past few years, the annual subvention of £12,500 from the Department of Education and Science, is likely to end a year and a half from now. What will happen then? Will the British Association founder altogether? Or will it find some other way of carrying on?

The first thing to be said about the latest misfortune to befall the British Association is that it is not a misfortune at all but an opportunity. By all accounts, the Department of Education and Science has taken the view that if there is a public need of a forum for the discussion of issues in professional science or even in the relationship between professional science and society at large, then the government has itself made provision for this need in the setting up of committees such as the Council for Scientific Policy. Although there is plainly a great difference between the functions and the styles of the two bodies, there is something in this riposte. At the same time, the Department of Education seems, in saying no to the association's most recent request for money, to have declared that if public funds are to be spent on educational objectives, they had better be spent through an agency more directly under Mrs Thatcher's thumb. Galling though it may be for the British Association, and the host of voluntary workers who have over the decades helped to sustain vigorous programmes of what may be called local education, this is also a fair if ungenerous point. Indeed, what the Department of Education and Science is saying is that it would itself prefer to organize the machinery for carrying out the functions for which it is directly responsible. As a principle of public administration, that point of view is quite impeccable, for then everybody can be sure that responsibility lies squarely where it belongs. But there is also a political reason for this rigour. Why should a government trust a voluntary body such as the British Association to do its bidding—and why should a voluntary body such as the British Association assume that it will never be in conflict with the government even on matters such as the education of the young and the enlightenment of the public?

The truth, of course, is quite the opposite. If the British Association has a place in British public life at all, it must surely stem from precisely the kind of independence that must affront politicians of all parties. At the beginning, in the early nineteenth century, the British Association came into existence because of the conviction of its founders that the institutions of the establishment, the government departments and the Royal Society included, were unable to tackle problems within the scientific profession and problems of the relationship between professional science and the wider community. In the half century that followed, the association built up a powerful record of achievement. Not only did it sponsor programmes of scientific research of great distinction—the Gassiot committee, one of the best known of the Royal Society's standing committees, is, for example, a legacy from the British Association—but it also served as a valuable means of awakening public interest in professional science. The principle that members of the association may be entirely unqualified as scientists is a relic of the best of nineteenth century liberalism. It is, of course, anomalous and disappointing that with the passage of decades, the involvement of the British Association with the problems which it first of all set out to tackle has if anything diminished even though the need has grown.

This is the analysis which the association should not shrink from at its present meeting in Swansea. Over the years, there has grown up a tendency to believe that everything would be different if only some sufficiently resourceful man could make impossible economies or wheedle improbable benefactions out of private industry. The job that needs doing, as much now as in the early nineteenth century, is, however, one that requires independence, both from government and from industry. In the past year or so, it is clear that a sufficiently alert organization such as the British Association could have helped to clarify questions such as the advisability of investing public money in the new accelerator at CERN (an issue which is still evenly balanced), the issue of what should be done about the organization of the research councils and the decision (scandalously delayed) on the future scale of university finance and the relationship between universities and the polytechnics. British public life, not merely British science, is impoverished by the lack of an informed and influential platform from which questions like these can be argued intelligently. Moreover, there is every sign that the scientific community would be prepared to invest not merely money but time in organizing such discussions. The fact that so many people troop devotedly and usually at their own expense to the annual meeting of the association is a sign of what wellwishers are prepared to do. To say that the British Association should become a kind of pressure group is to go too far, for these are not circumstances in which there is a single consistent point of view to be advocated. But these are circumstances in which intelligent public discussion is bound to be a social benefit, both for professional scientists and for the community at large. To let this potential role go by default would be a loss for British public discussion as well as a tragedy for the British Association.

But how is it possible to do ambitious things without a budget? This is what the accountants are bound to say. Undoubtedly there is much that could be done to turn this criticism by strictly business stratagems. At the same time, however, it is essential that means should be found for simplifying the association's constitution. The issue is not whether the sixteen sections into which the proceedings are divided should continue to exist, but whether there is some device for allowing the organization as a whole to act decisively when its own interests and those of its members call for something to be done. At the same time, ways must be found of convincing even busy working scientists that the association can do something to further the common cause of a healthy scientific community in an appreciative and responsive setting. In attempting all this, organization in the ordinary sense is much less important than publicity of the right kind, but everyone knows that to say as much will offend against the over-modest dissembling of the British Association's members. This is why it is to be hoped that the annual meeting in Swansea will find people prepared for the radical departures from past policy that the circumstances warrant.

The Next Step with Nuclear Explosions

THE French government has done the decent thing in bowing to public pressure and cancelling the series of nuclear explosions planned for the next few weeks in the atolls of the Tahiti group. For one thing, of course, it is intolerable that the government of France (like that of China) should continue to release large quantities of fall-out into the atmosphere without acknowledging that to do so is to create a public nuisance. This, after all, is a form of pollution that justifiably merits concern. Protests at the impending explosions from neighbouring regions in the Pacific may have helped to change the French government's course of action, but it is also probable that the time is coming when the Gaullist view of the utility of nuclear weapons is fading. It is a long time now since the strategic justification of the French independent nuclear force was argued with enthusiasm. For one thing, it has turned out to be a costly way of hoping to make sure that in the event of an aggression, it would be possible for the military forces of France "to tear an arm" off the enemy before sinking beneath a hail of nuclear explosions. Second, nuclear weapons seem increasingly irrelevant to the military problems of Europe, especially now that the gap between East and West has been bridged to some extent by the continuing civility of relations in central Europe and by the signature last week of the agreement between the Occupying Powers on the future of Berlin. Third, the French nuclear force tends to be a device within the framework of an increasingly united Europe (and the British nuclear force, now almost forgotten, would be equally an irritant if it were ever advertised). In short, the government of France would be well advised to wash its hands of nuclear weapons at this stage of the game. Enough has been done to show that the properties of nuclei are the same in France as elsewhere. Not nearly enough has been done to make use of them.

In these circumstances, the time has come rather to ask what arrangements will in future be made for regulating the uses made of nuclear weapons. It is getting on for a decade since the partial ban on nuclear tests was negotiated, and the International Atomic Energy Agency in Vienna is now up to its eyes—and some say buried—in the intricacies of policing that unsatisfactory agreement. In the year just ended, the agency took over responsibility for policing safeguards agreements at about a hundred nuclear installations. According to the annual report for the year just past, it spent a total of $1.7 million on the work, and the budget for the coming year, also now published, suggests that this will rise to $2.6 million in the next twelve months. As always, the agency's abiding problem in the implementation of the safeguards policy is the recruitment of sufficiently skilled people and the determination of the conditions under which they should work. Optimism at Vienna runs as high as ever, but it is hard for outsiders easily to accept that the agency would be able to police effectively safeguards for a world in which the number of nuclear power stations under its authority was counted not in tens but in thousands.

This is why it is heartening in the past few months that the subcommittee of the UN Assembly on Disarmament, meeting at Geneva, has taken up again the issue of extending the partial test-ban to include all experimental nuclear explosions, whether underground or in the air. Not merely would this device let the Vienna agency escape from the invidious position of policing the unpoliceable into which it has been jockeyed, but it would solve a host of other problems, from the proper balancing of risks and benefits in some of the newer developments of military technology—multiple nuclear warheads, for example. Moreover, an extension of the test-ban would help to ensure that the asymmetries incorporated in the present treaty, and which in particular allot privileged positions to countries which have already manufactured nuclear weapons for themselves, will not at some stage lead to demands among the non-nuclear powers that they too should have weapons to themselves. In this sense, the non-proliferation treaty remains what it has been since its conception (and what its preamble says it is)—a temporary expedient.

As it happens, technical developments are also favourable. The article on page 51 by Dr F. M. Anglin shows how it is now possible to discriminate more accurately than previously between earthquakes and nuclear explosions with less than the equivalent of 10,000 kilotons of energy. The idea (see page 8) is that even quite small explosions can be distinguished from earthquakes of comparable size by the high frequency parts of their seismic spectra. To be sure, nobody would pretend at this stage that all explosions can be distinguished unambiguously from naturally occurring earthquakes, but there has been an immense improvement in the technology of test detection and discrimination since the days when the non-proliferation treaty was first advanced.

Pure Che[illegible]stry Libr[illegible]
SCIENCE COLLEGE,
92. Upper Circular Road
CALCUTTA

Making Voters of Universities

THE United States Senate has done as much as any legislative body can to endear itself to academics. It remains to be seen whether it will be able to persuade other politicians in the United States, and especially the Administration, that the time has come to be generous once again to colleges and universities in the United States. Two weeks ago, more or less with acclamation, the Senate passed the draft of a piece of legislation intended to replace the Higher Education Act of 1965, and which would have the effect, for the first time, of making attendance at an American university into an assurance of a maintenance grant, at least for students whose parents are not too well off. But as well as helping needy students, the proposed legislation would help needy universities and colleges—those on the institutional breadline would be helped not merely with matching grants to accompany the student grant but also with special awards in times of trouble (see *Nature*, **232**, 518 ; 1971).

By backing this bill, the Senate has done three important things. First, for the first time, it has declared that higher education in the United States is something which the federal government must worry about. So far, apart from some of President Johnson's devices for helping universities in need by providing funds for libraries and other special facilities, the operations of the National Science Foundation and the other research bodies which for practical purposes support graduate education but only fitfully, and the scheme for guaranteed student loans introduced a year ago and still too new to be assessed, the United States Administration has traditionally taken the view that good colleges and universities will be able to swim and the others deservedly to sink. It will be interesting whether the Administration, Republican as it turns out, will accept an argument that has failed in previous Congresses to enchant its predecessors.

The Senate has also affronted a great many of those who believe that higher education, like the operation of supermarkets, should find its own level by proposing that a large amount of money should be spent on subsidies. On present estimates, the new higher education bill, if it ever becomes law by the summer of 1973 (when the present Act runs out), would provide $18,000 million over three years. Many of those who have accepted the principle that higher education should properly be a charge on the federal government have found their hands rising in horror at the prospect that the rate of public expenditure on universities and colleges should rise to such a point that it exceeds even the spending of the National Aeronautics and Space Administration and other similar agencies. But, of course, this is only one side of the coin. The better half of the case is with those who argue that if a government such as that of the United States can spend $5,000 million (at the peak in 1968) on getting to the Moon and related activities, surely it can find a comparable amount to ensure the intellectual future of American society and industry.

That, of course, is the rhetoric. The interest of what the Senate has done, so soon after a period in which universities of all kinds and research academics in particular have been acutely short of funds, is that it presents the Administration with an important political challenge. A year from now, when the Senate's bill should have found its way through yet another round of committees and should have struck a response from the House of Representatives as well (where similar ideas are waiting in the wings), President Nixon will be up to his neck in the election that will decide whether or not he will spend a second term at the White House. Although there is no strong tradition, in the United States or anywhere else, that higher education can become a political issue, next year may provide an exception. After all, the past few years have done very little to heal the breach between the present Administration and the universities which was created as much by brashness and bad manners as by malevolence in the early days of Mr Nixon's Administration. It is improbable that Mr Nixon will now willingly take the risk of giving offence in the universities even if he manages to return from Peking next spring with quite unexpected political triumphs. In all the circumstances, this may be an occasion when the Administration finds it prudent to let the Senate have its present bill.

---

100 Years Ago

PARIS

Académie des Sciences, August 21.—M. Faye in the chair.—M. Chabris has calculated the quantity of nitrate of ammonia yearly carried down to the soil for the nutrition of plants by means of the rain to be two pounds of nitric acid, and consequently, three pounds of nitrate of ammonia per acre.—M. Dumas contributed to the Academy a piece of bread, the provision for the army, which had been infected by *Oidium aurantiacum*. Such facts are not exceptional, principally in very hot weather, and may be detrimental to the public health, as the fungus spreads very rapidly, and it is very difficult to get rid of it. A special committee has been appointed to prevent the infection if possible. MM. Dumas, Baron Larey, Tulasne the botanist, and Pasteur, the celebrated author of so many works on spontaneous generation, are members.—M. Berthelot has examined most carefully a piece of carbon from the Cranbourne meteorite, an Australian stone, and shows by many scientific arguments that the Cranbourne carbon is quite unlike the Orgueil meteorite carbon (a French specimen). The Cranbourne carbon must have been acted upon by a high temperature in ultra-terrestrian space, and no trace of organic origin is to be found on it.—M. W. de Fonvielle sent through M. Leverrier a note establishing that meteoric phenomena analogous to the Marseilles phenomenon are not exceptional cases.—M. Bert, the former Prefect of Lille during the war, described some most interesting experiments on the effects of pressure in suffocating animals living in a confined space. The rapidity of death is not the same for every kind of animal. If the pressure is very high the death is not due to any mechanical effect or to the want of oxygen, but to the presence of carbonic acid, resulting from respiration. It is poisoned by the produce of its own lungs.—The Academy held a secret committee for the nomination of a free member. The list of candidates long delayed was at last published, and M. Belgrand is at the head. But the nomination will be contested.

From Nature, **4**, 380, *September* 7, 1871

OLD WORLD

POLLUTION

Swansea Syndrome

A GRANT of £11,000 has been awarded to Mr G. T. Goodman of the Department of Botany at the University College of Wales, Swansea, so as to continue his investigation of non-ferrous metals in mosses, grasses and soil. A first report of this study, in the region around Swansea, appeared in June this year (Goodman, G. T., and Roberts, T. M., *Nature*, **231**, 237; 1971). Now the Natural Environment Research Council is prepared to support an extension of the study to the rest of Britain.

The original discovery showed the presence of large amounts of metals such as zinc, lead, cadmium and copper in plants and soil to the east of the Swansea Valley. At one of the most heavily contaminated sites, for example, some mosses contained as much as 1,200 parts per million of zinc, 800 parts per million of lead and 40 parts per million of nickel. One bizarre accompaniment of the investigation was the discovery that several horses found dead in a field near Llansamlet in the spring of 1970 had large concentrations of lead in their systems, although it has not been possible to show beyond dispute that lead poisoning was responsible. Malnutrition is another possibility.

The appearance of the article by Goodman and Roberts has led to several decisions, not the least important of which is the setting up by the Welsh Office of a working party on pollution by metallic elements in and around the Swansea Valley. It is assumed that the high concentrations of metal elements in the region have been caused by windborne pollution from neighbouring slagheaps, but another possibility is that exhausts from chimneys are responsible.

The working party is interdisciplinary in outlook and the scientists under the chairmanship of Dr R. T. Bevan, Chief Medical Officer at the Welsh Office, include Dr D. H. Peirson of the Atomic Energy Research Establishment, Harwell, Dr S. R. Craxford of the Warren Springs Laboratory, Professor C. R. Lowe and Dr J. R. Glover of the Welsh National School of Medicine, Professor P. J. Lawther of the Medical Research Council and Mr G. H. Francis of the Agricultural Development and Advisory Service. The first meeting of the working party was held in July and the second is scheduled for next week. The brief given to the working party was to recommend to the Secretary of State for Wales the sort of research programme needed to be set up to extend the work of Goodman and Roberts with particular reference to any correlation that might be established between the presence of heavy metals in the environment and human and animal life.

A member of the working party said this week that several botanical sampling stations would be set up throughout the area and that direct air sampling would also be carried out. Soil and herbiage samples would be analysed and blood and urine samples would be taken from hundreds of people throughout the area. It is planned that this survey will take twelve months to complete.

The simplicity and effectiveness of the technique developed by Goodman and collaborators make it particularly suitable for determining heavy metal contamination over even a short period. In areas that are moss deserts—because of high pollution—it is possible to deposit moss samples for as little as two weeks and obtain information on the accumulated metals during this time. The inexpensive nature of this research—the original investigation was carried out on a £500 budget—makes it a particularly useful tool to monitor atmospheric pollution. The problems in the Swansea area, however, might turn out to be insignificant compared with those found elsewhere in the country but there is no doubt that the working party will highlight the effects and dangers of a high atmospheric heavy metal concentration.

EMPLOYMENT

Graduate Problems

FIRST reports from the universities suggest that the plight of applied scientists may not be as bad as was predicted earlier this year. But times are still hard for pure scientists. In April the Confederation of British Industry (CBI) predicted a cut of between 15 and 20 per cent in the largest traditional field of graduate employment—industry and commerce. At the same time, several companies cancelled their visits on the traditional "milk-round" to British universities.

Now that the degrees examinations are over, what is the situation? The university appointments boards emphasize that it may be too soon to know for sure, partly because some graduates are on holiday and not bothering to look for jobs and partly because they often do not let their appointments boards know after they have accepted a job. But the impression is that it has been a difficult year. Quite how difficult depends on where you look. At Oxford, Mr T. Snow, the Secretary of the Appointments Committee, said that "graduate unemployment is not a problem". But he does admit that there are fewer jobs, particularly for those who want specific careers. It is particularly hard for chemists who want to remain chemists.

The estimate of the size of the problem varies from university to university—at Sussex, for example, Mr I. H. F. Kerr, the Appointments Officer, described the situation as "very serious" and estimates that as many as 400 of the 900 ex-students may still be job-hunting. At East Anglia, the number still looking is "higher than last year, but not dramatically so", while at the University of Manchester, figures for mid-July show that the applied scientists—with the notable and unsurprising exception of aero-engineering — were managing as well as or only slightly worse than last year, but that the pure scientists are finding it very bad. Half of the physicists are unemployed, however, compared with a quarter last year, and two-thirds of the chemists compared with half last year.

Yet things are not disastrous and there are hopeful reasons. The employers' concept of a "graduate job" is beginning to change. Companies are considering graduates for jobs that people with A-levels would have had a few years ago and they are also more ready to accept applicants with any degree, not just in a specific field. Graduates are equally beginning to look at careers that formerly they would not have considered — chartered accountancy, insurance, banking and local authority work. And for the graduate who is prepared to be flexible there are still jobs available. The University of Manchester's latest Clearing House List has some 1,400 vacancies (although this is a drop of between one third and one quarter on last year). Many science students are turning to completely different fields — British Rail travel planning or the Civil Service, for example. The theory that a university degree is a passport to a certain sort of job seems now to be a dead duck. Today there are even graduate shift foremen in a steel firm near Cardiff.

But if the employment situation is difficult, what are the graduates doing? Many are still on holiday or doing temporary work, and will only start looking in the autumn when the situation may well be worse, not better. Others may be trying to postpone the decision by prolonging their education. There has been an increase of more than twenty per cent in graduate applications to teacher training colleges—nearly 14,000 have applied for an estimated 9,000 places. (The new requirement that teachers in secondary schools should have a Dip.Ed. may help to account for this.) Some appointments officers also suspect that more graduates are simply dropping out, saying that "no job is better than any job".

SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA

INDUSTRIAL RELATIONS

Jack Hill

THE dismissal of Mr Jack Hill's case against his employers highlights the predicament of up to 60,000 engineers throughout the country. Mr Hill, a sixty-three-year-old contracts engineer with the Newcastle upon Tyne engineering company of C. A. Parsons, was given a month's notice at the end of July for refusing to join the Draughtsmen's and Allied Technicians' Association (DATA) which, after a three-year battle, had won the right to a closed shop at Parsons. He sued the firm for wrongful dismissal.

Mr Justice Brightman, before whom the case was argued, concluded that under the present law he could not prevent the company from dismissing Mr Hill. He added that if the law had allowed, he would have granted the order. Under the Industrial Relations Act, which comes into force by stages from October, the action of C. A. Parsons would be illegal. Mr Hill is appealing against the decision, but it is likely that the appeal will be heard before the relevant clause of the new Act comes into effect. Until the appeal is heard Mr Hill retains his job.

There are thirty-eight engineers at Parsons in the same position as Mr Hill, but the United Kingdom Association of Professional Engineers (UKAPE), of which Mr Hill is a member, is only too aware that as many as 60,000 engineers in 5,000 companies around the country are in a potentially similar position. They are all a minority body of professionals in companies with large numbers of technicians belonging to unions such as DATA.

UKAPE sees itself as protecting the professional ethics and integrity of senior engineers and feels that some of the aims of the Amalgamated Union of Engineering Workers, of which DATA is a part, are directly opposed to that integrity. As things are, UKAPE seems to be able to say plenty but to do little—its membership is not all that large. But if those whom UKAPE considered as professional engineers—those with degree or equivalent status—are to protect what they consider to be their rights, then once the Industrial Relations Act comes into force they will have to join UKAPE if they are to have any say in negotiations between management and the proposed agency shops. Otherwise they will find themselves out in the cold, a minority without national support among a hostile and militant trade union. In such a situation, UKAPE could become a force to be reckoned with.

The circumstances of professional engineers are by no means unique. Chemists and physicists working in industry could find themselves in the same position. So far, to be sure, they have not had to deal with unions as militant as DATA, the members of which worked to rule twice and struck once in their efforts to establish a closed shop at Parsons.

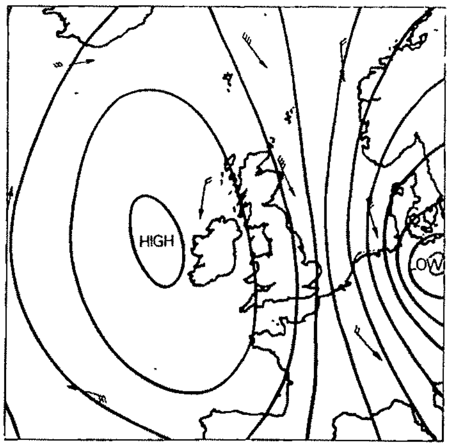
An Association of Professional Scientists and Technologists is being formed as a trade union (like UKAPE) although it will not be affiliated to the TUC (also like UKAPE) so that it can protect its members in the agency shops. But if Mr Jack Hill stands as one of the last—if one of the most spectacular—victims of the present law, it may transpire that his case is only the opening shot in a battle to come, for although the Industrial Relations Act will prevent closed shops in their present form, there are some fears that militancy will find other ways of achieving the same result.

RIVER FLOODING

London Sits and Waits

LAST week, five British Government departments issued a joint circular spelling out the consequences of the flooding of the Thames. It recommended all organizations likely to be affected to review their precautions and outlined the warning system at present in operation. London seems still to be in danger seventeen years after the Waverley Committee urgently recommended that it should be protected. Three hundred people died the year before, when the Thames Estuary flooded.

The Waverley Committee had good reason to be concerned. If the present level of defence was overtopped by 1 foot, serious flooding would result, with damage likely to be in the order of £1,000 million quite apart from the potential loss of life and the inconvenience of having London partially paralysed for up to nine months. The area theoretically in danger in the worst predictable combination of circumstances is that below the 23 foot contour. This is an area of 60 square miles and 1.2 million people, with 15 power stations, 4 sewage plants, 23 storm-water pumping stations, 56 telecommunications buildings, 46 miles of underground and 70 stations, almost all Government departments and the Houses of Parliament. The problem arises first because South-East England is settling so that the exceptional high water mark is rising at the rate of about 2.43 feet a century and second because of the power of the North Sea surges, usually caused by an intense depression from the Atlantic closely followed by a high pressure area. If the low pressure trough, on reaching North-East Scotland, swings south-east over the North Sea, the surge, amplified by the continental shelf, the comparatively narrow channel and strong winds, rushes down the North Sea and into the first convenient opening—the Thames. Every depression causes a surge, although usually a minor one. Should such a happening coincide with a high spring tide, and if the river were also full after heavy rain, the results could be disastrous.



Pressure conditions that will tend to induce Thames flooding.

At present there is a complicated five-stage system giving warnings ranging from a 12-hour-preliminary to a one-hour-final in Central London. The preliminary warning is given between 20 and 25 times a season, the three-hour Central London warning has been given only three times in the past five years. Given the present circumstances, the system seems as satisfactory as possible.

The government is at present paying 65 per cent (the Greater London Council is paying the other 35 per cent) of the £5.5 million needed to raise the riverside walls eighteen inches—the first time they have been raised since 1930. This will be completed in just over a year—the odds that the present level will be reached are about 1 in 10 in any year. With the new walls built, the odds of there being six inches of flood water in any one year will be 1 in 110. To provide what the GLC describes as an acceptable level of protection—7,000 to 1 in any year or 100 to 1 in a man's lifetime—the walls would have to be raised 6 feet, which is clearly unacceptable.

The alternatives—to build a barrage or a barrier—have been bandied about various committees and engineering companies since 1954. The government has recently approved the construction of such a barrier, which is now being designed, at Silvertown in the Woolwich Reach. Design is expected to take another eighteen months to two years, and construction a further five, at a cost of about £50 million.

NEW WORLD

Patent Policies Aim to Provide Incentives to Industry

by our Washington Correspondent

A POLICY designed to enhance the likelihood that research financed by the taxpayer will be commercially exploited was announced last week by the Office of Science and Technology. The new policy should delight private corporations, which now have the chance, under certain circumstances, to gain exclusive licences for exploiting government inventions, and it should also clear up a few vexing irregularities in the Federal government's attitude towards the granting of patents on inventions arising out of government sponsored research. Described by Dr Edward E. David, the President's Science Adviser, as a "new method of bringing flexibility to the market place", the new policy is the fruit of a study of federal patent policies carried out under the auspices of the OST by the Committee on Government Patent Policy.

The new policy, which replaces that spelt out by President Kennedy in 1963, contains the following major provisions:

- Heads of departments or agencies of the Federal government can in certain circumstances award exclusive licences to contractors, allowing them to offset risk capital in bringing an invention to the market place. The chief criterion for awarding such a licence is simply that commercialization of an invention arising from government-sponsored research "will best serve the public interest", and that the invention is unlikely to be exploited unless the company is given sufficient financial incentive.

Built into the new policy is also the provision that licences can be awarded for inventions that are the primary object of a contract. The previous patent policy had specifically prevented a contractor from gaining greater rights than a non-exclusive licence to such inventions, and the Federal Council for Science and Technology found that some agencies had misinterpreted this clause so that, for example, the Department of Health, Education and Welfare might be prevented from granting greater rights to a health invention.

Explaining the rationale behind the decision to enable departments and agencies to grant exclusive licences for the exploitation of government inventions, Mr. O. A. Neumann, executive secretary of the Committee on Government Patent Policy, said that studies have brought to light cases where inventions would not reach the market place unless a period of exclusivity was provided to the developer to recoup his private investment. Mr Neumann declined, however, to give details of such cases.

- If the Administration does decide to turn over principal or exclusive rights to an invention to a contractor, a licence to make, use or sell the invention will still be retained by the government, unless the head of the department involved determines that it would not be in the best public interest to do so.
- If the government itself decides to retain the principal or exclusive rights to an invention, it may also grant the contractor the right to a non-exclusive licence to manufacture and market the invention. But even in this case, the company's licence could later be revoked and replaced by an exclusive licence if the government decided that extra encouragement was needed to develop and market the invention.
- Finally, the new patent policy requires every department and agency to keep a close record of its licences, so that the effects of the policy can later be evaluated by the government Committee on Patent Policy. Pointing out that past government patent policies have been difficult to evaluate because of inadequately kept records of non-exclusive licences, Mr Neumann said last week "there is really no way for us to know what sort of activity we shall be getting into, and it is very difficult to see what the level of activity will eventually be".

Government departments and agencies are also divided over the question of what effect the new policy will have on their activities. NASA and the Department of Health, Education and Welfare, for example, point out that they have both in the past had the power to grant exclusive licences in exceptional circumstances, but each has only issued one or two such licences in the past few years. It seems likely, therefore, that departments and agencies will continue to allow commercial exploitation of inventions developed under government contract on a free-for-all basis as, for example, the Department of Agriculture did when a process to manufacture penicillin on a large scale was discovered under contract from the government. Exclusive rights are likely to be granted to companies only for inventions which would require large risk capital to bring them to the market place or where the market potential would be an insufficient incentive for the company to put up the development capital.

What the policy statement has accomplished, however, is to provide an explicit basis for the negotiation of government contracts which together account for some $15,000 million of federal money each year. About half of this expenditure is accounted for by the Department of Defense, which has in the past consistently assigned patent rights to the contractor when the contract was first negotiated, before any discoveries had been made. This practice has, however, come in for some attack in the past for giving away public property. What those critics will make of the new patent policy should be interesting.

DERMATOLOGY

FDA in Rash Again

by our Washington Correspondent

THE Food and Drug Administration may soon seek to extricate itself from a grave dilemma by allowing a highly toxic drug to be legally marketed for use against severe cases of psoriasis, a debilitating skin disease. The dilemma that the FDA faces is that whatever action it takes on the drug will result in strong criticism; if it allows the drug to be used against psoriasis, it will be assailed for not protecting the public against a drug that has already been shown to be highly toxic, but if it takes action against the manufacturer to prevent the drug from being sold as a dermatological preparation, it will be accused of taking out of the hands of physicians their only effective weapon against psoriasis.

The FDA has only itself to blame for its dilemma, for during the past four years it has turned a blind eye on use of the drug by dermatologists, and has taken no action against the manufacturer, Lederle Laboratories, for commercially promoting the drug for an unlicensed use. It has been fully aware of the drug's severe side effects, but has taken few steps to warn either the public or the medical profession about them.

Under the trade name 'Methotrexate' (MTX), the drug was licensed by the FDA in 1959 for use against leukaemia, but in 1963 the manufacturer notified the FDA that it was sponsoring experiments using the drug to treat psoriasis. The FDA has thus allowed the drug to have an experimental status for eight years, thereby violating its own rule that such situations should not be unduly prolonged.

Lederle Laboratories has notified the FDA of 71 different adverse side effects associated with use of MTX, ranging from disease of the kidneys, liver and blood, and ulceration, to nausea and bleeding from the nose and mouth, and the FDA is also aware that there have been at least 25 deaths associated with use of MTX to treat psoriasis. At the same time, however, some dermatologists have reported complete clearing of psoriasis symptoms from fifty per cent of patients who had the disease over as much as three-quarters of their body. Other treatments for the disease, which manifests itself as red, scaly patches which can be disabling if they cover a large area of the body, are largely ineffective.

Why cannot the FDA continue to ignore the problem as it has done for the past eight years? The chief reason is that the House Intergovernmental Relations Subcommittee which, under the chairmanship of L. H. Fountain oversees the work of the FDA, last month took the unusual step of holding public hearings on the FDA's handling of the drug. As a result, Mr Fountain has accused the agency of being "grossly remiss in not formulating and enunciating a firm but reasonable policy of enforcement", and last week he wrote to Charles C. Edwards, Commissioner of the FDA, urging him to write individual letters to physicians warning them of the hazards associated with MTX.

Fountain's letter, which was also signed by Mrs Florence Dwyer, ranking minority member on the committee, pointed out that the FDA has taken off the market drugs that are much safer than MTX, and that it would be inappropriate for the FDA to allow the drug to be marketed for treatment of psoriasis. The chief concern is that even if the FDA insists on safeguards such as strict supervision over the use of MTX, there is no guarantee that all doctors would be able to exercise the strict control that would be required, and that, in any case, adequate and well controlled studies of the efficacy and safety of the drug have not been carried out by the agency's scientists during the eight years that MTX has been on experimental trial. It is therefore argued that the FDA should warn every doctor by individual letter of the danger of using MTX to treat psoriasis, and that doctors should also be warned that continued use of the drug for unlicensed purposes could result in MTX being withdrawn from the market.

James D. Grant, Deputy Commissioner of the FDA, said in testimony before Fountain's subcommittee last month that the agency hopes soon to work out with the manufacturer an acceptable labelling for MTX which would permit its use only in severe cases of psoriasis. Such a compromise would in effect give legal sanction to the practice established during the past four years—a practice which Fountain said left him wondering whether the FDA understands its role to "enforce the law, not to debate, not to enter into agreements, not to anguish about violations but to act and terminate violations". He added that "if the FDA will not enforce the law, then the public does not need an FDA".

ALASKA

Pipeline Economics

While the Department of the Interior is busy revising its environmental impact statement on the proposal to construct an oil pipeline across the Alaskan wilderness, opposition to the plan is continuing unabated. The latest attack on the plan has come not from environmentalists, however, but from nineteen congressmen (eighteen Democratic and one Republican) who are concerned that the pipeline would exacerbate the disparity in oil prices that now exists between the west coast on the one hand and the midwest and east on the other. Led by Mr Les Aspin from Wisconsin, a noted opponent of the trans-Alaska pipeline plan, the congressmen (who all represent midwester or eastern states) have written a letter to President Nixon urging him to instruct Rogers Morton, Secretary of the Interior, not to issue permits for construction of the line until a full economic and environmental study of an alternative route through Canada has been carried out.

The nub of their argument is that present plans to pipe oil from the North Slope across Alaska to the ice-free port of Valdez, and then to ship it to the west coast for consumption, will decrease oil prices in the west and further increase the difference in oil prices between the west coast and the rest of the United States. A barrel of oil now costs 60c more on the east coast than on the west, and the letter to President Nixon suggests that the difference would be increased to about 140c if the pipeline were built. If oil were piped through Canada directly to the midwest and the east coast, however, the congressmen contend that the price differential would be wiped out. There is, however, no overriding ecological argument for rerouting the pipeline across Canada, although danger from spillage would be reduced since the Canadian route would not involve shipping, and unlike the trans-Alaska route, it is not located in an earthquake zone. The Canadian route would be about twice as long as the Alaskan route, it would cost considerably more, and it would take two or three years longer to build. In attempting to get the pipeline re-routed, its opponents, like the opponents of the SST, will therefore face considerable opposition from industrial interests. Among the signatories to the letter is Senator William Proxmire.

Short Notes

HEW Empire Attacked

Mr Paul G. Rogers, chairman of the House subcommittee on Public Health and Environment, hopes soon to introduce legislation designed to dismantle the unwieldy bureaucracy of the Department of Health, Education and Welfare. Speaking at a meeting of the American Hospital Association last week, Rogers said that health has a weak voice and leadership in the Federal Government because it has no secretary at cabinet level to argue for and coordinate the activities of the health industry, and accordingly he hopes to set up a department of health, outside the realms of HEW. Such a department, he suggested, would obviate the necessity for setting up specialist agencies such as the Conquest of Cancer Agency and the Office of Drug Abuse Control. Claiming widespread support for this proposal, Rogers pointed out that he knows of no organization that would oppose it, but even if he introduces the legislation as soon as Congress reassembles in September, it is unlikely to pass through the Congressional mill before next spring.

Professor for Life

The University of Chicago has appointed Charles L. Fefferman, this year's winner of the Prix Salem and a specialist in Fourier series, to the rank of full professor in the department of mathematics. The department has twenty-three other professors of mathematics, many of whom are better known than Fefferman, but what makes his appointment interesting is that at the age of 22, Fefferman is the youngest full professor in the history of the University of Chicago and probably of any other major university in the United States. He entered the University of Maryland at the age of 14, with the help of a special dispensation allowing him to be admitted without a high school diploma, obtained his BS degree in 1966, when he was 17, his PhD from Princeton in 1969, and was appointed assistant professor at the University of Chicago last year. Fefferman published his first paper at the age of 15, and has eight papers now in the press. His tenure of the professorship at Chicago is for life.

NEWS AND VIEWS

Detecting Underground Nuclear Explosions at Last?

IF the results of recent work in Canada are to be believed, it is possible to locate and verify explosions of more than 20 kton, fired in consolidated rocks, using seismometers in routine production. The first announcement of this achievement came at the twenty-fifth general assembly of the United Nations in December 1970, when the Canadian delegation presented a largely theoretical assessment of the feasibility capacity today for controlling a ban on underground testing of nuclear weapons. This study was based on information supplied in accordance with a resolution (2604A(XXIV)) of the general assembly in December 1969. At the Conference of the Committee on Disarmament in Geneva this summer, Canadian workers claimed, as a result of using this information in an extensive study of earthquakes and explosions, that the identification threshold for Eurasia is about 20 ktons referred to containment in consolidated rock. (To be sure, the deliberate evasion of detection is a separate question.)

Thirteen years ago this threshold of identification may well have satisfied the treaty makers, who seemed ready to negotiate an agreement on the basis of the 1958 Report of the Geneva Conference of Experts which concluded *inter alia* that some twenty to a hundred earthquakes a year would remain seismically undistinguishable from underground explosions of about 5 kton yield given a specially deployed network of control stations. Only earthquakes would be positively identified. Stations which now routinely communicate data could, the Canadian study now claims, be improved for a moderate cost such that the identification of 5–10 kton explosions would be possible with 90 per cent probability.

The criterion on which these conclusions were based is the difference between the amplitude spectra of earthquakes and explosions. The current method of measuring this difference is to apply corrections for distance, thereby normalizing the amplitudes of the P (short period) and Rayleigh (long period) waves to the magnitudes m_b and M_s respectively. Theoretical calculations presented at Beaugency in 1964 suggested that the ratio $m_b:M_s$ might be a method of identifying explosions when Carpenter (*Vesiac Report*, 4410-99-X, 119; 1966) found that the amplitudes of Rayleigh waves generated by explosions should be an order of magnitude less than those of earthquakes of equivalent body wave magnitude m_b. This result has been confirmed empirically by research at Lamont (*J. Geophys. Res.*, **71**, 4333; 1966), at UKAEA Blacknest (UKAEA *Special Report*, 46; UKAEA, 1965, and AWRE 0-67/66; HMSO, 1966) and at Ottawa (*Geophys. J. Roy. Astron. Soc.*, **17**, 1; 1969). A scientific article promised by the Canadian workers may remove the remaining uncertainties in the empirical observations, and the third part of the Cambridge series (*Geophys. J. Roy. Astron. Soc.*, **18**, 233 and 353; 1969) is expected to improve the theory.

So far as the Test Ban is concerned, however, it seems that the principal issue today is the difficulty of recording Rayleigh waves from small explosions. Compressional body waves (P) from underground explosions of 1–2 ktons have been detected and located at distances up to 80° by arrays of some twenty seismometers (AWRE 0-33/70; HMSO, 1970), but the Rayleigh waves required for identifying explosions of this size would not be recorded beyond about 10° by long period arrays of equivalent dimensions. This may be the reason why a new look is being taken at the more easily recorded body waves (P) in which interest flagged after the Novaya Zemlya underground explosions had reduced the value of the complexity discriminant (IASPEI, *Trav. Sci.*, Ser. A, Fasc. 24, 26; 1968). The Lincoln Laboratory of the Massachusetts Institute of Technology took the initiative by estimating spectral energies in selected bands of the P-wave train and found the ratio of high frequency (1.5–1.9 Hz) to low frequency (0.4–0.8 Hz) energy for earthquakes was generally lower than for explosions (IASPEI, *Trav. Sci.*, Ser. A., Fasc. 24, 25; 1968). Intuitively one might expect the seismic energy of explosions to be concentrated in the higher frequency band than for earthquakes but the discriminant worked satisfactorily in practice only for explosions in Central Asia.

On page 51 of this issue of *Nature*, Dr F. M. Anglin of the Seismology Division at the Department of Energy, Mines and Resources, Ottawa, has combined both the complexity and a modified form of the short period spectral criteria to give a satisfactory separation of earthquake and explosion samples taken from Eurasia. The methods for measuring complexity have all taken the ratio of some measure of amplitudes within the first few seconds of a short period P-wave train to the next few tens of seconds. Anglin finds the optimum condition by taking the rectified sum of the Yellowknife array elements phased to give the maximum P amplitude, and compares the first two seconds of the P signals with the following 33 seconds. In the frequency domain the principal difference from the Lincoln Laboratory procedure is to include an f_3 (Hz) term over a much wider band (0.32 Hz–5.0 Hz), and to sample the first 6 seconds of the P signal. The Lincoln Laboratory used the first 20–30 seconds.

The numbers representing these two parameters for twenty-seven underground explosions in five different areas of the Soviet Union and China, and fifty-one shallow focus earthquakes within 15° of one or other of the explosion epicentres, neatly separate the two classes independently of magnitude. Explosions with complex P-wave trains, like those detected from Novaya Zemlya, are rich enough in the higher frequencies to be included in the explosion population, and the frequency spectra of earthquakes with rather simple wave trains evidently peak at lower frequencies. There are only four samples (one explosion and three earthquakes) with magnitudes less than m_b 4.5, and because this is the magnitude range in which P-wave discriminants can make a significant contribution to the Test Ban problem, there is clearly still a long way to go. (It is important that the epicentral parameters of low yield nuclear explosions should be published.) The physical basis of both the complexity and spectral differences of seismic events is not explained, so it is impossible to predict what the result of a similar test on Nevada explosions and earthquakes would be; Lincoln Laboratory failed with these events.

OCEAN FLOOR

Standard Section

from our Geomagnetism Correspondent

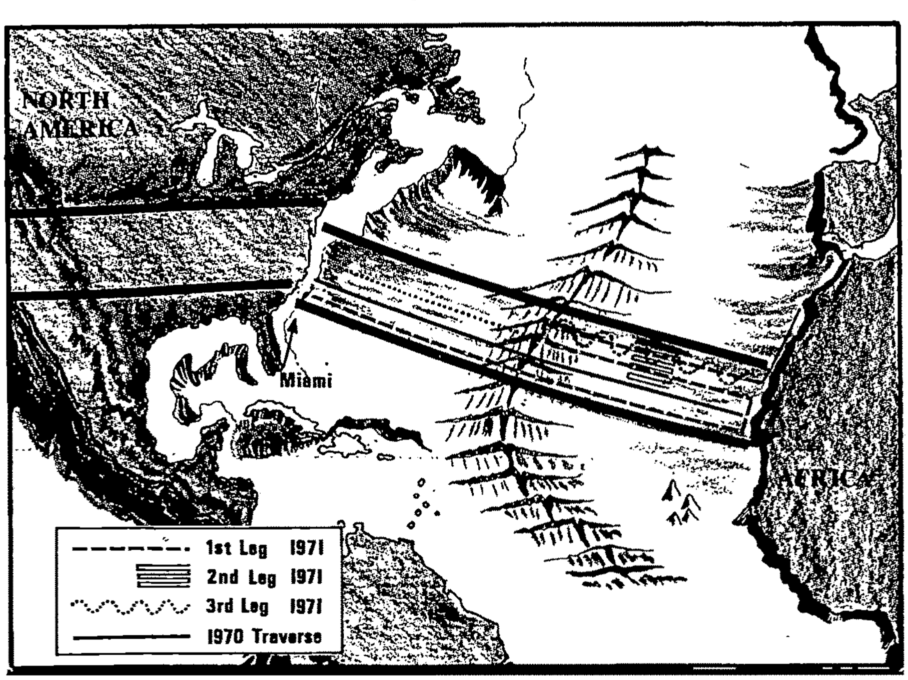
ALTHOUGH the third leg of the Trans-Atlantic Geotraverse was completed less than three months ago, some of the preliminary results have already been released by Dr Peter A. Rona, the project's chief scientist. The Trans-Atlantic Geotraverse (or TAG, in the inevitable mnemonic) was recommended in 1968 by the US National Academy of Sciences and the US Upper Mantle Committee as a desirable contribution to the International Decade of Ocean Exploration. Since it began in 1970 it has been under the direction of the National Oceanic and Atmospheric Administration which has put its own ship, the Discoverer, at the disposal of the project. According to Rona the general aim of the programme is to establish the "first standard crustal section across an ocean basin" and it certainly looks as though that will be achieved before the project is completed in 1974.

The section in question (see diagram) is a 200-mile wide, 3,500-mile long curved strip across the Atlantic from Cape Hatteras to Cap Blanc and is calculated to link points in Africa and North America which were immediately adjacent before the onset of continental drift. The western end of the traverse also links up with the Transcontinental Geophysical Survey section, the crustal section established across the United States in 1968 by means of topographic, gravitational, seismic, magnetic and rock-dating studies.

The first leg of TAG comprised three parallel tracks across the whole section. The second involved an intensive investigation of the foothills along the eastern slope of the mid-Atlantic Ridge; and, during the third, the ocean floor was sampled by dredge and corer from the mid-Atlantic Ridge to the African continental shelf. Although the project has an impeccable scientific basis in the academic sense (one of its listed aims is "a more thorough and valuable understanding of the past and present movements of the continents and rifting of the sea floor"), the emphasis is clearly on using the basic science as a guide to the assessment of the potential oil and mineral wealth of the deep oceans; and this is reflected in the first results to be announced.

Thus Rona reports that "extremely heavy" encrustations of manganese ore (which also contains copper, nickel and cobalt) have been found on exposed rocks in regions where Earth movements have caused fractures in the sea bed; and these deposits are believed to be thicker and more extensive than the better known manganese nodules found in other areas in recent years. Also under investigation by the TAG scientists—although no actual results have so far been given—is the relation between the rifting of a continent and the formation of heavy metals such as gold and platinum. Heavy metal ores are a feature of the Red Sea, a region thought to be in the early stages of rifting; and it is thus possible that the volcanic activity accompanying the formation of the mid-Atlantic Ridge may have left heavy metal deposits in the deep Atlantic beyond the continental shelves.

But of perhaps even greater significance are the large domes which have been found in the TAG section off north-west Africa and which resemble the oil-bearing salt domes in the Gulf of Mexico. The Atlantic domes which lie in 15,000–20,000 feet of water and some of which lie at least 800 miles off the African coast would, if their salt content is confirmed, be the first salt domes to be reported in the deep ocean and, as such, would represent the first indication of potentially significant marine oil resources beyond the continental shelves. The hypothesis which Rona and his colleagues are trying to test is that during its early development the Atlantic must have been a body of water almost completely surrounded by land and thus in which evaporation exceeded inflow. Under these conditions salinity would increase and salt would be precipitated, forming layers (and subsequently domes) into which would settle oil-forming plant and fish remains. As the Atlantic opened further, some of these deposits would be left in the deep ocean floor and not only on the continental shelf. So far, however, the validity of this hypothesis has not been confirmed.



ASTRONOMY

Universal Distance

from a Correspondent

How big is the universe? This was the theme of a conference on the galaxy and the distance scale held at the Royal Greenwich Observatory, Herstmonceux, from August 17 to 20 in honour of the Astronomer Royal, Sir Richard Woolley, who retires at the end of 1971.

The conference covered the whole range of distance determinations, from nearby stars to galaxies and quasars. Distances of galaxies are found by a series of intermediate steps involving galactic clusters, cepheid variables, ionized hydrogen regions and galaxies themselves as standard beacons; the latest results reported by Dr A. R. Sandage (Hale Observatories, Pasadena) give a Hubble time (time since the "big bang" if deceleration is neglected) of 18×10^9 years, exactly ten times the value estimated by Hubble in 1936, and a deceleration parameter $q_0=0.95\pm0.4$. This permits a universe that will either go on expanding indefinitely or fall back and oscillate, but probably excludes the steady-state theory ($q_0=-1$). The actual age of the universe then comes out to be about 13×10^9 years, which compares very favourably with the age of $(10\pm3)\times10^9$ years estimated for old stars in globular clusters on the basis of the theory of stellar evolution. The uniformity of the relationship between period, luminosity and colour of cepheid variables in different galaxies makes these estimates seem more firmly based than earlier ones.

Intermediate steps in the measuring process involve the distances of clusters and variable stars, corrections for absorption of light in interstellar space, the distance between the Earth and the centre of our galaxy and the distances to our two nearest extragalactic neighbours, the Magellanic Clouds. The distances of clusters depend on fitting their "zero-age main sequence" in the temperature-luminosity diagram to that of the Hyades cluster which is well determined by the moving-cluster method and confirmed—as was shown by Dr O. J. Eggen (Mount Stromlo Observatory, Canberra)—by entirely independent measurements of the temperatures, magnitudes and trigonometric parallaxes of nearby stars resembling the Hyades in age, motions and chemical composition. The absorption of light in interstellar space immediately surrounding our solar system was shown by Dr B. Strömgren (Copenhagen University) to be almost entirely concentrated in discrete small clouds or large cloud complexes, with almost no continuous distribution of dark matter between the clouds.

Going out to distances more comparable to the size of our galaxy, Sir Richard Woolley described the determination of luminosities of pulsating variables by the Baade-Wesselink method of comparing actual changes in radius deduced from velocity variations with the relative change deduced from variations in brightness and surface temperature. In combination with the theory of pulsation, this method leads to quite low masses for many variables which in turn suggests that they could have lost mass in the course of their evolution. Dr W. Becker (Basle University) described a procedure for building up a contour map of star density in the disk and halo of our galaxy by photometry in red, green and ultraviolet bands of the spectrum which enables faint stars of different luminosity and chemical composition to be sorted out. Dr A. Toomre (Massachusetts Institute of Technology) showed that the distortion of the central plane of our galaxy could be explained by tidal action of the Magellanic Clouds provided that the Earth's distance from the centre of our galaxy does not exceed 8 kiloparsecs (compared with the round figure of 10 kiloparsecs adopted by the International Astronomical Union some years ago). But an attempt to improve galactic constants by studying proper motions was found by Dr S. V. M. Clube (Royal Greenwich Observatory) to lead to inconclusive results.

Are the redshifts of quasars of cosmological origin, indicating enormous distances, or are they an intrinsic effect due to other causes? Arguments on each side were reviewed by Dr M. Schmidt (California Institute of Technology), who showed that the hypothesis of an intrinsic origin would require a revision of present-day concepts in physics, but that the cosmological hypothesis is not fatally in conflict with any results obtained to date.

X-RAY ASTRONOMY

Model for Sco X-1

by our Cosmology Correspondent

SINCE the discovery that Sco X-1 is a triple radio source with some features like those of a pulseless pulsar, there has been speculation that the source might indeed belong to the same family as pulsars (see *Nature Physical Science*, **229**, 218; 1971). K. Davidson, F. Pacini and E. E. Salpeter have now published a detailed model for the thermal X-ray emission from Sco X-1 in terms of a hot gaseous region surrounding a rotating neutron star (*Astrophys. J.*, **168**, 45; 1971). Although this model was developed before the recent flurry of activity which has resulted in the discovery of periodic variations in Centaurus X-3 and Cygnus X-1, these discoveries can, on the face of it, only add to the interest in models such as that of Davidson *et al.*

With all models of Sco X-1, the fundamental problem is the generation of thermal bremsstrahlung—the source has maintained a temperature of roughly 5×10^7 K for a decade, and it has been widely accepted that the basic energy source must be gravitational, with accretion of gas onto a white dwarf releasing energy as proposed by Prendergast and Burbidge in 1968. But the difficulty of explaining the detailed flaring and flickering of Sco X-1 at both optical and X-ray frequencies, together with the presence of intermittent periodic and quasi-periodic structure in the light curves from the source, makes it desirable that other models should also be studied. Davidson, Pacini and Salpeter have looked at models in which the energy source is a rotating neutron star, the inspiration for this stemming from the apparent success of the rotating neutron star model in explaining the energy generation mechanism operating in pulsars.

The model which is most successful has a dense shell of gas surrounding the central pulsar. Supported by particular and electromagnetic radiation far beyond the speed of light circle, at a distance of roughly 10^9 cm, this cocoon is heated to the observed temperature by the radiation it absorbs. For Sco X-1, the observations require that such a shell must be quite thick, the density in the line of sight being several g cm^{-2} so that the pulsar frequency fields do not penetrate the cocoon. Plausible values for the efficiency of the heating mechanism and the mass of the underlying star suggest a pulsar period of 3 ms and a gas cloud radius of 1.5×10^9 cm. But the surface field required is only 5×10^9 G,

Infrared Variations in Seyfert Galaxies?

ALTHOUGH measurements of the infrared emission of the Seyfert galaxy NGC4151 at 11 μm do not yield any evidence of significant flux changes with time, the possibility that some flux changes have occurred cannot be ruled out. That is the principal conclusion reached by Stein and Gillett in next Monday's issue of *Nature Physical Science*.

These results are of particular interest because of their importance for the radiation mechanisms at infrared wavelengths greater than about 10 μm. One model of Seyfert galaxies, for example, proposes that there is a central luminous source of short wavelength radiation surrounded by a cloud of particles that radiate at infrared wavelengths. This model would be untenable if there were fluctuations on a time scale of less than about a year. Variations of the order of a day would also place serious constraints on models involving non-thermal infrared sources.

Quite a large amount of evidence has been accumulated for time variations of the visible and radiofrequency flux from the nuclei of Seyfert galaxies and possible variations have also been reported at the near infrared wavelengths of about 2 to 3 μm; Stein and Gillett have now extended these measurements to 11 μm with photometric measurements of NGC4151 made during the two years up to June 1971.

They have found two pieces of evidence that seem to suggest variations on a time scale of less than a month. In both cases the three standard deviation upper limit of one measurement was much lower than the lower limit of the flux a few days later. But Stein and Gillett are quick to point out that deductions from this can be misleading, and they apply several statistical checks. They find, for example, that the standard deviation of the distribution of their twenty-nine measurements is only slightly greater than the average standard deviation of the individual measurements whereas, if variations had occurred, larger differences would be expected. The numbers of measurements lying within one, two and three standard deviations of the mean of the twenty-nine measurements are also close to the numbers expected if there is no variation. Similar conclusions were reached after analysis of measurements on a comparison star (γ U Ma).

which is much lower than the surface fields required for pulsars and allows the extremely rapid rotation to persist for millions of years.

Typical time scales which Davidson *et al.* expect to be associated with the motions of the components of their model range from the pulsar period, roughly 0.003 s, to the time scale for dynamic equilibrium, roughly 3,000 s. The orbital period of the gas is 30 s, and its cooling time is roughly 0.3 s, so that it must indeed be continuously excited for the system to be stable.

Most of the objections to this model are certainly no more severe than those which apply to other models for Sco X-1. Indeed, with the exception of the rather low magnetic field, the estimated parameters agree well with observations of this and other sources, and the cocoon seems a suitable source of erratic variations of the kind detected. Two crucial tests seem relevant. One or two such objects could be detected without a drastic re-thinking of the theory, but many more sources in the Sco X-1 mould would rapidly become a surfeit. And if Sco X-1 itself does show a continuation of the decline in activity and, possibly, temperature which has been hinted at by some recent observations, then it would seem to be too short lived for the Davidson *et al.* model—this would certainly be a new development, because the downfall of most models of the object so far has been their implausibly short time scales compared with the observed lifetime of Sco X-1.

MINICELLS

Exploiting Cytochalasin

from our Cell Biology Correspondent

ALTHOUGH minicells, small enucleate *Escherichia coli*, have been used occasionally by molecular biologists, it seems safe to say that they became available too late in the game. Many questions, which might with comparative ease have been answered by experiments with minicells, had already been solved by hard work and ingenuity with nucleate *E. coli*. But animal virologists look to be more fortunate for it seems, if the experiments of Prescott, Kates and Kirkpatrick (*J. Mol. Biol.*, **59**, 505; 1971) are anything to go by, that enucleate cells obtained by exposing nucleate cells in culture to cytochalasin B can be exploited to answer many outstanding questions.

Prescott and his colleagues have used enucleate L cells produced in this way to prove quite unambiguously that the uncoating and replication of vaccinia virus does not depend on the presence of a host cell nucleus. After exposing L cells to cytochalasin B, and in some experiments to actinomycin D as well to inhibit any RNA synthesis that the cytochalasin B might induce, and then washing the cultures and incubating them for 2 h in medium they added vaccinia virus and followed the fate of the infected nucleate and enucleate cells by light microscopy and autoradiography. Typical "factories" synthesizing vaccinia DNA develop in the enucleate cells and so the suggestion, originally made by Joklik in 1964, that the uncoating of vaccinia virus depends on a function coded by a host cell gene and activated by infection, has been neatly eliminated.

Joklik's suggestion stemmed, of course, from the finding that after the removal of the lipoprotein outer coat of vaccinia virus, the second step in its uncoating, the disruption of the inner "core" protein coat is specifically blocked by inhibitors of RNA and protein synthesis. This indicated that the synthesis and translation of messenger RNA was essential for the second stage of uncoating and in 1964 it seemed quite plausible that the gene(s) in question might well be cellular rather than viral. By 1967, however, Kates and McAuslan had shown that vaccinia cores with their protein coat intact can make and release messenger and Joklik's idea was beginning to look unnecessarily complex. Prescott's results are not altogether unexpected therefore and the chief interest in what he and his colleagues have to say lies in the method they have used.

Clearly, hypotheses which implicate directly the host genome in the replication of other animal viruses might be put to the test with enucleate cells. It would, for example, be interesting to know how far the replication of RNA tumour viruses proceeds in such cells. For that might reveal whether or not the stable association between a DNA provirus, which, as Hatanaka *et al.* have just shown (*Proc. US Nat. Acad. Sci.*, **68**, 1844; 1971), is probably made in the cytoplasm, and the host genome is essential for replication of these viruses.

Transfer RNA Precursor Sequenced

IN next Wednesday's *Nature New Biology*, Altman and Smith describe their latest investigations of the tyrosine transfer RNA precursor molecule of *Escherichia coli* and its maturation into tyrosine transfer RNA; their work is a remarkable example of the pitch of sophistication which has been reached in analyses of bacteria.

As Altman recently reported in *Nature New Biology* (**229**, 19; 1971) transfer RNAs, like ribosomal and probably messenger RNAs, are transcribed as part of a larger precursor molecule which is matured by cleavage. What Altman and Smith have now done is analyse the base sequence of the precursor of a tyrosine transfer RNA, prove that mutations which alter the amount of the mature RNA produced are located in a region of the precursor close to the cleavage point and detect a nucleolytic activity in extracts of *E. coli* which *in vitro* matures the precursor.

Obtaining strains which accumulate precursor involved a series of elegant genetic manipulations; with these achieved Altman and Smith obtained enough precursor to analyse its base sequence and they found several striking features. First, bases which, in mature transfer RNA, are modified are, in the precursor, unmodified. Second, it seems that the CCA sequence at the 3′ terminal end of the mature molecule is present in the precursor, contrary perhaps to expectation. Third, many of the oligonucleotides obtained after partial digestion of the precursor with T_1 ribonuclease correspond to those obtained after partial digestion of the mature molecule. This situation suggests that even in the precursor the secondary structure of the mature tRNA sequence has a clover leaf secondary structure. Finally, the 5′ terminal residue of the precursor is pppGp, which suggests that transcription *in vivo* as *in vitro* is initiated with a 5′ triphosphate purine nucleotide.

Altman and Smith then sequenced the precursor specified by a mutated tyrosine transfer RNA gene, which *in vivo* matures unusually rapidly. To do this, of course, they had to introduce other mutations which caused this precursor to accumulate. The sequence revealed that the mutation which leads to a rapidly maturing precursor changes a base four nucleotides removed from the cleavage point. Moreover, other mutations which cause the production of reduced amounts of mature transfer RNA but no accumulation of precursor also change bases close to the cleavage point. For these reasons Altman and Smith suggest that both the base sequence and the secondary structure at the cleavage point probably affect the rate and the precise point of cleavage and they propose models of the structure of the precursor which might account for these factors.

Finally, they have succeeded in detecting in extracts of *E. coli* a nucleolytic activity which *in vitro* cleaves the precursor at the correct point and yields a transfer RNA fragment with the same 5′ terminus as transfer RNA matured *in vivo*. This finding proves that modification of certain bases in the transfer RNA segment of the precursor is not essential for proper maturation. Indeed, modification may well occur *in vivo* after the precursor has been cleaved.

HAEMOGLOBIN

Monomers Monitored

from our Molecular Biology Correspondent

THE nature and strength of subunit interactions in proteins has an intimate bearing on the interconversion between states of high and low ligand affinity, which lies at the heart of control of activity. Here, as so often, haemoglobin is the archetype. That the tetramer is in dissociation equilibrium with dimer was established in the very dawn of protein history by the old masters, Svedberg and Pedersen. More recently debate has centred on whether, at sufficiently low concentration, the dimer will in turn give rise to monomer. The general view was that at the lowest observable protein concentrations the monomer remains below the level of detectability. Schachman and Edelstein, on the other hand, reported substantial dissociation into monomers, on the basis of sedimentation equilibrium measurements with scanner optics. This conclusion has now been repudiated by Kellett and Schachman (*J. Mol. Biol.*, **59**, 387; 1971).

Scanner optics for this system offer the advantage that by operating at the wavelength of the very intense Soret absorption band of the haemoglobin, measurements can be made at concentrations as low as about 10^{-8} M in subunits. Unfortunately, oxyhaemoglobin in such dilute solution is prone to oxidize to the ferric form, which then denatures, with irreversible formation of monomeric chains. This seems to have been the cause of the previous error. Kellett and Schachman's re-evaluation was made possible by the remarkable observation that methaemoglobin formation is inhibited by the presence of EDTA, which one can only suppose to function by sequestering traces of heavy metals that would otherwise catalyse the oxidation reaction. At all events, under these conditions the equilibrium distribution in the ultracentrifuge reveals that only oxyhaemoglobin dimer is present, and the same is true of other stable derivatives, such as carboxyhaemoglobin and methaemoglobin cyanide and azide. It may be noted that Bucci (*ibid.*, **55**, 401; 1971) also recently concluded that at concentrations down to 10^{-8} M, oxyhaemoglobin gave rise to no significant amount of monomer, for a complement fixation technique, sensitive at such low levels, revealed the absence of any hybridization between two different molecular species, such as would occur were there a finite degree of dissociation beyond the symmetrical dimer ($\alpha\beta$) stage.

In an accompanying article, Kellett (*ibid.*, **59**, 401; 1971) tackles the much more demanding problem of dissociation of oxy and deoxyhaemoglobin under the influence of high salt concentrations. The difficulties are both experimental, in regard to the anaerobic manipulation of the deoxygenated solutions, and theoretical, in terms of the thermodynamics of the three-component system of protein, water and salt. Kellett has devised an analytical procedure which yields the dissociation constant for the dimer-tetramer equilibrium in terms of a buoyant weight, extracted directly from the sedimentation distribution. The upshot is that whereas the dissociation of oxyhaemoglobin to dimers (though not monomers) is strongly promoted by salt, deoxyhaemoglobin remains tetrameric even at the lowest observable concentrations in 2 M sodium chloride or iodide. Qualitatively the finding of such resistance of the unliganded protein to dissociation is not new, but Kellett's results make it clear that its dissociation constant is not less than three orders of magnitude lower in 2 M sodium chloride than that of oxyhaemoglobin. Evidently then, resistance to dissociation is a feature of the "constrained" (low-affinity) structural state. This has a number of implications in relation to functional schemes that have been propounded, and also explains the apparently anomalous failure of the cooperativity (Hill constant) to diminish in high salt, when, it was thought, the protein was dissociated to dimers.

It is interesting to find that some putative evidence for haemoglobin monomers in a quite different context has now been annihilated. The haemoglobin-binding protein of serum, haptoglobin, attaches to haemoglobin at sites on the α-chains. Nagel and Gibson found that when haptoglobin was partly saturated with haemoglobin α-chains, β-chains were taken up, and this process occurred at a faster rate than when whole haemoglobin was added in place of β-chains. This result was put down to a slow dissociation of the tetramer, by way of the known $\alpha\beta$ dimers, to single β-chains, which then rapidly associated with the haptoglobin-bound α-chains. Boyd, Smith and Andrews (*Canad. J. Biochem.*, **49**, 891; 1971) prepared the α-chain-haptoglobin complex, and added to it an excess of mouse haemoglobin. By gel filtration they then separated out of the mixture, first, a haemoglobin-haptoglobin stoichiometric complex, second, mouse haemoglobin, and, finally, human α-chains. Evidently then, the initially bound α-chains are displaced. Now it is known that mouse and human haemoglobins hybridize satisfactorily. Boyd *et al.* conclude therefore that monomers do

---

Supermassive Rotors and Active Galactic Nuclei

A LONG standing model for quasars and active galaxies postulates the presence of supermassive differentially rotating stars, supported by radiation pressure, at the centres of these objects. In spite of other suggestions, including the possibility of pulsar-like emission from a supermassive rotating object, this relatively simple model remains a likely explanation for the energy source in quasars and galaxies. But the model is only superficially simple—although it is clear from the most naive calculations that a supermassive rotating disk satisfies the broad requirements of the primary energy supply in highly energetic sources, quantitative calculations concerning the evolution of such disks remain inadequate. It is only within the past two years that it has been shown that disks of this kind are unstable against fragmentation, and although this complicates further calculations the prospect raises some interesting conjectures regarding the ultimate fate of the disks. E. E. Salpeter, of Cornell University, has now provided an outline of the successive fragmentations which should accompany the slow evolution of a supermassive star. This approach, presented in next Monday's *Nature Physical Science*, raises several questions which point the way for further development of the model.

It seems that, so long as the disk is radiation dominated, fragmentation must occur. This process will be repeated in the subdisks formed, until small objects supported by hydrodynamic forces are formed. Because the descendants of the original disk will form a hierarchical grouping after several fragmentations, radiation escaping from the system will suffer successive redshifts as it leaves first the subgroup from which it originates, then the group of subgroups in which that subgroup lies, and so on.

One source of electromagnetic radiation could be pulsar-like activity, which would occur just before the fragmentation of any disk which carries a magnetic field.

The major difficulties in extrapolating this simple picture to more realistic models concern differential rotation and the detailed nature of the gravitational and pulsar radiation emitted. Gravitational radiation, for example, might result in selective changes in the orbits of the fragments until a stable, symmetric pattern with no gravitational radiation is formed.

not enter into the scheme at all. Whereas β-chains can add directly to the complex, the reactive species in haemoglobin is the $\alpha\beta$ dimer, and the slow step is then the dissociation of each α-chain from the haptoglobin to allow an $\alpha\beta$ unit to bind in its stead.

UPPER MANTLE

Tasman Conductivity

from our Geomagnetism Correspondent

THE Tasman Geosyncline, which lies along the southern part of the east coast of Australia, is a long structure generally some 200–300 km wide and includes two major topographic features, the Australian Alps and the Main Dividing Range, as well as the three major cities of Canberra, Melbourne and Sydney. Geologically it comprises folded and metamorphosed rock belts of Ordovician to mid-Devonian age and includes the major granite batholith which forms Mount Kosciusko, at 2,219 m the highest point of the Australian continent. Seismic refraction studies have shown that the crustal rocks beneath Canberra are about 40 km thick but thin to about half of that in the coastal region. The eastern boundary of the geosyncline is not particularly clear although seismicity extends to the continental shelf. The western boundary, on the other hand, is rather different; seismological studies have indicated quite a clear boundary to the current tectonically active belt. Little, however, is known about the region immediately to the west of the geosyncline in spite of its proximity to the three principal Australian cities. Outcrops are poor there and the only major structure mapped, and that superficially, is a sedimentary basin.

To learn something about the behaviour of the upper mantle, both beneath the geosyncline and to the west of it, Tammemagi and Lilley (*Geophys. J.*, **22**, 505; 1971) have made four magnetotelluric soundings at 150 km intervals roughly along the latitude of Canberra—at Moruya on the east coast, Spring Valley near Canberra almost on the crest of the belt, Wagga Wagga slightly to the west of the geosyncline and Griffith well to the west. The magnetotelluric method itself is not new, although in this case automatic equipment was operated at each site for at least four weeks, a rather longer than usual period, to ensure that enough events were recorded. In spite of the long history of the method only a handful of magnetotelluric measurements have ever been made in Australia and none at all in what could be regarded as Australia's best known geological region.

Three events or periods of activity, including at least one magnetic storm, were frequency analysed for each site and for signals in the period range 10^3 to 10^4; and the usual curves of apparent resistivity (of the upper mantle) were constructed. As is well known, the interpretation of magnetotelluric data is frequently hampered by the inherent ambiguities, especially where the frequency range is limited and where the curves of apparent resistivity show anisotropy. In this particular work, three of the four stations were troubled by anisotropy. At Moruya, on the east coast, ocean-induced anisotropy is clearly present. The data are most consistent with a two-layer conductivity model although because of the anisotropy a large number of detailed models within this classification are possible. At Wagga Wagga and Griffith the situations are similar except that three-layer models are more appropriate. But at Spring Valley, close to the crest of the geosyncline, there is no anisotropy. A two-layer conductivity model is again most appropriate here although a range of electrical conductivity structures is possible.

But although anisotropy and the limited frequency range have prevented Tammemagi and Lilley from making detailed conductivity models, one important fact has emerged from the data —this is that in the depth range 100–300 km the electrical conductivity under the stations Moruya and Spring Valley is an order of magnitude higher than that under Wagga Wagga and Griffith.

In other words, the conductivity is higher under the Tasman Geosyncline than under the region to the west of it. In view of the correlation between electrical conductivity and heat flow discovered in Utah and Colorado by Reitzel *et al.* (*Geophys. J.*, **19**, 213; 1970), Tammemagi and Lilley interpret this as a temperature difference in the upper mantle between the two regions. Using the relationships presented by Tozer (*J. Geomagn. Geoelect.*, **22**, 35; 1970), they calculate that at a depth of about 200 km the temperature difference should be about 200° C; but whatever the precise figures, the existence of a

---

Helping Human Adenovirus to Replicate

SEVERAL human adenoviruses infect only abortively African green monkey kidney cells—the virus particles penetrate these cells and are uncoated; viral RNA is made, the viral genome replicates and at least some viral proteins are synthesized, but very few progeny virus particles are produced. In these monkey cells it seems that either some crucial viral proteins are not synthesized or the transport of the viral proteins from the cytoplasm where they are made to the nucleus where progeny virus particles are assembled is blocked. By coinfecting the cells with simian virus 40, however, these blocks are removed and the yield of adenovirus is greatly increased. In other words, SV40 provides some helper function which permits human adenoviruses to complete their replication in non-permissive monkey cells. In next Wednesday's *Nature New Biology*, Henry, Slifkin and Merkow report experiments which shed some light on this intriguing phenomenon.

Henry *et al.* infected with human adenovirus 2 coverslip cultures of non-permissive African green monkey kidney cells and permissive VERO and HeLa cells, which support the complete replication of this virus. Some of the monkey cell cultures were also coinfected with SV40 either immediately or within 24 h and 5-fluorodeoxyuridine (FUdR) was added to some of the coinfected cultures. This drug blocks the synthesis of "late" SV40 proteins but does not impair the SV40 helper function(s). At 24 or 45 h after infection 2 min pulses of labelled amino-acid were fed to the cultures which were then chased with cold medium. The synthesis and transport of protein made during the pulse could then be measured by autoradiography.

In brief Henry *et al.* found, using an immunological precipitation assay to differentiate between viral and host proteins, that some 60–70 per cent of the viral protein made in permissive cells is transported into the cell nuclei. In the monkey cells infected by adenovirus 2 alone only about 20 to 30 per cent of the protein labelled during the pulse is in the nucleus some 2 h later, the remainder is still in the cytoplasm concentrated about the periphery of the nucleus. By contrast, in monkey cells coinfected with SV40 and exposed to FUdR about 50 per cent of the adenovirus protein is transported to the nucleus. Further, the amount of viral protein made in the coinfected cells is significantly greater than the amount made by the cells infected with adenovirus alone.

It seems therefore that some SV40 gene product(s) not only enhances the synthesis of adenovirus proteins but also promotes the transport of these proteins into the nucleus where they are assembled into capsomeres and capsids. It is probably this second helper function which is chiefly responsible for increasing the yield of progeny virus particles; its molecular basis, needless to say, awaits elucidation.

temperature contrast is presumably reflected by, either as cause or effect, the tectonic activity within the geosynclinal region.

The possible presence of temperature variations naturally raises questions about the heat flow in the area although, unfortunately, very few measurements have been made. There is, however, a published heat flow value of 2.0–2.2 μcal cm^{-2} s^{-1} at Spring Valley and one of 1.1–1.2 μcal cm^{-2} s^{-1} at Moruya which suggest that towards the middle of the geosyncline at least the heat flow is higher than average in keeping with the enhanced mantle temperature. By the same token, heat flow to the west should be lower, though not necessarily lower than the world average. The only heat flow determinations to the west are several hundred kilometres away at Broken Hill which is itself in a tectonic region, the Adelaide Geosyncline. Tammemagi and Lilley thus predict a heat flow trough between the two geosynclines and, should such a trough be proved, look forward to confirmation of their interpretation of the magnetotelluric data.

CENTRAL NERVOUS SYSTEM

Useful Brain Cells

from our Cell Biology Correspondent

BIOCHEMISTS debating whether or not to take the plunge and tackle some of the biochemical problems posed by the development and differentiation of the central nervous system should be encouraged by what Seeds has to say in the latest issue of the *Proceedings of the US National Academy of Sciences* (**68**, 1858; 1971). For he has shown that dissociated mouse embryo brain cells, when put in rotation cultures, form themselves into aggregates which continue to undergo at least some of the biochemical changes that occur during the normal postnatal development of the intact mouse brain.

Seeds chose to follow the activity in his aggregates of three enzymes, choline acetyltransferase, acetylcholinesterase and glutamate decarboxylase, which are associated with the process of nerve transmission and which greatly increase in activity in the mouse brain during the first three to four weeks of postnatal life: the central nervous system of mice is unusually poorly developed at birth and both the formation of synapses and the growth of dendrites as well as biochemical development occur after birth. Seeds finds that the time course and pattern of increase in the activity of choline acetyltransferase and acetylcholinesterase in intact brains and in the aggregates of embryonic brain cells in culture are encouragingly similar.

Moreover, morphological differentiation also occurs in the aggregates; Seeds mentions, for example, the presence of numerous synapses. Although nobody suggests that these aggregates can be used to solve all the questions posed by the development of the central nervous sys'tem they at least offer a most promising starting point.

Brain cells also have attractions for biochemists interested in cytoplasmic microtubules, and Bryan and Wilson (*ibid.*, 1762) report that these organelles in embryonic chick brain cells are polymers of two species of protein not one. Using colchicine as a label, several groups have isolated microtubule subunits from a variety of different cells. The best characterized of these, isolated from adult human brain, appears to be a dimeric molecule comprising two polypeptide chains weighing about 55,000 to 60,000 daltons, and it has generally been assumed that the two polypeptides in each dimer are identical. Bryan and Wilson, however, find that the two polypeptides of the dimer protein of chick brain microtubules can be separated by polyacrylamide gel electrophoresis after they have been reduced and acetylated. Furthermore, although these two polypeptides have the same molecular weight, about 55,000, they differ significantly in their content of six amino-acids.

From this and similar studies of microtubules from other sources Bryan and Wilson conclude that the basic subunit of these organelles is an $\alpha\beta$ dimer, rather than an $\alpha\alpha$ dimer or a mixture of $\alpha\alpha$ and $\beta\beta$ dimers.

CHEMOSYSTEMATICS

New Committee

A JOINT Committee on Chemotaxonomy has recently been formed by the International Union of Pure and Applied Chemistry (IUPAC) and the International Association for Plant Taxonomy (IAPT) to look into all aspects of the organization of international collaboration in chemosystematics. The committee invites comments from interested persons; these should be sent to the chairman (Dr W. F. Grant, Genetics Laboratory, Macdonald Campus of McGill University, Ste. Anne de Bellevue 800, Quebec, Canada) or to the secretary (Dr T. Swain, Royal Botanic Gardens, Kew, Richmond, Surrey, England).

Polyadenine and Haemoglobin Messenger

LAST year, Lim and Canellakis (*Nature*, **227**, 710; 1970) discovered tracts of polyadenine, some fifty to seventy bases long, in the ribonuclease digests of purified rabbit globin messenger RNA. Since then similar tracts of poly A have been detected in RNA fractions, isolated from the polysomes of HeLa and ascites cells, which are also presumably messengers. It seems therefore that the presence of sequences rich in adenine may be a general feature of many, perhaps even all, eukaryotic messengers. Because of their restricted sequences it has been generally assumed that these tracts are not translated but occur on either, or on one side of the coding sequence in the messenger, and in *Nature New Biology* next Wednesday Burr and Lingrel report direct evidence that this is the case.

After labelling the 3′ terminal residues of rabbit globin messenger RNAs with tritiated borohydride they digested the molecules with either T_1 or pancreatic ribonucleases and isolated the 3′ terminal fragments. After T_1 digestion these fragments elute from 'Sephadex' columns in the region at which oligonucleotides with eight phosphate residues appear. The terminal base of this fragment is adenine. The 3′ terminal fragments obtained after pancreatic ribonuclease digestion, which cannot contain pyrimidines, are five and six bases long and, because the first guanine residue cannot be within nine bases of this terminus, Burr and Lingrel conclude that these messengers have at their 3′ termini a tract of five or six adenine residues and from the known amino-acid sequences of the rabbit globins it is clear that these bases are not translated.

The sequences Burr and Lingrel have analysed are considerably shorter than those detected by Lim and Canellakis, and there are several possible explanations of this difference. Much longer poly A sequences may exist at the 5′ ends of these molecules; the short sequence Burr and Lingrel have determined may be part of a much longer adenine rich tract or the poly A tracts may be labile and partially lost either during the extraction of the messenger or indeed *in vivo* as the molecules age. Needless to say, the function of poly A in eukaryotic messengers is as yet a matter of speculation and, as anybody who reads what Burr and Lingrel have to say will realize, there is no shortage of ideas.

The Doomsday Syndrome

JOHN MADDOX

THE past few years have been quite remarkable for the way in which there has flourished a rich vein of commentary on the place that science and technology occupy and should occupy in the modern world and for the way in which the underlying theme of this commentary has tended to be that science and technology will quickly bring society to its knees. Even quite short memories are sufficient to show that this development flies in the face of the view of the social function of science and technology that was current and commonly accepted as recently as twenty years ago. Then, and for the best part of a century before, it was supposed that social progress must rest on technical progress of some kind or another. The great philanthropists of the nineteenth century, for example, were constantly linking their schemes for liberating people from unpalatable and degrading occupations with the hope that mechanical alternatives would be found for jobs of work that were manifestly unsuitable for people. They were buoyed up by the encouragement of the nineteenth century liberals and by the legacy of the earlier utopians—people like Godwin, for example—who took the view that there would be no limit to the achievements of the human spirit so long as people could be liberated from the need for drudgery so as to keep alive and from the restraints of the environment in which they found themselves and which was, almost by definition, hostile. It is hard to imagine a more complete contrast than that between the way in which technical developments used to engender optimism and the way in which the prospect of technical innovation has now become a kind of threat.

The signs of this new attitude are now plain to see. First of all, the commentators are conspicuous and what they have to say is arresting. Several arguments have been used in the past few months to heighten the sense of impending disaster. The rapid growth of the world's population is, for example, one of the most common. And, of course, it is by now well known that the population of the world cannot continue to grow at the present pace for decade after decade. On present estimates, the world's population probably falls only little short of 4,000 million, and so many of the people now alive are either children or still in the child-bearing age group that it seems unlikely that contraceptive devices, however fully and ardently employed, will allow the world's population to stabilize at less than 7,000 million. This population should be reached by the turn of the century. It goes without saying that the most spectacular growth will be in the countries still at an early stage of their economic development so that, by the year 2000, countries such as Brazil and Indonesia will be roughly comparable in the size of their populations with the United States and the Soviet Union.

The prospect of this pattern of growth has apparently caused great consternation in the past few years. The most vociferous prophet has been Dr Paul Ehrlich, who has held quite openly that what he calls "the population bomb" must spell disaster for society at large, not merely for those unfortunate communities where the pace of growth is greatest. And what kind of disaster? Dr Ehrlich says quite categorically in his best-known book that the world will run out of food—that the mid-seventies will be times when famine is rife and when tens of millions of people will die each year of starvation. This view, itself an echo of the doctrines which Malthus put forward in the earlier versions of his distinguished tract on population, has been taken up by all kinds of commentators, from people like Lord Caradon to a host of popularizers. In the circumstances, it seems almost an indecent question to ask whether disaster is as near as all this talk suggests.

In the first place, however, it is a surprising fact that the population of the world has grown less quickly than its production of food. The Food and Agriculture Organization of the United Nations, in its time one of the most fervent beaters of the calamity drum, has kept statistics of food and population going back to the early nineteen thirties. The remarkable feature of these statistics is the way in which food and population appear to march hand in hand. Although there are obviously ups and downs in the statistics of food production, chiefly because of fluctuations of climate from one year to another, the production of food per head of population has actually increased by five per cent between the late thirties and the late sixties. And now, as everybody knows, many developing countries are faced with the prospect not of famine but of the social and economic problems of marketing harvests of cereals which so far exceed the past performances of peasant farmers that it is necessary quickly to develop the commercial machinery long familiar in the agriculture of nations in which subsistence agriculture has long since disappeared. In other words, whatever the prophets of doom may say, it does seem that famine will not be the most obvious cause of calamity in the next decade. On this point, it appears, the doomsday view seems entirely inappropriate.

To say all this does not imply, of course, that the pace of growth of the world's population brings no unfortunate or unforeseen consequences, or that nothing needs to be done about it. It is, however, important that these problems should be seen in their historical perspective. Students of population are impressed with the way in which it seems to have taken the human race from the beginning of its evolution two million years ago to the beginning of the nineteenth century to reach 1,000 million, a further century to add a second thousand million to the total roster and merely half a century since then to double the population of the world. On the face of things, this is much more than exponential growth—the time constant is continually decreasing. Yet there are good reasons for thinking that the growth of a human population is much more complicated and subtle than the growth of a population of bacteria in a set of Petri dishes.

In Britain, for example, the mediaeval population was kept in check by a succession of infectious diseases, from plague to influenza. The agricultural revolution in the eighteenth century so increased the productivity of the land that the population was able to grow quite quickly, and there is plenty of evidence that from the second half of the eighteenth century on, the birth rate in Britain was much less than it might

The pages which follow are intended as contributions to the wider understanding of the interest and excitement in contemporary science on the occasion of the 133rd annual meeting of the British Association at Swansea from September 1 to 8.

have been if all women of child-bearing age had produced their full biological quota of children. In other words, it looks as if it is quite possible for a growing population to limit its rate of growth even without the full apparatus of modern contraceptive techniques. The way in which the population of Britain took another spurt forward in the second half of the nineteenth century is a little more puzzling. Part of the explanation is that children died less frequently in infancy, and therefore survived to have children of their own, but it also seems that comparatively prosperous families were able deliberately to decide to have larger families.

This brief history is relevant to what is happening now elsewhere in the world. First of all, the rapid growth of the world's population in the past thirty years is almost directly attributable to the way in which better medicine and better insecticides (used against the mosquitoes which carry malaria, for example) have enabled hard-pressed communities to keep their children alive until middle age. In some quarters, quite misguided, this is held to be a proof that medicine and insecticide technology can be "blamed" for the growth of the world's population. The truth is, of course, quite different. In present circumstances, there is good reason to think that one of the conditions that must be satisfied before rapidly growing populations take the steps to limit the pace of growth which seem to have taken place in the eighteenth century in Britain is that there must be a still further improvement of medical care in infancy. In South America, India and South-East Asia, it seems to be quite commonplace that mothers when asked how many children they would like to have provide higher answers when the infant mortality is high. For reasons which are obvious and which cannot be dismissed, they take the view that they must have enough babies to make sure that one at least will grow up to be an adult male. But it is clear that prosperity is another condition to be met, as recent experience of a falling birth rate in places such as Hong Kong and Singapore bears out. As it happens, all this is quite familiar and, to say the least of it, it is surprising that the prophets of doom have made so little of this to them unpalatable evidence.

What this implies is that there is every reason to expect that social forces, already apparent in the recent demographic history of Britain, will in due course help to control the populations of developing countries. To be sure, it is important that steps should be taken to encourage these developments. If the true objective of those who preach calamity is to bring about a stable solution, they would be much better advised to advocate those kinds of social innovations that might encourage stability than to complain that famine is only just around the corner. In doing so, of course, they would be driven to recognize that one of the ironies of the contemporary problem of population growth is that the countries where the population is growing most quickly are so compelled to invest resources in schools and medical facilities that they have very little for the kind of industrial investment that might in the long run make them economically prosperous, itself a precondition for population stability. The moral in this is no doubt that foreign aid of the right kind could make an important contribution to the stabilization of the world's population. The modern equivalent of Malthus's position is that population growth brings impoverishment. It is irresponsible of the doomsday men to shirk these subtleties, and to settle for a kind of biblical choice between survival and calamity.

The alarm which attends the public discussion of the population problem crops up everywhere. Pollution, for example, is now so widely held to be a threat to the survival of orderly society that there is a danger of a serious distortion of social priorities. DDT and the other chlorinated hydrocarbons which have functioned as insecticides since the war are good examples. The materials are persistent, and are toxic to animals which concentrate them. It seems to be well established that birds such as peregrine falcons and eagles are particularly affected by these insecticides. At the same time, however, nobody would suppose that the damage done to eagles in, say, North America by insecticides is anything like as great as the damage caused by the illegal shooting of eagles by farmers in the Dakotas and elsewhere in the Middle West of the United States. And, moreover, there is no evidence at all that these insecticides have been damaging to people. To be sure, experiments in the United States, admittedly inadequate and only preliminary in character, have shown that the chlorinated hydrocarbons in very large doses can increase the incidence of tumours in mice, but there is as yet no sense of what bearing this may have on human health, although it does seem clear that the long range effects of DDT and similar materials are unlikely to be more damaging than a great many other familiar chemicals in the environment, and certainly less damaging than some of those which have in the past few decades been eliminated altogether—the use of carbon disulphide in vulcanizing plants, for example. Although the persistence of DDT argues for close control of the way in which it is used, it is surely entirely misguided that an over-zealous scorn for DDT and related chemicals should rob the world at large of the benefits they may have in the prevention of malaria, in the improvement of precisely the kinds of agricultural productivity that bear on the problem of how to feed the growing population and which are, in any case, a good deal less damaging to the environment than some of the materials—sodium arsenate, for example—which they replace. To be sure, it is always possible to hope that more subtle methods of controlling insect populations, ranging from the deliberate infection of insect populations with parasites to the use of pheromones for baiting insect traps, may in due course help to control populations of unwanted insect pests without causing serious side effects. But it is only proper that those who advocate the abolition of DDT on the grounds that it will bring calamity should warn the agriculturists whom they would deprive that biological control is still a technique applicable only in particular circumstances.

Population and pollution are, however, only two of the spectres which perplex commentators on the relationship of science and technology with society. Other people fear that carbon dioxide in the atmosphere will eventually produce such a powerful greenhouse effect that the Antarctic ice cap will be melted but, in reality, nobody can be sure that the effect will be that predicted and in any case the accumulation of carbon dioxide in the atmosphere is by no means the inexorable process that the doomsday men suggest. Other recent fears—the current alarm about the way in which exhausts from supersonic aircraft may destroy the ozone layer in the atmosphere and thus expose people to dangerous doses of ultraviolet light—are similarly unfounded, but this does not undermine their usefulness in the hands of those who seem anxious to demonstrate that the world is about to end.

In the long run, the most interesting feature of the present wave of gloomy speculation is likely to be the sudden emergence of what will, no doubt, be considered to have been a wave of fashion. Most probably, the real threat of global catastrophe posed by nuclear weapons in the forties and more especially the fifties is a part of the explanation why there should now be such concern that global calamity might arrive. But, paradoxically, the threat of nuclear war on a catastrophic scale is now in retreat. That the fifties should have left behind such a sense of bewilderment about the way to strike a balance between the useful applications of technology and the unwanted side effects which it can produce is a social phenomenon of great interest. Is it possible that the explanation is to be found in the sharp contrast between the doomsday view now prevalent in advanced societies and the optimism about science and technology still to be found where prosperity is, for the time being, less in evidence?

An extract from an address to Section X of the British Association.

Science, Statistics and Society

A. W. F. EDWARDS

Department of Medicine, University of Cambridge

Statistical techniques primarily developed for the natural sciences are finding increasing application in the social sciences. At a time when there is little agreement among statisticians about the properlogical basis of statistical inference, the use of equivocal methods for the interpretation of scientific data of social relevance, and the misunderstanding of some of the basic tenets of inductive inference, may have grave social consequences.

STATISTICAL theory has been developed, principally over the past hundred years, in response to the needs of astronomers and physicists and, more recently, to the needs of medical, biological, and agricultural research workers. The body of methods which has thus been created is now increasingly finding application in the social sciences, with the result that techniques originally invented for the reduction of astronomical observations, or for the testing of hypotheses in controlled experimental conditions, are being applied in areas which are socially sensitive. But are they the appropriate techniques? Are they sufficiently clearly understood to contribute positively to the analysis of data in the social sciences, or do they obscure the train of thought by substituting rhyme for reason?

As the "centre of gravity" of research moves from the physical, through the biological, to the social sciences, statistics (in its inductive, as opposed to purely descriptive, role) becomes of increasing importance as the filter through which the information contained in the data will pass. But in the minds of most non-statisticians this filter is a black box, filled with mysteries, even though provided with labelled inputs and outputs. The black box has recently adopted the dimensions of a computer centre, complete with packages of statistical programs available on demand to all who conform to their input requirements. Though admitted to be full of mysteries, it is widely supposed that the black box operates on principles generally accepted by statisticians. It comes as a shock to discover that the statistical world is in a ferment of disagreement over these principles. Not even does the concept of a null hypothesis and the associated simple test of significance, that hallmark of statistical numeracy demanded by the editors of progressive journals, pass unchallenged.

Controversy in Statistical Inference

As an example, consider the following question set in a university examination for a BA in Sociology[1]:

"Let μ be the true IQ of student X. It is known that his scores on test Y are normally distributed with mean μ and standard deviation 4.00. The test is administered to him on occasion Z and he scores 125.

(i) What is the maximum-likelihood estimator of μ?

(ii) Test the hypothesis that $\mu = 120$.

(iii) Comment upon the statistical bases of the methods used to solve (i) and (ii)."

A well informed candidate might answer as follows:

"(i) The maximum-likelihood estimator of μ is the sample mean; in this case the estimate is therefore 125.

(ii) See (iii).

(iii) The statistical basis of (i) is in dispute. Some hold that it is reasonable to contemplate a 'true' value, and that the maximum-likelihood estimator is to be preferred because it has optimum properties under repeated sampling, too complex to be discussed here. Others hold that the concept of a 'true' value is vacuous, and that the maximum-likelihood estimate is to be taken because it offers the best fit to the data, in the sense of maximizing the likelihood. It should be noted that still others reject the use of maximum-likelihood on either justification.

"There are at least six points of view on (ii):

(*a*) The question does not admit a statistical solution.

(*b*) A test may be constructed on Neyman–Pearson principles, according to which the hypothesis will be accepted or rejected, as in a decision procedure, the justification being in terms of repeated sampling.

(*c*) A test may be constructed on Fisher's principle in which the probability of the observed result, or one more extreme, on the basis of the null hypothesis, is used to judge whether that hypothesis is to be rejected. It might be 'one-tailed' or 'two-tailed'.

(*d*) The fiducial distribution of μ may be found, and a statement of fiducial probability made about the null hypothesis.

(*e*) A Bayesian position may be adopted, in which a prior probability distribution for μ, representing knowledge about μ prior to the observation, is compounded with the likelihood function derived from the observation, to form a posterior distribution for μ. There is no complete agreement over a suitable prior distribution, nor over the most suitable form of test once the posterior distribution has been found.

(*f*) A pure likelihood position may be adopted, in which the null hypothesis is compared with the most likely alternative hypothesis using the likelihood ratio.

"In view of the unresolved arguments over the applicability of these methods, I am unable to answer (ii) to my satisfaction, though it should be noted that since the distribution is specified as normal, some of the methods would give very similar answers, a circumstance that has helped to obscure important logical distinctions."

There is, of course, no time to teach such a critical approach. "In the behavioural sciences in general the overwhelming practice both by researchers and those responsible for statistical training has been to ignore the issues raised by the critics and to continue doing things as before."[2]

In an earlier article[3] I examined some of the logical problems of the conventional approaches, concluding in favour of the use of likelihood as the basis of the calculus for inductive inference[4]. In the present article I air some of the more elementary points in probability and statistics which are often misunderstood, hoping thereby to encourage those who already harbour suspicions about many conventional arguments not to allow themselves to be dazzled by statistical brilliance.

The Null Hypothesis

No argument should be accepted simply because it relies on some orthodox statistical practice. Statistical techniques need to be viewed as aids to, and not substitutes for, thought. They will not help if they are not clearly understood, and they may seriously hinder. Take, for example, the null hypothesis, widely acclaimed in the social sciences as the *sine qua non* of objective data analysis, but viewed with suspicion by others as providing "the basis for the simplest decisional procedures which have so effectively colonized the more gullible sciences"[5]. Not so many years ago responsible people arrived at sensible conclusions from bodies of data by an intuitive process that can only be described as "judgment", in which their training, their experience, and their unique way of thinking each played a part. Such a subjective approach is now viewed with scorn; instead it is argued that we should adopt the objective statistical approach of setting up a null hypothesis and testing it. But how are we to choose the null hypothesis?

A notorious example is provided by the current debate on the inheritance of intelligence, in which it appears at first sight that a suitable null or "empty" hypothesis is that intelligence in man contains no inherited component. But since data on the inheritance of intelligence are difficult to acquire, and the subject of much special pleading, a "rejection of the null hypothesis at the 1% level of significance" is hardly to be expected. Repeated non-rejection of the null hypothesis is too easily interpreted as indicating its acceptance, so that on the basis of no prior information coupled with little observational data, the null hypothesis is accepted.

Far from being an exercise in scientific objectivity, such a procedure is open to the gravest misgivings. What used to be called prejudice is now called a null hypothesis, and what used to be called judgment is now called prejudice. For may we not, with equal absurdity, adopt the null hypothesis that all variations in intelligence are genetic in origin? Thus we find that not only are the statistical methods appropriate for testing a null hypothesis in dispute, but the very concept of such a privileged hypothesis is a potential danger.

If we base our actions—such as social policies—on such an approach, we lay ourselves open to the tyranny of null hypotheses, and the prejudices of those who choose them. Thus it would be "natural" to advance the null hypothesis that family allowances have no effect on family size, but—in the absence of evidence on the point—it would be absurd to act as if this hypothesis were true just because it has been designated "null". A further example is provided by discussions of whether violence and obscenity on television corrupt: the absence of positive evidence is sometimes taken to justify the assumption that they do not.

In the more abstract world of pure scientific research, in which such statistical techniques evolved, questions of prejudice less frequently arise, and are generally less important when they do. But in other fields the adoption of methods which are so open to misuse is to place a dangerous weapon in uncritical hands: "The enthusiast whose sole interest in science is to find support for a preconceived social policy, not only deceives himself, and perhaps others who are not aware of his bias, but inevitably brings some discredit upon all other workers in the same field"[6].

Moral and Metrical Equality

One of the characteristics of our age is a preoccupation with equality. In its moral sense, "the condition of being equal in dignity, privileges, power, etc., with others"[7], surely no justification is needed, yet there is a tendency to seek one by predicating moral equality on "metrical" equality, "the condition of being equal in quantity, amount, value, intensity, etc."[7]. There is thus often a strong motive for assuming metrical equality solely on the basis of no evidence to the contrary, an assumption to which some statistical theories lend support.

In the approaches which depend on the null hypothesis, scientific respectability is apparently conferred on the concept of equality, for if we have no information on the variability of a statistical population, an apparently "empty" hypothesis is that all the members of that population are the same in respect of the attribute in question. On many socially important problems it is regarded in some quarters as irresponsible to challenge the null hypothesis with data, yet in truth the hypothesis itself is an exceptionally strong one in most applications, one which no thinking scientist would accept were it not for the influence of current statistical practice.

In the Bayesian approach a similar role is played by Laplace's *Principle of Indifference* applied to beliefs. Ignorance is represented by an equality of probability, a postulate which many cannot accept because they hold that a probability statement is invariably informative, and that the only permissible expression of ignorance is no statement at all[4].

The predication of moral on metrical equality, and the unwarranted assumption of the latter, are weak points in a common argument; the first is an example of the best causes attracting the worst arguments, whilst preoccupation with the second, and hence a concern with relative rather than absolute standards, heralds an inflation which is not only monetary.

The assumption of equality may indeed have precisely the opposite effect to that which it is intended to have by its well meaning proponents. Thus, returning to the question of intelligence, if we assume that each child has the same intellectual potential whatever his parentage, and if this turns out to be a false assumption, those at either extreme of the Mendelian lottery will not receive the special treatment necessary to increase social equality. As has been observed on more than one occasion recently, any attempt to allocate university places or degree classes in such a way that each group (be the grouping by class, race, creed, sex, or any other category) is represented in proportion to its numbers may have most undesirable effects. For should any such group in fact be less well endowed genetically than another, it will soon become common knowledge that a first-class degree (say) is of a lower standard when the holder is a member of that group. Membership of a particular group will then be seen to be relevant to the assessment of the individual, which is precisely the situation we wish to avoid. Perhaps these matters were more clearly understood before the advent of statistics. In the sixteenth century it was well known in Cambridge that the intellectual reputation of Trinity College was founded on the fact that its fellowships were not restricted by place of birth: in most other colleges there was a restriction on the number of fellows from each county, in an attempt to ensure national equality[8]. The system fell into disuse.

Causation, Correlation, Classification

The confusion of thought contained in the failure to distinguish the concepts of causation and correlation is so elementary that it would not need stressing were it not for its grave consequences. It is a distinction which statisticians are invariably at pains to make clear, and hence differs from those misconceptions which have their origin in statistical theory. The confusion of the two concepts is a major source of prejudice, especially in race relations.

At the same time as techniques, now regarded with suspicion by many statisticians, are sweeping through the social sciences, some of the more straightforward consequences of probability theory are neglected. Perhaps the most important of these is the fact that there are two ways by which we may acquire information about an individual Suppose I speak to you on the telephone and we arrange a meeting, never having met each other before. I know from your voice that you are a man, and from your name that you are an Englishman. Even though this is all the information I possess about you, it enables me to make a statement about your height, thus: the probability that you are between 5 feet 8 inches and 5 feet 8.5 inches tall is 0.8, if I take as my datum the fact that Englishmen are normally distributed in height with a mean of 5 feet 8 inches and a standard deviation of 2.5 inches.

Such a statement is poised half way between complete ignorance and complete knowledge of your height; that is why it is phrased in terms of probability: "It has been often recognized that any probability statement, being a rigorous statement involving uncertainty, has less factual content than an assertion of certain fact would have, and at the same time has more factual content than a statement of complete ignorance. The *knowledge* required for such a statement refers to a well-defined aggregate, or population of possibilities within which the limiting frequency ratio must be exactly known. The necessary *ignorance* is specified by our inability to discriminate any of the different subaggregates having different limiting frequency ratios, such as must always exist"[9].

The important point is that my statement refers to *you*. It is not true to assert—to take a recent example from the discussion on education—that "characteristics of social groups have no implications for the kinds of individual differences which are the concern of education"[10]. For such a statement to be valid, either the social groups must not differ in respect of the characteristic, or the logic of probability theory is at fault. The first point needs to be demonstrated. The process of induction outlined above is very old: indeed, the whole point of giving objects names at all is to classify them, and hence to convey information about them. If I say "I own a dog" you know certain things about the animal I keep in my house, though you have not seen it.

The second way in which we acquire information about an individual is by direct measurement. Thus, when I meet you, I may judge your height "to the nearest inch", or, if I carry a measure, "to the nearest tenth". But this new information does not annihilate the old; indeed for over two hundred years we have possessed a theorem which enables us to combine information from these two sources: Bayes Theorem.

The technical details of the theorem cannot now detain us, but reading sociological articles leaves a distinct impression that this most important theorem has not yet penetrated into the thinking of social scientists. In their concern to treat the individual as an individual they deny the relevance of group characteristics; but this is to deny Bayes Theorem, and hence to suppress some relevant information. Objectivity is not to be achieved by the suppression of information.

One of the consequences of the theorem is that, as individual measurements become more reliable, they become more important relative to the "prior" information derived from group characteristics, as is to be expected: a doctor who books a maternity bed for one of his patients on the basis of standard tables for the duration of pregnancy is making the best use of the meagre information at his disposal; but when the patient telephones to say that labour has started, the new information far outweighs the old.

The problem of the applicability of Bayes Theorem is a deep one. It is clearly appropriate in scientific situations such as those described above, where information must not be discarded. But it is excluded when considering criminal cases in courts of law, in which guilt is pronounced only if it is "beyond all reasonable doubt". If the direct evidence is so meagre that the prior information, such as provided by previous convictions for the same type of offence, is relatively important, then there will still be "reasonable doubt" about the guilt of the accused. Besides which, justice requires that every case be decided "on its merits", since the accused may be a reformed character for whom evidence of previous convictions would be irrelevant. He should have the benefit of the doubt.

It is in the assessment of the merits of the individual that the operation of Bayes Theorem needs the closest scrutiny. Thus in considering applicants for a post, we should ideally evaluate the suitability of each and every one with such diligence that any prior information is of negligible importance in our final decision. In practice, the effort is too great. We therefore invent qualifications, such as degrees and certificates, and only consider applicants who hold qualifications we consider appropriate: professors are almost invariably appointed from among graduates (George Boole was an exception). An inevitable cycle then starts. From being an indication of suitability for a post, a degree becomes a prerequisite. It thus becomes desirable in itself; demand exceeds supply; expansion occurs to meet the demand, and the supply is soon more than adequate. The surplus graduates are aggrieved that they cannot find employment of the kind that graduates formerly enjoyed. An inflation has taken place because the original purpose has been partially forgotten, and the cry is raised for the abolition of the system.

The consequences of misunderstanding some of the basic tenets of inductive inference, and of uncritically accepting and applying statistical recipes whose foundations are still in dispute, are grave. Quantitative work in the social sciences suffers and inappropriate modes of thought are introduced into otherwise rational discussion.

We need not applaud the extreme reaction of Hogben[11] to the penetrating advances that have been made this century towards a better understanding of statistical inference, but we would be foolish not to heed his warning: "Laboratory experiments will have to stand on their own feet without protection from a façade of irrelevant computations. Sociologists will have to use their brains. In my view, science will not suffer".

[1] Quoted by Rosenbaum, S., *J. Roy. Statist. Soc.*, A. (in the press).

[2] Morison, D. E., and Henkel, R. E. (eds.). *The Significance Test Controversy—A Reader*, (Butterworths, London, 1970).

[3] Edwards, A. W. F., *Nature*, **222**, 1233 (1969).

[4] Edwards, A. W. F., *Likelihood* (Cambridge University Press, in the press).

[5] Edwards, J. H., *Nature*, **221**, 593 (1969).

[6] Fisher, R. A., *The Genetical Theory of Natural Selection*, Clarendon Press, Oxford, 1930).

[7] *Shorter Oxford English Dictionary*, (Clarendon Press, 1959).

[8] Gray, A., *Cambridge University: An Episodical History*, (Heffer, Cambridge, 1926).

[9] Fisher, R. A., *Statistical Methods and Scientific Inference*, (Oliver and Boyd, Edinburgh, 1956).

[10] Richards, M., Letter to *The Times* (July 2, 1970).

[11] Hogben, L., *Statistical Theory*, (Allen and Unwin, London, 1957).

Fossilmanship in East Africa

SARAH BUNNEY

Recent discoveries in the Plio-Pleistocene deposits of East Africa may call for a re-examination of man's evolutionary history.

HAD a cantankerous camel ridden by Richard Leakey not stalled one August day in 1969 in the desert heat of northern Kenya, palaeoanthropologists might have missed one of the most interesting discoveries of recent years—a skull, intact but for its teeth and jaws, of an early hominid more than two and a half million years old. But for the bad humour of the camel, as Leakey recounted later in the *National Geographic Magazine*[1], he and his party would not have broken camp earlier than was planned and would probably not have come upon the skull lying in a dry stream bed in the Ileret area on the east side of Lake Rudolf. The fossil resembles[2] *Australopitheicus boisei*, the robust form of australopithecine known from East Africa and, by coincidence, Leakey found it almost ten years to the day after his mother, Dr Mary Leakey, discovered the first representative of this species in Olduvai Gorge, farther to the south.

Hunting for fossil man thus has its lucky moments—and the Leakeys certainly seem to have their share of them—but as the pages of *Nature* for the past three or four years show, this activity requires not only a remarkable flare for knowing exactly where to look for fossils but also a willingness to work sometimes under the most inhospitable conditions in what is often unexplored territory; recent palaeontological expeditions to East Africa have made history in more ways than one.

The rewards have, nonetheless, been rich. Richard Leakey's chance discovery in 1969 of the robust australopithecine skull in the Rudolf area and the associated hominids and fossil vertebrates collected in that year and in the previous year's season were but foretastes of what was to follow in 1970. The results of this last season's work, described earlier this year[3], added not only another fifteen or so hominids to the Rudolf list (including what may prove to be specimens of an early representative of *Homo*), but also extended the vertebrate collection to some six hundred specimens[4].

Richard Leakey is wisely, though tantalizingly, reticent about the taxonomic affinities and dates of these hominids until more detailed anatomical and geological analyses have been made, but there now seems little doubt that this part of East Africa will be as significant in the study of human origins as Olduvai Gorge in Tanzania made famous by Richard's father Dr Louis Leakey.

Being Plio-Pleistocene in age, the Rudolf deposits (see Fig. 1), and those in the Omo Valley in Ethiopia[5,6], extend back in time from those at Olduvai by another two million years and, together with the other finds of australopithecines elsewhere in Kenya, they help to fill in the gap between the *Ramapithecus* stage of human evolution, of Miocene times (see the recent reviews of this subject[7-9]), and the early Pleistocene *Australopithecus* remains from Olduvai and the South African sites. It might therefore be thought that the jigsaw of human evolution was neatly being pieced together. But is as becoming clear from the preliminary results of the Rudolf expeditions[3], not all the pieces are fitting into the puzzle in quite the way that palaeoanthropologists have been led to expect and it seems likely that Richard Leakey's interpretations of the Rudolf material will be as contentious as those his father made regarding the *Homo habilis* specimens from Olduvai.

Coexistence

The chief point of interest in the reports to date[2,3] of the Rudolf investigations are Richard Leakey's suggestions that more than a million years ago the genus *Homo* coexisted

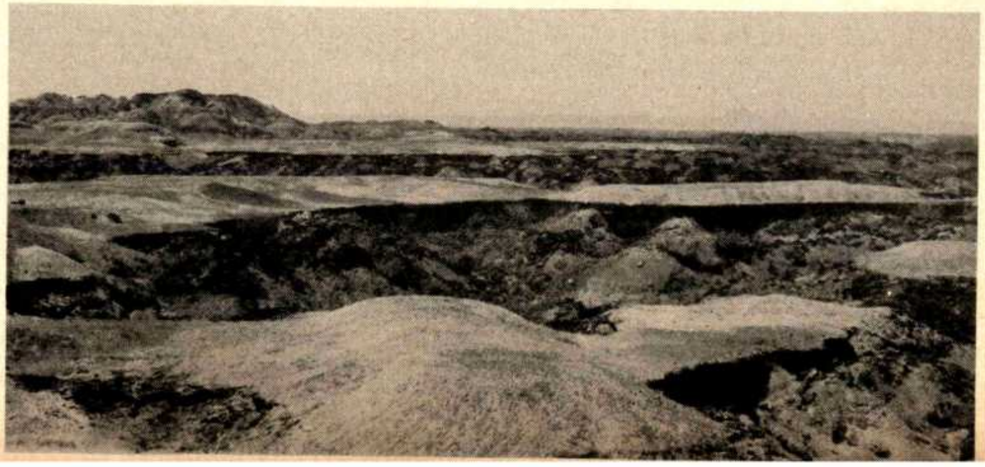


Fig. 1 Pleistocene exposures in the Koobi Fora area, East Rudolf.

with *Australopithecus* in this part of East Africa at the beginning of the Pleistocene, and therefore that the two genera may well have been quite separate and distinct at this time. He also revives the idea that the differences in size and morphology between the gracile and robust types of australopithecine (both of which have been found in stratigraphically similar deposits in the Lake Rudolf area) relate to sexual dimorphism and the two are thus not separate species or subspecies as is usually held to be the case.

Richard Leakey bases his claim for *Homo* at Lake Rudolf on three specimens—two mandibles (both are fragmentary but KNM–ER–730 is more complete than KNM–ER–731) and an incomplete femoral shaft (KNM–ER–737). Further discoveries and more detailed examinations are obviously required before this claim will be seriously considered by other workers—what is now needed, in particular, is some agreed definition of a complex of morphological features characteristic of the line of evolution leading to man—but if these specimens are accepted as *Homo*, and KNM–ER–730 seems a particularly good candidate for inclusion in the genus, they may be the best evidence so far of the genus outside Java where *Homo erectus* is known. Richard Leakey has so far published no dates for his *Homo* sites. It seems very likely that they are more than a million years old, but it is not clear from the reports whether they are as old as the age of 2.61±0.26 million years which is the date given by Leakey[2] for some of the Rudolf hominids including the specimen (KNM–ER–406), *Australopithecus* aff. *boisei*, mentioned earlier.

This is not the first suggestion that *Homo* and *Australopithecus* were contemporaneous in some parts of Africa. For several years, Louis Leakey has claimed that a more advanced form of hominid, the famous *Homo habilis*, coexisted with *Australopithecus* in the Olduvai Gorge locality for about a million years and that this form and not *Australopithecus* was responsible for fabricating the primitive tools recovered from Beds I and II, known as the Oldowan culture. Only recently[10], he has published an account of another *Homo habilis* specimen (OH 24) which was discovered in 1968 in Bed I at Olduvai. The fossil, nicknamed "Twiggy", was found in a very crushed state and its inclusion in *Homo* is likely to be questioned by the several workers who regard *Homo habilis* as an East African variant of the gracile *Australopithecus africanus*, a species which was known first from the South African sites.

There have been similar doubts regarding the material from Swartkrans in South Africa, once known as "*Telanthropus capensis*" and subsequently reclassified by Professor T. Robinson as *Homo erectus*. According to Robinson and some other workers, this material is evidence of the coexistence in the early Middle Pleistocene of a more advanced hominid than the robust australipithecine. *A. robustus* (formerly *Paranthropus*) known in large number from that site. But this claim has been questioned by some authors who hold that the so called *Homo erectus* material, like *Homo habilis*, is simply another variant of the polytypic *A. africanus* population of the time. This issue was reopened last year with the description by Dr. C. K. Brain and his colleagues[11] of a reconstructed skull from Swartkrans which they claim is a representative of the genus *Homo* but to which they assign no specific name. The niceties of this taxonomic problem, like those of *Homo habilis*, will no doubt continue to be argued about by Wolpoff[12] and others for several more years to come.

Part of the reason for the current confusion over early hominid taxonomy lies in the fact that although there is now plenty of cranial material, particularly teeth and lower jaws, postcranial remains of *Australopithecus* are rare. It is only through detailed analyses of such bones that some idea can be obtained of the gait and stance of the creatures. One of the criteria used for distinguishing *Homo* from the pre-human stage is his upright bipedalism, and the limb bones of early hominids which can provide clues to the origin of bipedal locomotion are therefore particularly valuable. The hominid limb material from Lake Rudolf, claimed by Richard Leakey[3] to be representative of both the *Australopithecus* and *Homo* forms, should thus be very useful.

Until the Rudolf discoveries, a complete femoral shaft of an australopithecine was unknown, although femoral ends of robust forms had been recovered several years earlier from Swartkrans (SK 82 and 97) and from Olduvai (OH 20) (see ref. 13 for a discussion of these), and a reconstruction of a femur of a gracile form has been made from material recovered at Sterkfontein. The Rudolf material should therefore allow useful comparisons with this australopithecine material, with the femur of the supposed *Homo* from Rudolf, and also with postcranial material of undisputed *Homo erectus* such as that recovered in 1970 from the Middle Pleistocene Bed IV at Olduvai and described recently in *Nature* by Dr M. H. Day[14]. This last material (OH 28) was found in association with an Acheulean industry and is the first direct association of tools with *Homo erectus*. This association naturally does not necessarily imply that *H. erectus* was responsible for the industry, but Mary Leakey[15] thinks it likely because there is other evidence at Olduvai of a possible link between early Acheulean hand axes from Bed II and *Homo erectus*. Day has also been able to compare[14] OH 28 with femora of approximately the same age from Choukoutien in China and with the femora claimed to be of the same age from Trinil in Java.

Antiquity

In spite of the considerable confusion over the fine distinctions between *Australopithecus* and *Homo*, the new work in East Africa is confirming the view that there were marked morphological variations between populations of early hominids and that these variants can be broadly grouped into gracile and robust forms which persisted relatively unchanged for several million years. For just how long they persisted is being brought out by some of the recent discoveries in Kenya and elsewhere. Until a few years ago, australopithecines were thought to have evolved as a distinct group only about a million years ago; even with the redating in the early 1960s of the timing of the beginning of the Pleistocene epoch the antiquity of these hominids was thought to be only as much as about 2 to 3 million years. With the discoveries of new Plio-Pleistocene deposits in Kenya and Ethiopia, however, and more reliable radiometric dating techniques it is becoming clear that *Australopithecus* had evolved by the end of the Pliocene.

The oldest undisputed australopithecine material known to date seems to be the portion of a lower jaw with one molar intact found by Professor B. Patterson's team at Lothagam Hill near the south end of Lake Rudolf in Kenya. This fossil is thought to be a representative of the gracile form of australopithecine and its date of 5.5 million years puts it well and truly in the Pliocene. Patterson and his colleagues also have the distinction of finding part of a hominid humerus, which likewise probably belonged to a gracile form in deposits at Kanapoi in Kenya. The date of this specimen was put at 2.5±0.2 million years when it was described in 1967 by Patterson and Professor F. C. Howell, but more accurate dating of the lava flow overlying the deposits in which the fragment was found and comparisons of the mammalian fauna with those from the Omo Valley and elsewhere suggests that the Kanapoi hominid is between 4.0 and 4.5 million years old. In their turn the Omo Valley hominids seems to have a range of between 2.0 and 4.0 million years[5,6]. At Rudolf the dating has not been completed, but the age already given to the robust skull KNM–ER–406 antedates the earliest hominid

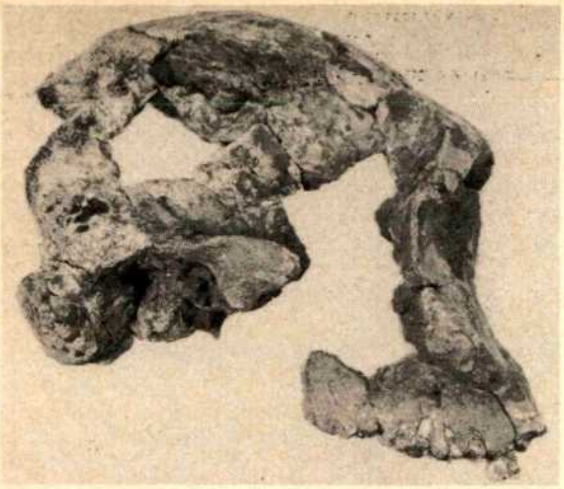


Fig. 2 An almost complete right half of a cranium of an australopithecine collected in 1970 from the Ileret area, East Rudolf.

from Olduvai by about 0.8 million years and similar robust types from Swartkrans and Kromdraai *A. robustus* (≡*Paranthropus*), by about 1.5 million years.

Whether australopithecines older than 5.5 million years will be found is another matter, though Bishop *et al.*[16] claim to have recovered the upper molar tooth of a hominid from the 9 to 12 million-year old Ngorora Formation in the Baringo District in Kenya. Louis Leakey has described this specimen as having some affinities to *Kenyapitheus* (equivalent to *Ramapithecus* of Simons and Pilbeam) and some to both *Homo* and *Australopithecus*. Judgment on this find will no doubt be reserved until further discoveries are made from this locality. It is nonetheless clear that the fossiliferous seciments at Baringo are going to be particularly useful in unravelling the evolution of vertebrates during the Pliocene.

It was during the Pliocene that many African mammalian groups had their origin and diversification; other groups, by contrast, became extinct. Comparisons of faunas from different localities can thus provide useful information about the evolution of mammals in Africa and the dating of one faunal assemblage can form a standard of comparison for other faunas. This obviously has a bearing on the correlation of important hominid-bearing sediments. Maglio has already produced a tentative correlation[17] for some Plio-Pleistocene deposits in East Africa based on the remains of fossil elephants found in them. Isolated teeth of Proboscidea are common in these sediments and are often well preserved because of their large size and thick enamel covering and are therefore useful stratigraphical indicators. Maglio has also compard the rich collection of vertebrates from Lake Rudolf[4] with the assemblages known so far from the Omo Shungura Formation and from the lowermost beds at Olduvai. Two of the species of elephant recovered at East Rudolf include the earliest known occurrences of the *Elephas* and *Loxodont* lineages and indicate that the Koobi Fora I beds at this locality are slightly older than the Kanapoi and Lothagam Hill deposits. There is also an indication[18] at East Rudolf of an older Mio-Pliocene sedimentary and volcanic complex in the Suregei Plateau, and this may provide crucial faunal remains for comparisons with those from Baringo. There is a chance too that some very early hominids will turn up here.

Robust and Gracile

As has been stated, the oldest unequivocal hominid is that dated at 5.5 million years from Lothagam Hill. This is a gracile form; the first sign of the coexistence of this type with the robust form comes later in time at Omo where Howell and his colleagues[5] have found fragments of two australopithecine variants which resemble *A. africanus* and *A. boisei* and which are between 3 and 4 million years old. The contemporaneity of robust and gracile forms has subsequently been confirmed by the results of Leakey's three seasons in the Lake Rudolf area. (The question of *Homo* in these deposits was discussed earlier; whether it is *Homo* or a variant of the gracile form does not alter the concept of two coexisting populations of early hominids.)

There has, of course, been much discussion about the relationships of the types of robust australopithecine—the northern *A. boisei* and the southern *A. robustus*. It is held by some workers, for example Tobias[19], that the East African robust form was ancestral to *A. robustus* and that the two were members of an over specialized line of early hominids which became extinct in the Middle Pliestocene. The genus *Homo*, according to this theory, evolved from the less specialized gracile *Australopithecus*. It has also been suggested that the size and morphological differences between the gracile and robust types relate to sexual dimorphism, but this view has not been widely accepted because it does not explain why at some sites there is a predominance of one type only and it is considered unlikely that just one sex, if the australopithecines were indeed widely sexually dimorphic, would be preserved at these sites. The recovery by Richard Leakey and his team of both types from the same stratigraphic levels should reopen this thorny problem. As already mentioned, Leakey believes that the two types represent the two sexes of the same austalopithecine species and that this population need not necessarily have been in competition for resources with his supposed *Homo* population; *Homo* and not *Australopithecus* would have been responsible for the Oldowan culture (see Fig. 2) recovered in 1969 from the Koobi Fora area (with a date of 2.6 ± 0.26

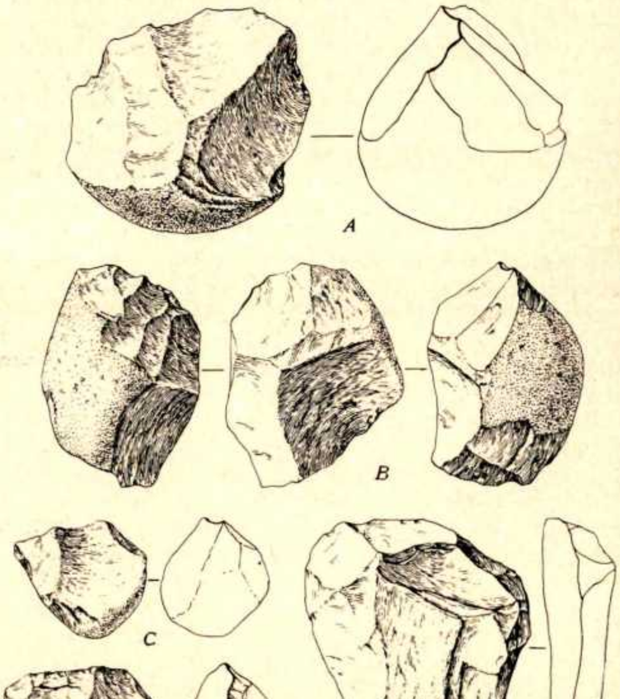


Fig. 3 Oldowan culture collected in 1969 from the Koobi Fora area, East Rudolf.

million years, it is the oldest collection of unequivocal artefacts known).

If *Homo* and *Australopithecus* did share a habitat at Rudolf in the early Pleistocene and the gracile form was indeed the female of the robust form, long established ideas in the palaeoanthropological world really would be upset. It would certainly require the re-examination of the theory that the gracile australopithecine was ancestral to *Homo*, and raises the interesting possibility that all the early Pleistocene australopithecines and not just the robust forms were members of a side branch of human evolution and doomed to extinction in the Middle Pleistocene.

The suggestion that *Homo* and *Australopithecus* were evolving in parallel in East Africa between three and one million years ago is not likely to be generally accepted at present, but it is one that deserves serious discussion. In this connexion, the recently described robust australopithecine cranium dated at between 1.1 and 1.2 million years from Chesowanja in the Baringo District is interesting. This specimen is described by Carney *et al.*[20] as less specialized than either the *A. robustus* or *A. boisei* types, and they claim that it possesses certain features characteristic of *Homo*. The cranium, unfortunately, is badly preserved and its significance in the evolution of man is questionable.

It is to be hoped that more important finds will turn up in the next few years. Who knows what might be recovered in the Lake Rudolf and Baringo area. Has the early hominid ancestor of *Homo* yet to be found?

[1] Leakey, R. E. F., *Nat. Geog. Mag.*, **137**, 712 (1970).
[2] Leakey, R. E. F., *Nature*, **226**, 223 (1970).
[3] Leakey, R. E. F., *Nature*, **231**, 241 (1971).
[4] Maglio, V. J., *Nature*, **231**, 248 (1971).
[5] Howell, F. C., *Nature*, **223**, 1234 (1969).
[6] Brown, F. H., and Lajoie, K. R., *Nature*, **229**, 483 (1971).
[7] *Nature*, **219**, 1820 (1968).
[8] Tattersall, I., *Man's Ancestors : An Introduction to Primate and Human Evolution* (Murray, London, 1970).
[9] Pilbeam, D., *The Evolution of Man* (Thames and Hudson, London, 1970).
[10] Leakey, M. C., Clarke, R. J. and Leakey, L. S. B., *Nature*, **232**, 308 (1971).
[11] Brian, C. K., *et al.*, *Nature*, **225**, 1112 and 117 (1970).
[12] Wolpoff, M. H., *Nature*, **230**, 398 (1971).
[13] Day, M. H., *Nature*, **221**, 230 (1969).
[14] Day, M. H., *Nature*, **232**, 383 (1971).
[15] Leakey, M. C., *Nature*, **232**, 380 (1971).
[16] Bishop, W. W. and Chapman, G. R., *Nature*, **226**, 914 (1970).
[17] Maglio, V. J., *Nature*, **225**, 328 (1970).
[18] Vondra, C. F., *et al.*, *Nature*, **231**, 245 (1971).
[19] Tobias, P. V., *Olduvai Gorge*, **2**, (Cambridge University Press, (1967).
[20] Carney, J., *et al.*, *Nature*, **230**, 509 (1971).

Pure Che stry Library
SCIENCE COLLEGE
Upper Circular Road
CALCUTTA

Problems of Artificial Fertilization

R. G. EDWARDS

UNTIL recently, opportunities for scientific and clinical studies on human conception have been extremely limited, largely because of the inaccessibility of the ovary and the difficulty in obtaining preovulatory oocytes. One egg per month was a discouraging target and experience had shown that even this single egg was difficult to recover because ovulation could not be predicted with any degree of accuracy. In the past two or three years, considerable progress has been made with the clinical and scientific problems associated with the study of human fertilization and cleavage. The opening came with the discovery that human oocytes would undergo their final stages of maturation—those occurring just before ovulation—when removed from their follicles and placed in a suitable culture medium. These stages could be timed with considerable accuracy, and the availability of this human material provided opportunities for studies on meiosis, ovulation and fertilization[1]. Oocytes matured *in vitro* thus provided the material for studies on human fertilization *in vitro*, which was achieved two years ago.

Patients Involved

When fertilization had been accomplished using these oocytes, the emphasis of the work changed to include studies on patients in order to realize the potential of these studies for human infertility. There were various reasons for this extension into clinical studies. While oocytes matured *in vitro* provide good material for fertilization, they are unsuitable for studies on cleavage because observations in animals has shown that the resulting embryos fail to develop normally[2]. For normal development to full term, oocytes must be collected after completing, or almost completing, their maturation in the ovary (see Fig. 1). Endocrine methods were thus needed to induce follicular growth and oocyte maturation in the patients, the preovulatory growth of oocytes had to be timed exactly from the estimates made from cultures and surgical methods had to be developed for the collection of the oocytes just before ovulation. The achievement of these objectives has opened the possibility of growing and analysing cleaving embryos in culture, replacing them in the mother, and studying the processes of implantation. Each of these projects opens fundamental opportunities for the study of human conception. What progress has been made in these various areas of investigation?

The surgical methods for collecting human oocytes are perhaps almost as refined by now as they ever will be. Laparoscopy has proved to be a simple and very safe procedure permitting the necessary manipulations to be carried out on the ovary[3]. Oocytes are collected from more than one-half of the available Graafian follicles, and although the proportion could be improved the loss of one-half of the oocytes in this way is not holding up further developments in any serious manner. The timing of oocyte maturation and ovulation are also under fine control. None of the many patients so far examined has ovulated before the expected time, and many of the oocytes were fertilized soon after

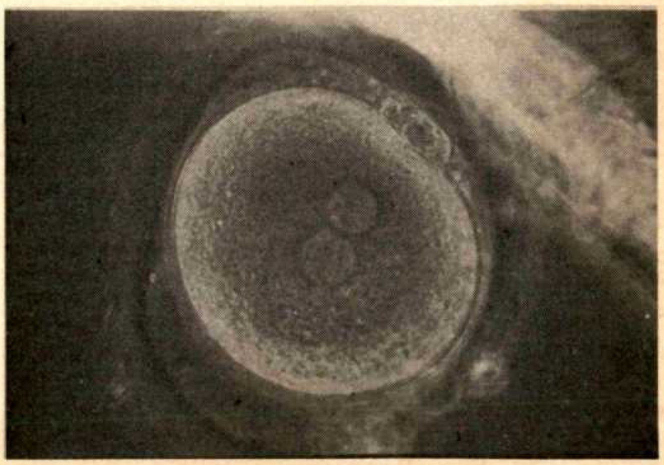


Fig. 1 Human pronucleate stage.

collection, showing that they were certainly preovulatory. The major problems in the initiation of follicular growth and oocyte maturation concern the response of the patients to the endocrine treatments, which are based on the use of human menopausal gonadotrophins on amenorrhoeic women[4]. Some of the patients have few or no preovulatory follicles after the treatment.

There are well known difficulties with the method—for example, the great variation between patients or even between successive treatments of the same patient[5]. Three or four preovulatory follicles are the minimum required to provide sufficient oocytes to work with. Measurements of the excretion of oestrogen in the urine of the patients during treatment provides some indication of their response to the treatment, particularly as a safeguard against overstimulation, but its usefulness as a measure of the growth of preovulatory follicles remains to be decided. There is some scope for improvements in the hormone treatment. The amount of gonadotrophin being administered to the patients is modest in comparison with that use for the treatment of amenorrhoea, partly because the patients have a normal menstrual cycle which might heighten their response. Simply raising the dosage of gonadotrophins should induce a greater response in most patients. Alternatively, the use of compounds such as clomiphene, to induce the release of endogenous hormones, might prove superior, cheaper, and more acceptable than the injection of gonadotrophins. Clomiphene is widely used in the treatment of female hypopituitarism, and should work well with cyclic patients. The recent purification of the gonadotrophin releasing hormones of the hypothalamus could well offer another alternative, provided that the dosage and response can be controlled.

Human Ova

Studies on the fertilization of mammalian ova *in vitro* have moved quickly in the past two or three years after a decade of uncertainty. Belief in the necessity of uterine spermatozoa had become almost dogmatic as a result of experiments involving mostly rabbit ova. Even the demonstration that hamster ova could be fertilized *in vitro* by epididymal spermatozoa[6] had scarcely shaken this fixed belief. When the conditions needed in the culture medium for hamster fertilization were defined, the work was extended to human material, using oocytes matured *in vitro* and washed, ejaculated spermatozoa. Fertilization was established using the same criteria defined for studies with animal ova[7]. Moreover, similar studies are now being extended to other species such as the mouse and cow, and even in rabbits spermatozoa have now been found to penetrate through the membranes of the ova. The culture media are simple: a basic Tyrode's solution is supplemented with bovine serum albumin, pyruvate, and bicarbonate to a *p*H of 7.6. Pyruvate is obviously an energy source, and the albumin presumably serves as a source of nitrogen or to stabilize cell membranes. The significance of the high *p*H, or of the contributions of the cumulus cells surrounding the ova, remain unexplained.

Media suitable for fertilization and for cleavage are rarely identical. The development of media to support the cleavage of human ova has not relied on experiments with mammalian ova to nearly the same degree. Simple defined media had been developed for the cleavage of mouse ova, following the recognition of the importance of pyruvate as an energy source during these early embryonic stages[8]. The initial attempts to culture human embryos utilized these media, but cleavage progressed only to the 8-celled stage[9] (Fig. 2). More complex media developed for routine tissue culture have proved much more successful. A medium composed of Ham's F 10 supplemented with calf or human serum, at a *p*H of 7.3 under a gas phase of 5% O_2, 5% CO_2 and 90% N_2, has supported development to the blastocyst stage[10]. Several blastocysts, each having 100 or more nuclei and several mitoses, have been grown under these conditions (Fig. 3). The inner cell mass, trophoblast and blastocoelic cavity were well defined, and some blastocysts expanded in culture. The blastocoel appeared to form from the secretions of large cells that accumulated at one pole of the morula. Modified body fluids are also reported to support the cleavage of human embryos[11]. According to one report, oocytes matured *in vitro* had to be fertilized by human spermatozoa previously incubated in the uterus of a monkey[12]; the abortive cleavage of the embryos might have been a consequence of maturation *in vitro* or some other cause.

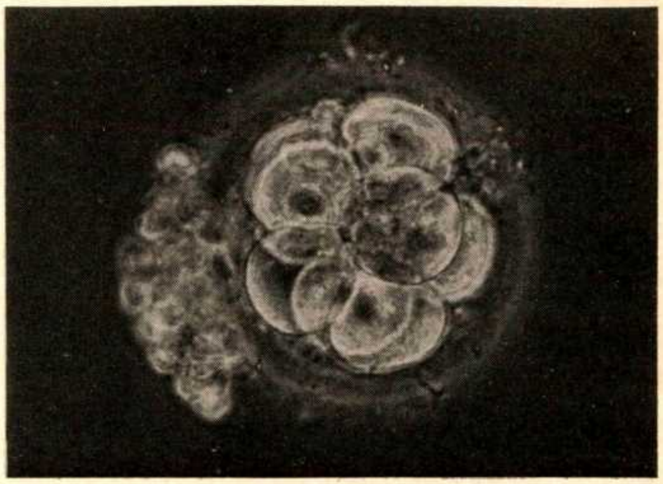


Fig. 2 Human eight-celled embryo.

Where Next?

Further development beyond the blastocyst stage must surely be the next study. This raises the question of whether the embryos will be able to implant in the mother, that is, if the uterus has been sufficiently stimulated by the endocrine treatments used to induce follicular maturation. Another question concerns the capacity of the embryos to implant. The indications are good: the pronounced differentiation of many embryos in culture augurs well for their continued development. Conditions in the mother also seem to be satisfactory: most patients have had a secretory endometrium, excreted suitable amounts of pregnanediol, and their induced cycle has resembled a natural cycle[3].

What is known of the capacity of the embryos for normal development? The criteria for cleavage are now well recognized, and provide the first safeguard. Several of the embryos have possessed a chromosome complement that appeared to be diploid, thus excluding the possibility of triploidy. Trisomy has not been excluded. Yet the more that

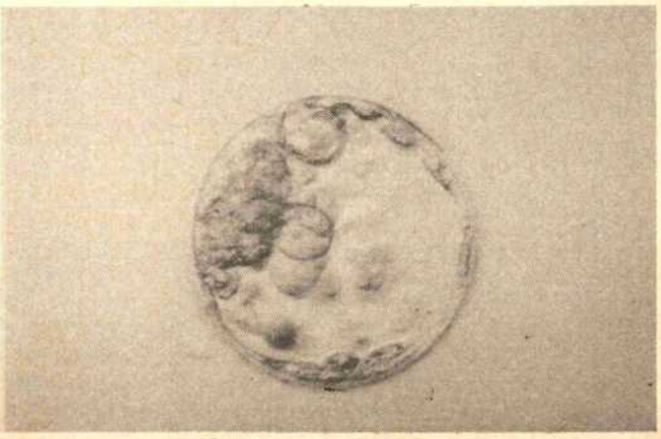


Fig. 3 Human blastocyst.

is learnt about natural human pregnancy, the more obvious becomes the conclusion that many embryos conceived naturally are trisomics, triploids or mosaics destined to perish in the first trimester. The possibility of trisomy in cultured embryos thus becomes less important, and especially since Down's syndrome can be identified in foetuses by amniocentesis. Nor should the problem of teratogenic development present undue difficulties, because widespread experience in animals and man has shown that agents known to be teratogenic in later stages of pregnancy are lethal to preimplantation embryos.

The immediate social consequences of the successful reimplantation of human embryos would be the cure of some forms of infertility, notably where the wife has occluded oviducts or the husband is oligospermic. There should be no criticism in giving these couples their own children: comments about overpopulation seem to be highly unjust to such an underprivileged minority.

The next development could well be the sexing of embryos before transfer. Rabbit blastocysts were sexed from the presence or absence of sex chromatin in small pieces of trophoblast excised by microsurgery[13]; the embryos recovered after a few hours in culture and some of them developed to full term in recipient females. The sex of all foetuses had been predicted correctly; this was the first occasion that the secondary sex ratio had been controlled in any mammal. Most efforts in this direction had been devoted to the attempted separation of X and Y spermatozoa, so far without any convincing success.

Improvements are now needed in the methods for sexing blastocysts. Two approaches are currently feasible: the identification of enzyme activity or of antigens determined by sex-linked genes. The activity of glucose-6-phosphate dehydrogenase (G6PD) and hypoxanthine–guanine phosphoribosyl transferase (HGPRT) have been studied in mouse embryos; it is not yet certain that these genes are sex-linked in mice as they are in man. Assuming that they are sex-linked, female embryos might be expected to possess twice the activity of male embryos. Unfortunately, levels of G6PD were dominated by the massive amounts of maternal enzyme "inherited" from the oocyte, and which declined steadily during cleavage[8,14]. The activity of HGPRT in embryos increased after fertilization, indicating that this enzyme was being synthesized during these early stages and, perhaps more important, that the sex chromosomes are active at this time[15]. This enzyme might thus be present in different amounts in male and female embryos. Evidence for sex-linked antigens is restricted to one report showing that the sex ratio of mouse offspring can be altered by interfering with the normal immunological processes of pregnancy[16]. Sexing blastocysts would be an excellent approach to the control of sex-linked mutant genes in man by avoiding the birth of affected boys. Strict control would have to be exerted over sexing if it could be demonstrated that widespread parental choice would seriously disturb the sex ratio in human populations.

Studies on human conception open new approaches to human problems including preventive medicine and family planning. More knowledge about the growth of ovulatory follicles, the secretions of the blastocyst or the components of capacitation could lead to the development of novel methods of contraception. Rational discussion is needed about the emerging social issues involved in these studies. Much of the debate so far has been unreal in the sense of being far removed from the actual clinical problems involved: constant reference to nuclear cloning is an example. Earlier remonstrations about the disposal of tiny blastocysts appear almost irrelevant when foetuses of four months' gestation or older are being aborted for social reasons. Alleviating infertility has even been criticized because more consideration is needed about the survival of the family unit in our society! Realization of the benefits that could accrue to many people should lead to more rational discussion and conclusions.

[1] Edwards, R. G., *Lancet*, ii, 926 (1965).
[2] Chang, M. C., *J. Exp. Zool.*, **128**, 379 (1955).
[3] Steptoe, P. C., and Edwards, R. G., *Lancet*, i, 683 (1970).
[4] Hack, M., Brish, M., Serr, D. M., Insler, V., and Lunenfeld, B., *J. Amer. Med. Assoc.*, **211**, 791 (1970).
[5] Crooke, A. C., *Brit. Med. Bull.*, **26**, 17 (1970).
[6] Yanagimachi, R., and Chang, M. C., *J. Exp. Zool.*, **156**, 361 (1970).
[7] Edwards, R. G., Bavister, B. D., and Steptoe, P. C., *Nature*, **221**, 632 (1969).
[8] Brinster, R. L., Chap 17 in *Biology of the Blastocyst* (edit. by Blandau R. J.) (University of Chicago Press, 1971).
[9] Edwards, R. G., Steptoe, P. C., and Purdy, J. M., *Nature*, **227**, 1307 (1970).
[10] Steptoe, P. C., Edwards, R. G., and Purdy, J. M., *Nature*, **229**, 132 (1971).
[11] Shettles, L. B., *Nature*, **229**, 343 (1971).
[13] Gardner, R. L., and Edwards, R. G., *Nature*, **218**, 346 (1968).
[14] Brinster, R. L., *Biochem. J.*, **101**, 161 (1966).
[15] Epstein, C. J., *J. Biol. Chem.*, **245**, 3289 (1970).
[16] Lappe, O. O., and Schalk, M. J., *Transplantation*, **11**, 491 (1971).

Assembly of Tobacco Mosaic Virus Particle

SCIENCE COLLE
Upper Circular
CALCUTTA

P. J. G. BUTLER
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

TOBACCO mosaic virus (TMV) is one of the best characterized viruses and consists of a long rod, which is composed of a helical array of identical protein subunits with the single stranded RNA lying in the helical groove between adjacent turns of the protein helix (for review see ref. 1). TMV was the first virus to be reconstituted from its RNA and protein[1] and has frequently been used as an example of the self-assembly process, and the protein alone is capable of forming rods which are structurally indistinguishable from the virus helix (see ref. 2). However, the protein also has many other polymorphic forms and this property complicates the understanding of the protein aggregation and has led to different theories being suggested to account for this process[2,3].

Recently, in a concerted attack on this problem in the MRC Laboratory in Cambridge, the structures of these different aggregates have been determined, using the electron microscope and X-ray diffraction techniques, and the conditions in which they occur defined[4]. The results support the ideas put forward some years ago by Caspar[2] that all the aggregates are the result of the same specific interactions as are found in the virus but slightly modified in the different polymorphic forms. The results also allow the mechanism controlling which protein aggregate will occur to be determined[5]. One of these aggregates, the disk, is found only in the conditions in which virus assembly occurs and it has been shown that growth of the virus does not occur by the addition of single protein molecules but rather by the incorporation of a whole disk at once[6]. The polymorphism of TMV protein is thus the basis for the rapid and specific formation of the virus from its protein and RNA.

Protein Aggregates

The aggregation of TMV protein can occur in one of two modes. The first to be structurally understood was the helix, in which the arrangement and conformation of the protein subunits is identical to that in the virus. The aggregation of the protein in this mode can be described as "quasi-crystallization" and the resulting rods are of indeterminate length, usually very large. The second mode can be described as "two-layer" aggregation and aggregates of this type are stabilized by an additional "pairing" interaction, which is not present in the helix, between the subunits vertically above each other. The movements of about 5 Å of the outer ends of the subunits, involved in this pairing, have been seen by 3-D image reconstruction from electron micrographs[7]. Because of the detailed geometry of the TMV protein subunit and of the intersubunit interactions, the layers curve round and the ends meet to form a closed torus consisting of two rings, each of 17 subunits (Fig. 1). It is this structure which is called the "disk".

The growth of the two-layer aggregates, from the trimer to the 32-mer, can be described as linear condensation polymerization, with each subunit adding with the same binding energy. The formation of the trimer, which begins the association process, and the addition of the final two subunits to close the disk cannot be described so simply. However, the energies of these various interactions have been calculated[5] and the resulting quantitative model does predict the observed sedimentation patterns.

A further complication with the two-layer aggregates is that under favourable conditions for the bonding interactions (at high ionic strength) the disks can stack on each other, to give even larger aggregates. This can result in a series of discrete, large aggregates in equilibrium with a range of small aggregates and gives rise to the sedimentation patterns which have caused much argument in the past. Still further complication is caused by the readiness with which the aggregation of TMV protein can become irreversible, presumably by some slight damage to the protein, and hence give aggregates which are no longer in equilibrium with the rest of the mixture. One such structure, the stacked disk rod, consisting of long rods of disks stacked on each other in a polar fashion, has recently proved important in the elucidation of both the structure[4,7] and control of formation[5] of the two-layer aggregates, just because of its size and the irreversibility of the disk aggregation within it, but even this had earlier caused considerable difficulties in the interpretation of experiments in which it had occurred.

The bonding in these two modes is very similar, with the pairing perturbation in the two-layer aggregates as the only major difference[4]. The importance of this pairing in limiting the growth of the aggregates to a single direction and thus causing the formation of a discrete aggregate, the disk, rather than the indeterminate sized helix, lies in the assembly process for the viral rods, which I shall discuss later.

When the conditions in which any particular aggregate contributes significantly to the equilibrium are determined (Fig. 1 of ref. 4), the *p*H is found to be the controlling variable for the mode of aggregation. As hydrophobic bonding is the driving force for aggregation[3], increasing both the temperature and the ionic strength will favour aggregation and cause large aggregates to form, but neither has a major effect on the mode of aggregation. The exact *p*H at which the transition between the modes occurs depends slightly on the temperature and ionic strength, but it is always about *p*H 6 to 7, with the helical aggregation predominating below the transition and the two-layer aggregates above. This transition *p*H is found to correspond to the *p*Ks of two carboxyl groups, which titrate abnormally in the virus[2] but not in the stacked disk rods[5], and in the titration of the protein helix an abnormally sharp release or uptake of protons correlates with the disaggregation or formation of the helix[8,9].

Control of Aggregation

The control effect can be seen qualitatively when the *p*H of a solution of the protein helix is raised and the turbidity, due to the long rods, disappears immediately as the rods dissociate to give the small protein aggregates (called the "A-protein"). If the *p*H is lowered again soon afterwards, the turbidity returns as the protein subunits reform helix. An important variation occurs if the protein is kept above the transition *p*H, in conditions where it forms disks, for long enough for the disks to be formed before the *p*H is lowered. In this case short, imperfect, helical rods are formed within 5 seconds, and these then anneal over a period of about a day to give long, perfect helices again. Close examination of the imperfect helices shows that they are composed of two turn segments of helix stacked on top of each other, with their ends not in register[4], each segment appearing to be formed from a single disk by it having dislocated under the influence of the high proton concentration.

Since the subunits in any single disk must change their mode of aggregation together, any such transition is inherently cooperative and can be considered to be allosteric, with the protons as the allosteric effectors. In this case the effectors act by protonating two carboxyl groups, to give two carboxyl–carboxylate pairs. This abolishes the repulsion between the charged carboxyl groups and concomitantly the pairing distortion of the two-layer aggregate, enabling the subunits to take up the strictly equivalent positions of the helix. Were it not for the carboxyl–carboxylate pairs the bonding in the helix would presumably be more favourable than that in the disk, but the unfavourable interaction of these groups acts as a negative switch to prevent helix being the most favoured aggregate under all conditions.

This negative, and energetically unfavourable, switching is in contrast to the position that is found in the cases where ionic interactions have been investigated in detail in other proteins. In haemoglobin, which in many ways forms a close parallel with the situation in TMV protein, salt bridges are formed between the chains in the deoxy form but not

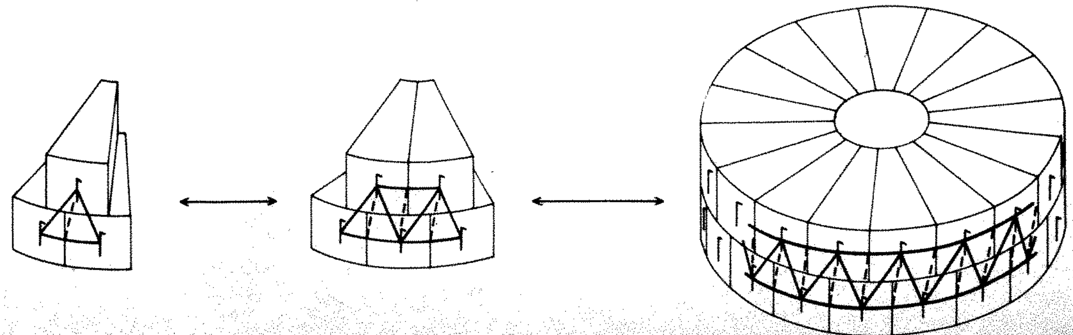


Fig. 1 Model for two-layer aggregation to form disks[5]. Subunits associate laterally in a linear fashion with polymerization in the axial direction limited by the pairing deformation[4] (shown by the dotted line). Other intersubunit contacts are shown by solid lines.

in the oxy form[10]. These bridges have to be broken to allow the quaternary structure change, which also involves a movement between the chains of about 5 Å, brought about by the ligand, to go from the deoxy to the oxy structure[10,11]. In both haemoglobin and TMV protein the allosteric transition is also influenced by other ligands, diphosphoglycerate in the case of haemoglobin and with TMV protein the viral RNA.

Protein and RNA

TMV has 16⅓ protein subunits per turn of the helix and one problem in its assembly is the nucleation of the helix, as a single ring of subunits joined side to side would be readily broken before it had grown to form a small helix. With the growth of a two-layer aggregate (Fig. 1) this problem would not occur as a subunit in either layer could bond to two subunits in the other layer as well as to its neighbours in the same layer, to give a relatively strong, cross-strutted structure. It has also been seen, from the effect of the *p*H drop, that a disk is capable of being transformed into a small helix. In the relevant biological process this is brought about by interaction with the viral RNA rather than by protonation[6]. This interaction occurs between a special sequence of about 50 nucleotides at the 5′-hydroxyl end of the TMV RNA (ref. 6) and two protein disks[9], possibly as shown in Fig. 2a. After the disks have formed short helices, entrapping the first turn of the RNA (Fig. 2b), the initiation is complete and growth can ensue. In general, growth will occur with the RNA protruding from one end of the growing nucleoprotein helix, and the first step will be the interaction of this with a further protein disk which dislocates to become helical and "unrolls" onto the top of the nucleoprotein helix to enclose one turn of the RNA (Fig. 2c). A further turn of the RNA can be enclosed by the second turn of the protein helix by intercalation through the "ruck" which is formed at the end of the first turn, where the RNA protrudes sideways (Fig. 2d).

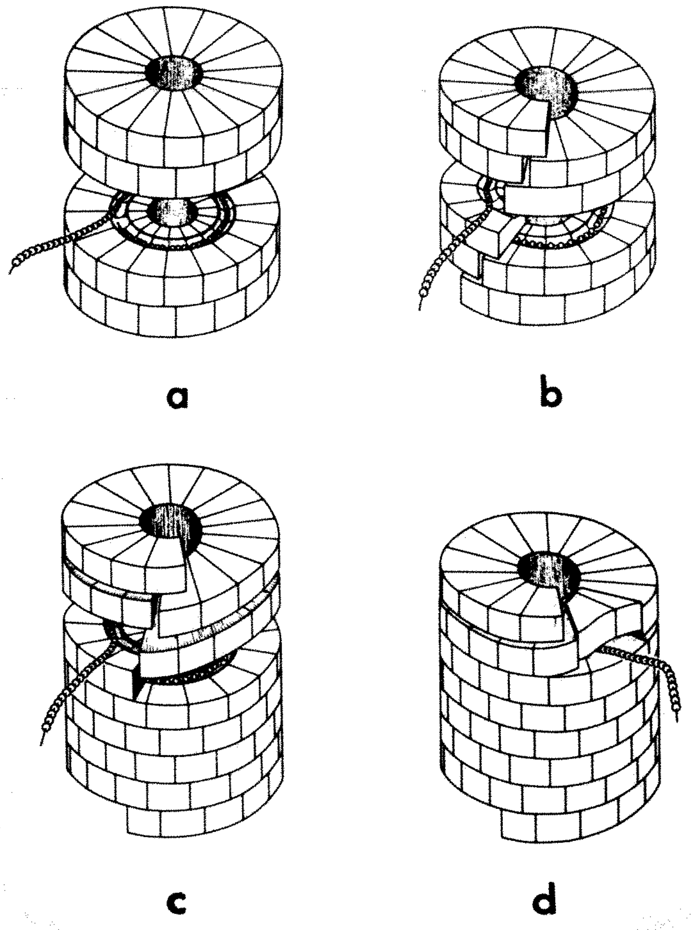


Fig. 2 Model for TMV assembly from disks and RNA[9]. Initiation—a: 5′-hydroxyl end of TMV RNA interacts with two disks; b: disks dislocate to form short helices enclosing end of RNA. Growth—c: dislocated disk "unrolls" to entrap turn of RNA; d: second turn of RNA intercalates through "ruck" formed at end of first turn of helix.

Because of the special nature of the initiation reaction it is very fast and the subsequent growth is relatively slow, unlike most polymerization reactions where the initiation is the slow step. The rate limiting step of the TMV assembly is the actual rearrangement of the protein subunits from the disk into the nucleoprotein helix and is fast enough for the whole assembly to be complete within a few minutes.

General Remarks

The possible role of the disks in nucleation of TMV assembly was foreseen[6] and, indeed, provided the motive for our early experiments with disks and the RNA, but although in hindsight the continued growth with disks as the protein source has several advantages, it was completely unexpected since everyone expected that the virus would grow by adding single subunits at the end of the growing helix, rather like crystal growth along a screw dislocation. The problem of helix initiation has already been discussed and the biological solution for this, using two disks, confers a high degree of selectivity for its homologous RNA onto the protein. This comes about because each protein subunit interacts with three nucleotides in the RNA, and hence 51 nucelotides in all are involved in a single turn of the nucleoprotein helix. In the initiation step these nucleotides bring about the transition of two disks into helix, involving 68 protein subunits, and this apparently requires an especially favourable interaction. By contrast, the use of disks for the further growth will allow even unfavourable nucleotide sequences to interact and to be coated, as the cooperative transition of the protein subunits will result in the favourable interaction nearby overcoming the local unfavourable effect.

Although the formation of disks is slow, taking several hours at normal protein concentrations, these could be formed before the RNA is synthesized and then be available to coat and protect it very rapidly in the plant cell. The optimum conditions for the formation of disks (*p*H 7, ionic strength about 0.1) are about those which might be expected in the cytoplasm of a tobacco plant cell, as must be the case if this mechanism is the true biological one.

Clearly it would be a disaster for the virus if its protein alone could form a stable structure over a wide range of conditions, and so not be available to interact with the viral RNA. The role of the polymorphism of the protein is to prevent the occurrence of this disaster, so that instead of being an adventitious and bothersome feature it is now seen to be the controlling factor for the rapid and successful growth of the virus. This polymorphism is itself controlled by a negative switch which prevents the very stable helix being the dominant form of the protein alone over all moderate conditions, but which can be switched on by the viral RNA to give just this stable product as the virus.

[1] Caspar, D. L. D., *Adv. Prot. Chem.*, **18**, 37 (1963).
[2] Frankel-Conrat, H., and Williams, R. C., *Proc. US Nat. Acad. Sci.*, **41**, 690 (1955).
[3] Lauffer, M. A., and Stevens, C. L., *Adv. Virus Res.*, **13**, 1 (1968).
[4] Durham, A. C. H., Finch, J. T., and Klug, A., *Nature New Biology*, **229**, 37 (1971).
[5] Durham, A. C. H., and Klug, A., *Nature New Biology*, **229**, 42 (1971)
[6] Butler, P. J. G., and Klug, A., *Nature New Biology*, **229**, 47 (1971).
[7] Finch, J. T., and Klug, A., *Phil. Trans. Roy. Soc.*, *B*, **261**, 211 (1971).
[8] Klug, A., and Durham, A. C. H., *Cold Spring Harbor Symp.*, **36** (1971, in the press).
[9] Butler, P. J. G., *Cold Spring Harbor Symp.*, **36** (1971, in the press).
[10] Perutz, M. F., *Nature*, **228**, 726 (1970).
[11] Perutz, M. F., *Cold Spring Harbor Symp.*, **36** (1971, in the press).

Vintage Year for Tumour Virology

JOHN TOOZE

REVIEWING the results of a year's work in an area of research as active as tumour virology is an invidious as well as a difficult task, for with such a wealth of new information choice is nothing short of embarrassing and sins of omission are all too easy to commit. But no one would, I believe, argue against giving pride of place to reverse transcriptase, the enzyme which Temin and Mizutani and Baltimore (*Nature*, **226**, 1209, 1211; 1970) simultaneously discovered in the particles of four animal and avian tumour viruses, for the significance of the discovery of this enzyme extends far beyond tumour virology. Reverse transcriptase is, of course, the enzyme which can use the single stranded RNA genomes of RNA tumour viruses as templates for the synthesis of first an RNA.DNA hybrid and then a double stranded DNA.

With so many research groups now aboard the bandwagon, it is hard to believe that the enzyme was discovered less than eighteen months ago. It is perhaps even harder to believe that its existence was predicted by Temin as long ago as 1964, when he speculated about the way in which RNA tumour viruses transform cells which have DNA genomes. Temin argued that to become stably associated with the genome of a cell, and to maintain the cell in a transformed or malignant state, the RNA genome of a tumour virus must be transcribed into a double stranded DNA molecule, which he called the provirus. Once the DNA provirus carrying the genes which cause transformation had been made, it could integrate into the cellular genome and be inherited along with the cellular chromosomes. Indirect evidence suggested that transformation by RNA tumour viruses depends on DNA metabolism rather than RNA metabolism and the discovery of reverse transcriptase, an enzyme with the necessary capability, conclusively vindicated Temin's heterodox ideas; it also, of course, provided countless of his colleagues with new research programmes.

Within weeks of the announcement of the discovery of reverse transcriptase, Spiegelman and his colleagues had screened as many different sorts of tumour viruses as they could lay their hands on and had found the activity in all of them (*Nature*, **227**, 563; 1970); today the tally exceeds thirty. Moreover, two viruses—visna virus, which causes a slowly progressing neurological disorder in sheep, and progressive pneumonia virus, which causes a slowly evolving disease of sheep lung have been shown not only to contain reverse transcriptase but also to possess previously unsuspected oncogenic potential; they transform cells *in vitro* (Takemoto and Stone, *J. Virol.*, **7**, 770; 1971). In short, reverse transcriptase as Temin and Baltimore defined the enzyme, that is an enzyme which can use natural single stranded RNA as a template for the synthesis of double stranded DNA, has been found in every RNA tumour virus so far examined.

Tumour virus genomes are not the simplest of RNAs to work with and not surprisingly, Temin (*Nature*, **228**, 424; 1970) and Spiegelman (*Nature*, **228**, 430; 1970) and their colleagues, and other groups, soon began using as templates for reverse transcriptase synthetic RNAs, DNAs and hybrids of RNA and DNA which have a simple and known base composition. Some of these synthetic templates were discovered to have another very great advantage; they were used by the enzyme more efficiently, by almost two orders of magnitude, than the viral genome. For a few weeks if not months this finding caused considerable excitement, for with such very efficient templates for reverse transcriptase at hand it seemed reasonable to hope to detect the enzyme in human cancer cells, supposing they had been transformed by putative human cancer viruses. And sure enough, several groups, for example Gallo and his colleagues (*Nature*, **228**, 927; 1970), found enzyme activities in human leukaemic cells which could use synthetic nucleic acids as templates for the synthesis of DNA; transiently the way seemed open to tracking down human cancer viruses by following the reverse transcriptase footprints they had so obligingly left behind them.

But the bubble soon burst. For although human cancer cells undoubtedly contain a DNA polymerase activity which can be programmed by synthetic double stranded RNAs and hybrids of RNA and DNA, it soon became apparent that such activities are by no means restricted to cancer cells; they are present in normal cells. Todaro and his colleagues, for example, have not only shown that (*Nature*, **229**, 318; 1971), but, using mouse 3T3 cells infected with mouse leukaemia virus, they have separated the reverse transcriptase specified by the virus from the cellular enzyme which can be programmed by synthetic hybrids to make DNA of RNA and DNA (*Nature New Biology*, **231**, 163; 1971). The salutary lesson of this episode was simply that synthetic nucleic acids are not specific templates for reverse transcriptase; they are most useful for characterizing the enzyme once its existence in a cell or virus has been proven in experiments with its natural RNA template, but synthetic nucleic acids cannot be used to establish the enzyme's existence.

Human Cancer Virus

This particular exploitation of reverse transcriptase in the search for human cancer viruses thus ended abruptly in disillusionment. More recently, however, Moore and his colleagues (*Nature*, **229**, 611; 1971) have detected a virus in samples of human milk which in structure closely resembles mouse mammary tumour virus. This murine virus causes mammary cancer in mice and its existence inspired Moore *et al.* to search for a human counterpart. The human milk virus they found seems to be more prevalent in families or populations with a particularly high incidence of breast cancer; moreover, it contains reverse transcriptase which can use as template the endogenous single stranded RNA genome, as Schlom *et al.* have shown (*Nature*, **231**, 97; 1971). Human milk virus is, therefore, the most likely candidate for the distinction of being the first human cancer virus to be identified.

The central role of a reverse transcriptase activity in Temin's provirus hypothesis has been touched on briefly (see above). But what experimental evidence is there to prove that the reverse transcriptase which everyone is assaying in animal tumour virus particles plays any role in the process of transformation ? As more information about the properties of this enzyme and the drugs which inhibit it is being accumulated a trickle of circumstantial evidence which implicates reverse transcriptase in transformation is appearing. Streptovaricins, for example, which block reverse transcriptase also, according to Brockman and his colleagues, prevent the transformation of mouse fibroblasts by mouse sarcoma virus. Furthermore, streptovaricins delay the induction of leukaemia in mice by murine leukaemia virus. Such indirect evidence, however, can always be interpreted in more than one way and the experiments Hanafusa's group reported (*Virology*, **43**, 313; 1971) provide more convincing evidence of the role of reverse transcriptase in transformation. Before the enzyme had been discovered, his group had isolated a variant of Rous sarcoma virus which appeared to be completely uninfectious and, unlike wild type virus, failed to transform chick fibroblasts. After the discovery of reverse transcriptase Hanafusa *et al.* tested their variant and found it lacked activity. The obvious conclusion is that the variant carries a mutation which inactivates reverse transcriptase and renders the virus uninfectious.

To bolster this interpretation of the properties of their variant, they infected chick fibroblasts with the uninfectious sarcoma virus and an avian leukaemia virus which can replicate in these cells but does not transform them. After such double infections the fibroblasts were transformed and yielded progeny sarcoma virus capable of transforming other fibroblasts. However, the fibroblasts transformed by these progeny sarcoma viruses yielded not infectious progeny virus but uninfectious virus identical to the original variant. The most plausible explanation of these results is as follows: the variant lacks reverse transcriptase and as a result is uninfectious and unable to transform; in the presence of an avian leukaemia virus the sarcoma virus genome can use the reverse transcriptase specified by the leukaemia virus and can as a result transform cells; the progeny sarcoma virus particles produced by cells infected with these two viruses are infectious and transforming because they can pick up reverse transcriptase specified by the leukaemia virus; the cells transformed by the progeny sarcoma viruses carrying the leukaemia virus enzyme cannot yield infectious, transforming progeny, however, because the sarcoma virus genome cannot specify its own reverse transcriptase.

These experiments appear, therefore, to prove that reverse transcriptase is essential for establishing transformation, just as the provirus hypothesis of Temin predicts, and they also indicate that once a cell has been transformed by a provirus reverse transcriptase is not essential for the maintenance of transformation. Of course, if transformation depends on the integration of provirus DNA, made by reverse transcriptase, into the host genome several other enzymes must be involved in the process. The two nucleases and the DNA ligase which Temin's group have detected in tumour viruses, in addition to the transcriptase (*Nature New Biology*, **230**, 232; 1971), may be there to accomplish the recombination of provirus and host DNA.

While Hanafusa's experiments indicate that reverse transcriptase is required only during the first step of transformation, Martin (*Nature*, **227**, 1021; 1970) has reported the isolation of a mutant strain of Rous sarcoma virus which carries a lesion in a viral gene, the product of which is required continuously to keep the cell in a fully transformed state. Martin exposed Rous sarcoma virus to nitrosoguanidine and then screened the mutagenized stocks for viruses which transform chick fibroblasts at 35° C but fail to transform cells at 41° C. One of the mutants he has isolated has these properties: cells transformed by the virus at 35° C have all the properties of typical transformed cells; when, however, the temperature of the cultures is raised to 41° C the cells rapidly assume many of the characteristics of untransformed cells growing in culture. If the temperature is lowered again to 35° C the cells once more appear to be transformed. Clearly this mutant carries a temperature sensitive lesion in a viral gene which must be continuously expressed to maintain transformation *in vitro*.

Whether or not Martin can show that this mutation also affects the growth of sarcomas induced in chicks in the same way in which it affects the transformation of cells in culture remains to be seen. If he can prove that the mutant affects tumours *in vivo* the identification of the product the mutated gene specifies should answer many of the outstanding questions of tumour biology.

Oncogenes

Although RNA tumour viruses and some DNA viruses (see below) are able to infect and transform susceptible cells in culture and induce tumours in susceptible animals, very few natural cancers have the epidemiological characteristics of infectious diseases. For this reason Huebner and Todaro postulated that genes capable of causing malignant transformation, so called oncogenes, are part and parcel of every animal's genetic make-up. Carcinogens, they envisage, act by switching on oncogenes or in the case of tumour viruses by doing this and also introducing more copies of oncogenes into a cell. Such an all embracing hypothesis is hard to test rigorously but Hanafusa *et al.* (*Proc. US Nat. Acad. Sci.*, **67**, 1797; 1970), Vogt and Früs (Second Lepetit Colloquium, 1970) and Weiss during the past year have provided perhaps the most convincing evidence so far to support the oncogene story. In short, they have been able to isolate an avian leukaemia-type virus (it has yet to be proven that this virus can induce leukaemia) from the cells of apparently healthy chick embryos and Weiss has even induced the production of this virus in the cells of wild Malayan jungle fowl. These observations suggest that the cells of all chickens, irrespective of whether or not the birds are destined to develop leukaemia, have the genetic potential to specify an avian leukaemia virus.

Inevitably, RNA tumour virologists in their first flush of enthusiasm after the discovery of reverse transcriptase have stolen most of the limelight from their colleagues working with the DNA tumour viruses, which outside the laboratory are unlikely to be important carcinogens. But it would be mischievous to imply that the DNA tumour virologists have been gathering moss. Both Eckhart and Dulbecco (*Proc. US Nat. Acad. Sci.*, **67**, 1775; 1970) and Benjamin (*Proc. US Nat. Acad. Sci.*, **67**, 394; 1970), for example, have characterized conditional lethal and host range mutants of polyoma virus which carry lesions in a gene or genes, the product(s) of which are required continuously to maintain a cell in the transformed state. These mutants are equivalent to that of Rous sarcoma virus isolated by Martin (see earlier) and it is now clear that the integration of a tumour virus genome into the genome of a host cell is not sufficient although it appears to be necessary to maintain transformation. And the recent experiments of Wall and Darnell (*Nature New Biology*, **232**, 73; 1971) dispel any remaining doubts about the integration of polyoma virus DNA into host cell DNA. For they have shown that in polyoma transformants messenger RNA molecules are made which contain both cellular and viral base sequences in the same molecule.

But perhaps the greatest stir among DNA tumour virologists during the past twelve months has stemmed from experiments with agglutinins or lectins, glycoproteins which are obtained from plant seeds and which cause cells to clump or agglutinate. It now seems clear that although both normal cells and cells transformed by DNA tumour viruses and some chemical carcinogens bind more or less the same amount of any given agglutinin (Ozanne and Sambrook, Livingstone and Cline, *Nature New Biology*, **232**, 155, 156; 1971) the transformed cells are more readily agglutinated than normal cells. Moreover, cells transformed by the temperature sensitive mutant Eckhart has characterized behave like normal cells when grown at the non-permissive temperature. These findings indicate that the surface of a transformed cell differs from that of its untransformed counterpart and that this change is probably controlled by the transforming gene of the virus. Changes in the cell surface on transformation no doubt account for many of the characteristic properties of transformed cells including their failure to respond to contact inhibition of growth when maintained in culture. And *in vivo* the ability of cancer cells to metastasize no doubt stems from some change in the cell surface. Clearly much future research in tumour virology will be concentrated on the chemistry and structure of the surface of untransformed and transformed cells as well as on reverse transcriptase, the genetics of tumour viruses and the search for human tumour viruses.

Glaciation and the Stones of Stonehenge

G. A. KELLAWAY
Institute of Geological Sciences, 5 Princes Gate, London SW7

More geological evidence can now be brought to bear on the problem of the origin of Stonehenge. Spreading ice sheets rather than human activity could have brought the rocks to Salisbury Plain.

FOLLOWING the discovery of glacial deposits in north Somerset[1], the search for other evidence of regional glaciation has continued. In 1969, shortly before the M5 motorway sections at Clevedon became available, Dr A. Whittaker of the Institute of Geological Sciences found abundant fragments of white Chalk scattered on the steep landslipped slopes of Brent Knoll, Somerset. The Knoll is an isolated hill composed chiefly of Jurassic clay rising out of the coastal marshes between the Mendips and the mouth of the River Parrett and attaining an altitude of 457 feet (140 m) above O.D. This was the first intimation that Cretaceous Chalk might exist in the Drift deposits of Somerset. The source of the Chalk fragments is still uncertain. However, in the light of subsequent developments it seems that they may have been derived from submarine outcrops, or from outliers of Chalk (now perhaps destroyed), the material having been deposited by an ice sheet entering Somerset from the south or west.

Late in the summer of 1970, I found Chalk-bearing Drift deposits at Holwell near Nunney [ST 72554523] in the eastern Mendips where temporary exposures were seen while ground was being cleared for a new quarry. Here reddish brown sandy clay up to 3–4 feet (1 m) thick rests on Jurassic limestone (Inferior Oolite). The deposit is thin and patchy and has been partly decalcified, but the deeper pockets contain rounded and subangular fragments of hard white Chalk thought by Mr C. Wood to have come from the lower or middle part of the formation. Flint, chert, sandstone, quartzite and Carboniferous and Jurassic limestone pebbles are present, suggesting that the material is a relict till.

These and other glacial deposits situated on limestone uplands at the eastern margin of the Mendips at an altitude of about 500 feet (152 m) above O.D., lie in the proximity of gorge-like melt-water channels transecting the Mendip ridge at Nunney and East Cranmore. Their discovery at once suggests that ice could have blocked the western approaches to the Vale of Pewsey and the low lying ground described by F. W. Harmer[2] as "Lake Trowbridge". He believed that water from this lake was responsible for the downcutting of the Avon Gorge at Clifton.

Spread of Ice

During the most recent glaciation (Devensian, or Weichsel) ice from the uplands of South Wales reached the Vale of Glamorgan locally, but the main mass of ice in the Irish Sea did not cross the Pembrokeshire peninsula. It may, however, have extended somewhat further south in St George's Channel than was formerly supposed[3]. Most of the lowlands of South Wales and the greater part of the west and south of England, with the possible exception of Dartmoor and some other high ground, were unglaciated. Winter snowfall may have been heavy in the south-west, permafrost more strongly developed in the south-east of England. The Devensian ice of the Midlands seems to have come no further south than Wolverhampton, thus the greater part of the lower Severn Valley and the Cotswolds were not glaciated. Thick deposits of Head formed in Devon and Cornwall, the Mendips and Cotswolds, and were represented in Wessex and south-eastern England by the familiar "Coombe Rock".

Conditions during the two glaciations preceding the Devensian were very different. The penultimate (Wolstonian, or Saale) glaciation seems to have affected most, if not all, of South Wales[4] and in the deep valleys of the Pennant Sandstone uplands it is difficult to make a distinction between Devensian and Wolstonian glacial features and deposits[5]. In Pembrokeshire, the Vale of Glamorgan and Somerset the effects of the Wolstonian Glaciation are however recognizable.

Using information accumulated during the past decade the position of the main streams of ice moving eastwards along the Bristol Channel during the Wolstonian Glaciation can now be outlined. In the far west Lundy Island is said to have been overriden by the Wolstonian ice sheet[6], and glacial deposits were laid down at Fremington and Barnstaple[7]. Further east the great flow of ice which passed over Pembrokeshire was constricted as it moved up channel, confined on the north by the high ground of the South Wales Coalfield[5] and on the south by Exmoor and the Brendon Hills. Crossing the Severn Estuary, one arm of the ice moved up the broad Flax Bourton valley leading to Bristol[1]. Another stream, after dividing round the western Mendips, moved eastwards, crossed the lowlands of Somerset and invaded West Wiltshire. The northern part of the ice flow carried rocks which were derived principally from the South Wales Coalfield and the

Severn Estuary. In the central region erratics which can be matched with rocks from the Lake District, North and South Wales are found. In the south the erratics come mostly from Devon and West Somerset. Considerable quantities of marine and estuarine material torn or scooped up from the exposed floor of the Bristol Channel were carried into Central Somerset and the Clevedon area. Cretaceous flint and chert is abundant in almost all the glacial deposits, and has found its way into the younger raised beach deposits and the Devensian Head. Unlike the much older "Anglian" or Elster Glaciation the Wolstonian ice appears to have introduced no sarsen stones, and where this glaciation was strongest the Tertiary sarsen erratics carried by the older "Anglian" ice have been cleared away or thrust to one side.

"Anglian" Glaciation

The question remains, however, whether the glaciation, which seems to predate the so called "25-foot" raised beach of the Weston-super-Mare district and may therefore be comparable in age with the Chalky Boulder Clay glaciation of the Vale of Moreton, was responsible for the introduction of erratics to Salisbury Plain. The Wolstonian ice would have been capable of conveying Pembrokeshire material to the region east of the Severn Estuary and Bristol Channel. There are, however, indications of an older "Anglian" glaciation of the West of England and Bristol Channel which was much more extensive and carried very large Scottish erratics weighing as much as 50 tons[8]. This glaciation was probably responsible for the introduction of Welsh erratics to Salisbury Plain (Fig. 2). It equates with the ancient Plateau Gravel or "Northern Drift" ice sheet which carried Bunter pebbles and other northern erratics into the Cotswolds and the Upper Thames Valley and which preceded the formation of the Hanborough Terrace of the Upper Thames and Evenlode Valleys[9]. The magnitude of the glaciation can be judged from the fact that the upper surface of the ice-sheet in the vicinity of Cheltenham stood at well over 1,000 feet (305 m) above present sea level. One of the first comprehensive studies of the problems presented by the fragmentary record of this great glaciation, of which little evidence has survived further north, was made by Sandford[10,11].

The "Anglian" glaciation of the eastern part of the English Channel is thought to be of the same age as the "Anglian" glaciation of Devon and Cornwall where the presence of big "foreign" erratics has been attributed to the action of "coastal ice". The situation of the big erratics found at Croyde in North Devon cannot be explained in this way without assuming that the sea level was as high or higher than at present; this during a major glaciation. As Taylor has pointed out[12,13], the presence on the north coast of Devon of rocks from South Devon can hardly be accounted for by the drifting ice theory without arousing incredulity.

The name "Anglian" is in many ways unsuitable because of its link with the East Anglian succession. It should therefore be clearly understood that, as used in this account, the term refers to the ice sheet which invaded the coastal region of Hampshire and Sussex from the west carrying sarsens and big "foreign" erratics[14,21]. Erratic pebbles from these or comparable glacial deposits were later incorporated in the marine gravels of the Slindon (or 100-foot) raised beach of West Sussex[15]. Others found their way into the younger solifluction and beach deposits of the coastal plain.

The very low sea level accompanying the "Anglian" glaciation of southern England and the English Channel was therefore separated by the high Slindon (Tyrrhenian) sea level from that of the Wolstonian (Saale) glaciation. During the period of falling sea level marking the retreat of the shore-line from the 100–300 foot level intensive piping and disturbance of the Slindon raised beach and the formation of very thick solifluction deposits (the Main Coombe Rock of Sussex) took place.

It is generally considered, however, that the ice of the Wolstonian glaciation did not extend into the central and eastern parts of southern England. Salisbury Plain, though dissected by melt-water from the Wolstonian ice, does not seem to have been covered by an ice sheet at this time. The removal of the Clay-with-flints which was noted by Reid[16], probably took place during the "Anglian" glaciation. The detailed movements of the various ice masses invading southern England during this glaciation are as yet unknown. They may well have been complex, and the directions shown in Figs. 1 and 2 should be regarded as only an approximation.

It seems likely that the older highly cryoturbated Plateau Gravels of the Hampshire Basin represent disturbed glacial outwash from the "Anglian" ice since they are composed of material derived from the Clay-with-flints, and the local Mesozoic and Tertiary rocks. Small and medium-sized sarsen boulders are present, and Palaeozoic rocks also occur particularly in the western districts.

Irish Sea Erratics

Turning to the question of the known eastern limit of Irish Sea erratics, pebbles of porphyritic igneous rocks together with flint, chert and "flinty slate" were found in the nineteenth century on Flat Holm island in the Bristol Channel[17]. The Geological Survey collection includes a fragment of a "soda-granite" from the same locality, presumably part of a pebble of Welsh origin. The desirability of re-examining Flat and Steep Holm has been evident for some time and in May 1971 I paid a brief visit to the islands in company with Mr Brian Hawkins of Bristol University and Dr Brian Kelk of the Institute of Geological Sciences.

On the south side of Flat Holm the storm beaches are composed almost entirely of local Carboniferous Limestone (Goblin Combe Oolite and Clifton Down Mudstone) but on the north side the limestone pebbles are mixed with Chalk flint, greensand chert, Old Red Sandstone and a wide range of igneous and metamorphic rocks which have been identified by Mr R. K. Harrison and seem to have been derived from South Wales, North Wales and the Lake District. The erratic tracks fit with the evidence from South Wales where Bowen[4] has recently summarized the work of Griffiths[18] demonstrating the dispersal of Pembrokeshire and other rocks carried by ice from the Irish Sea, probably in Wolstonian times. On the other hand, the Flat Holm beach material includes very little rock from the mainland coasts and the most likely explanation is that the pebbles are derived from a submerged deposit of till, probably lying off the north coast of the island. Further discussion is postponed until this has been located and other reported occurrences of erratics have been investigated.

Here it may be useful to observe that reddish brown till with Chalk flints, greensand chert and rocks from South Wales was found in 1970 at or below sea level at Kenn near Clevedon, though in this case the glacial deposits are buried by Holocene mud[1]. Other residual masses of glacial sand and gravel ("Burtle Beds") rise out of the marshes in central Somerset at Catcott Burtle, Westonzoyland and Othery[19]. In these outwash deposits, previously described as marine, the remains of marine, estuarine and freshwater organisms as well as the bones of land animals occur. The associated erratics are derived chiefly from West Somerset and North Devon. They include Devonian or Old Red Sandstone, Carboniferous, Bunter and Jurassic rocks, Cretaceous chert and flint. These are associated with pellets of soft red and green Triassic mudstone, some mixed with marine shells and remoulded in ice, others, lumps which may have been rounded while in a frozen condition. At least one mega-erratic is thought to have existed in the Westonzoyland area[20]. This was known as the "Devil's Upping Stock". Unfortunately it was cut up for ornamental stone and its petrological affinities are therefore unknown.

Scattered on the Dorset, Marlborough and Berkshire downs are isolated groups of Tertiary sandstone erratics known as sarsens. The name is probably derived from the Anglo-Saxon *sar* (troublesome) and *stan* (a stone), and, as Chatwin[21] has pointed out, it is significant that a farming people should have paid particular attention to them.

Many differing views have been expressed as to their ultimate source and mode of emplacement. A full discussion of these problems must await more detailed description. For present purposes, however, it may be said that in certain areas, notably Sussex and Somerset, they behave as would any other erratics and are associated locally with erratics of "foreign" rocks which seem to have travelled with them. The Wolstonian ice of the Bristol Channel does not seem to have picked up Tertiary sandstone, possibly because it did not traverse the extensive tracts of submerged Tertiary rocks found in the English Channel and the western approaches. Nor were sarsens carried by the "Northern Drift" ice of the Cotswolds and Severn Valley.

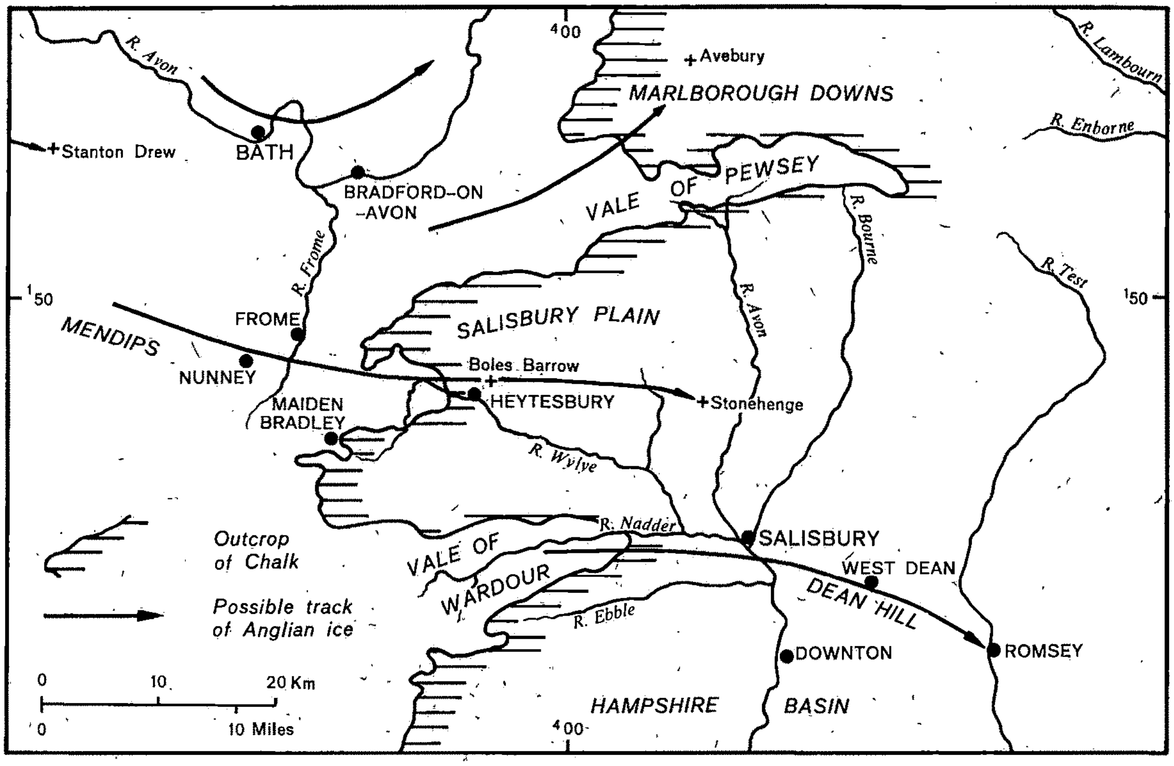


Fig. 1 Sketch map of Salisbury Plain and its environs showing principal localities referred to in the text. Ice entering Wiltshire from the west may subsequently have been deflected by sarsen-bearing ice from the English Channel moving in a SW–NE direction.

South of Bristol and Bath sarsen erratics are found on high ground in the eastern Mendips and Broadfield Down, mostly in areas which were not eroded by the Wolstonian ice. In Sussex, on the other hand, the sarsen erratics were obviously brought in by ice from the English Channel and the most likely sources of the sandstone, which is not found in the local rocks on shore, are the Tertiary formations which are known to cover large areas of the sea floor.

Sarsens and Stonehenge

For the archaeologist the sarsen stones and their origin are matters of particular interest, since the two greatest megalithic monuments in the British Isles, Avebury and Stonehenge, are built wholly or in part of sarsen sandstone. As long as it was believed that the sarsens were the remains of a relict Tertiary sandstone "lowered" into position by the dissolution of the chalk, progress in understanding the distribution of the sarsens and the glaciation of southern England was impeded.

One of the most mysterious features of the great megalithic monument of Stonehenge is the presence of the foreign stones, or "bluestones" as they are popularly described[22]. It has long been known that these are not of local origin and there has been intense speculation about their presence on Salisbury Plain. Since the bluestones of Stonehenge are considered to have come from Pembrokeshire, the discovery of evidence for the glaciation of the region east of the Bristol Channel is clearly of more than local geological significance. H. H. Thomas[23] who was the first to recognize in Pembrokeshire the probable source of the bluestones, believed that there was no valid evidence to support the theory advanced by Judd[24], that they were conveyed to Salisbury Plain by ice. At this time the existence of glacial deposits at Fremington in North Devon had only tentatively been recognized by Maw (1864)[25] and their glacial origin was not generally acknowledged until much later[18,26,27]. On the other hand, the clearly expressed views of Harmer[2] respecting the glacial origin of the gorges of the Bristol Avon were widely ignored or frankly disbelieved by most geologists working in the area. Thomas therefore concluded that ice from the Irish Sea did not reach Devon and Somerset and went on to say that the available evidence "permanently disposes of the idea of glacial transport for the Foreign Stones of Stonehenge".

The effect of this pronouncement on archaeology and, indirectly, on English glacial geology has been considerable. H. H. Thomas explained the presence of the foreign stones on Salisbury Plain and the wide variety of rocks represented among the bluestones by suggesting that they were originally erratics gathered up locally and used in the construction of a great stone circle which he thought might have been built at

Cil-maen-llwyd on the south-eastern slopes of the Preseli mountains[23]. In this way he accounted for the presence of relatively soft sheared volcanic rocks which would not otherwise have been used for building, particularly where hard dolerite and rhyolite are freely available as they are at Mynydd Preseli. According to this theory the stones were subsequently removed from this "venerated stone circle" and conveyed by its votaries to Stonehenge. The archaeological evidence for the "venerated stone circle" has since been discredited[28,22]; but belief in the human transport of the stones has persisted, though the theory of overland transport preferred by H. H. Thomas has been replaced by carriage by water.

One outstanding item in this problem is the Altar Stone, shown by Thomas to have been derived originally from the Senni Beds of Breconshire or, alternatively, from the Cosheston Sandstone of southern Pembrokeshire. Even if we suppose that all the Old Red Sandstone material found at Stonehenge came from South Pembrokeshire, there is still no means of establishing Mill Bay or Milford Haven as the immediate source of the material used at Stonehenge. Not only does the Old Red Sandstone of south Pembrokeshire cover a fairly large area on land, it also extends beneath the sea and it has long been known that the whole region has been strongly glaciated. It may be that Neolithic man picked up a 7 ton boulder of Cosheston Sandstone on the foreshore of Milford Haven. On the other hand, ice could have quarried the bedrock or picked up a boulder from a very much wider area, and deposited it anywhere between south Pembrokeshire and Salisbury Plain. It is certain that neither the Altar Stone nor some of the other sedimentary rocks recognized by Judd[17] at Stonehenge came from the Preseli Mountains. As Judd pointed out, many of the Palaeozoic rocks found at Stonehenge would have been too soft or fissile to have been of any value as a building material. The inference is plain. Even if the hard igneous rocks were worth transporting to Stonehenge, the softer stones have still to be accounted for, and glacial action is the only reasonable solution.

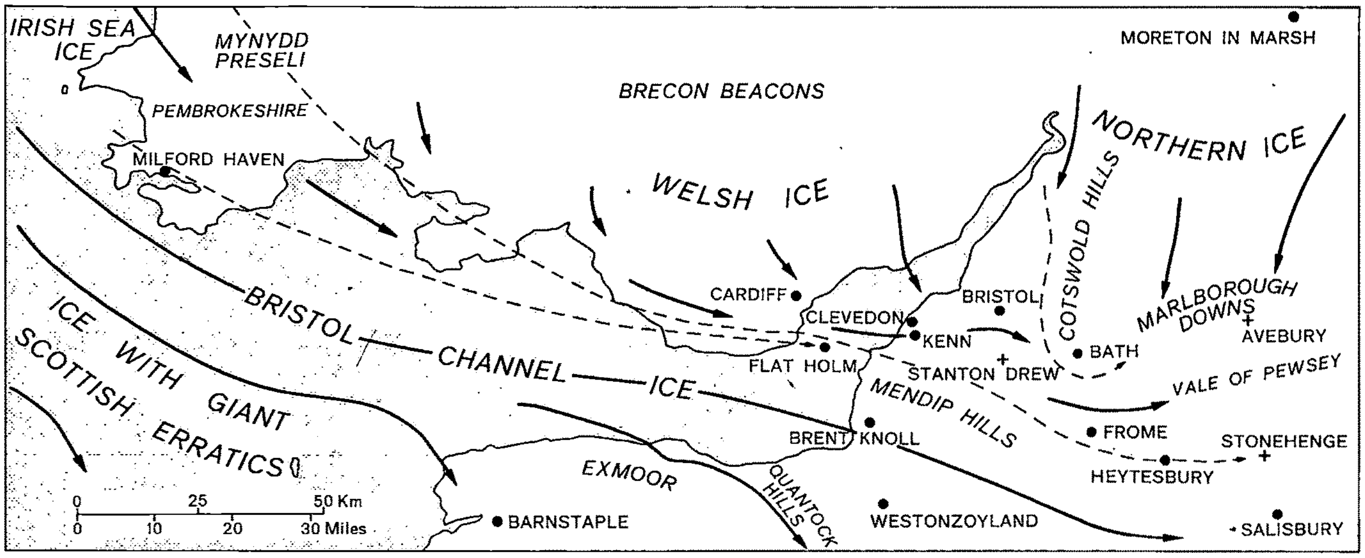


Fig. 2 Bristol Channel region showing generalized erratic "tracks" and inferred direction of flow of the "Anglian" (Elster) ice. Exmoor may have formed a small centre of local ice accumulation, the Mendip and Cotswold Hills were largely overridden at the height of the glaciation. The northern limit of the sarsen erratics is given roughly by a line joining the southern end of the Quantock Hills, Stanton Drew, Bath and the northern edge of the Marlborough Downs.

Apart from the bluestones at Stonehenge, only two other large pieces of igneous rock from Salisbury Plain are known to exist. Both are preserved in Salisbury Museum. One is a boulder weighing about 9 ton found in company with sarsen stones in the Neolithic long barrow known as Boles Barrow near Heytesbury [ST 94214677]. The other is a smaller stone from Lake, south of Amesbury in the valley of the Salisbury Avon. Both appear to be petrologically similar to the dolerites from Mynydd Preseli and those at Stonehenge. The stone from Lake may, however, be a piece detached from one of the Stonehenge bluestones in comparatively recent times and for present purposes is best ignored.

If the bluestones of Stonehenge were conveyed in ice from Pembrokeshire it might reasonably be expected that some ice-scratched or polished erratics would have been found in Wiltshire. Many of the bluestones used at Stonehenge have been dressed or shaped by hand, others show rough or matt surfaces which convey no useful information beyond the fact that they have undergone some weathering. The Boles Barrow stone does appear to retain part of its original surface on which the harder crystalline knots or patches have been faceted. This may be a relic of glacial smoothing, but much of the polish on the stone is due to handling.

The relative age of Boles Barrow and Stonehenge is said to be uncertain, though the former is attributed to the Windmill Hill culture and Stonehenge to the Beaker culture. Archaeological evidence shows that the foreign stones were certainly on Salisbury Plain in Neolithic times[29] and that the bluestones of Stonehenge were originally used in the construction of an earlier monument, possibly at the Cursus[30].

Other Means of Transport

Following rejection of the theory of glacial transport, carriage of the stones on boats or rafts by way of the strongly tidal Severn Estuary and Bristol Avon has come to be the most widely accepted hypothesis. Nevertheless the Neolithic and Bronze age rivers differed considerably from those of the present day, and the Bristol Avon has been virtually converted into a man-made channel by centuries of excavation, dredging and control by embarkments, lock gates and weirs. It seems to me questionable whether river transport of such massive stones was practicable above Bathampton in Neolithic times even if the serious navigational hazards of the Severn Estuary and the lower Avon had been surmounted.

Why should the builders of Stonehenge carry lumps of slate, quartzite, greywacke, flagstone, soft-sheared volcanic ash and Old Red Sandstone to Stonehenge when ample supplies of hard dolerite were available at Mynydd Preseli? The strange mixture of stones—Old Red Sandstone, dolerite, rhyolite, volcanic ash, calcareous ash—described by Atkinson[22] from the west side of the bluestone circle, and the presence of Chilmark Stone from the Vale of Wardour in the packing of stone holes, certainly supports Judd and Thomas in their conclusion that the stones were originally erratics. The reasons for thinking that these were collected from a "venerated stone circle" and conveyed overland are, as we have seen, no longer acceptable. The suggestion that Mynydd Preseli was a "sacred mountain" leaves unexplained the use of Cosheston Sandstone and other Old Red Sandstone rocks from unsanctified south Pembrokeshire. Yet, if the stones did not come from one "venerated stone circle" or "sacred mountain" the motives for conveying them 150 miles (241 km) to Stonehenge while ignoring local resources, are incomprehensible. For large masses of suitable stone (Great Oolite Limestone) remain to this day, within 20 miles (32 km) of Stonehenge on the landslipped slopes of the Avon valley between Bathampton and Bradford-on-Avon, the identical defile through which the Pembrokeshire rocks are supposed to have been conveyed by Bronze Age man to Salisbury Plain.

In view of recent developments a re-examination of the Stonehenge rocks has been commenced. Whatever may be the outcome of these investigations there is bound to be an element of doubt about some of the earlier conclusions. While it is true that some or all of the igneous bluestones could well have come from Mynydd Preseli, comparable rocks are known in the Ordovician terrain near Cader Idris[23,31]. There may also be offshore areas of similar Ordovician volcanic and associated intrusive rocks in the Irish Sea and St George's Channel[18].

This does not affect the interpretation of the glacial history since it can be demonstrated that ice flowed out of the Irish Sea into the Bristol Channel carrying rocks from a very wide region. However, it has a critical bearing on the theory of human transport which depends on the material having been derived from a limited area on land. If it could be proved that Neolithic man actually quarried the crop of the spotted dolerite at Carn Meini or worked the frost-shattered debris on Mynydd Preseli the theory of human transport might be credible. No such evidence has been produced. Neolithic flint miners dug shafts and galleries in the chalk in Sussex and East Anglia but their tools, like those used at Stonehenge, were deer horn, bone, flint, sarsen and wood, of little value for quarrying hard massive rocks. In practice, Neolithic man therefore used lumps and boulders, or tabular erratics and slabs that he could split with the aid of fire and water.

Frost-wedged fragments, though not strictly erratics, could also have been used at Stonehenge, but even if this could be established it would not prove transport by human agency, since some of the Stonehenge erratics may well have been broken down *in situ* by frost action on Salisbury Plain.

Archaeological attention has been diverted from the effects of glaciation and concentrated on the petrology of the igneous bluestones in the belief that it is possible in this way to prove that they were conveyed by man directly to Stonehenge. Without questioning the conclusions of Thomas[23] concerning the original source of the igneous bluestones used at Stonehenge, it must be admitted that there are circumstances when the use of specific "indicator rocks" to pinpoint individual localities can be misleading. Of very few rocks can it be said that they are unique and confined to one small area and that no other occurrences may exist on the sea floor or beneath drift deposits on land. Pebbles and fragments may be secondarily derived, glacial erratics can be introduced by an early glaciation and moved again during a later one. Errors in determining provenance are one of the hazards of using erratics but the risks have to be accepted if progress is not to cease

Erratics in Megalithic Monuments

If Stonehenge and Boles Barrow are constructed of, or incorporated, large erratics deposited by the "Anglian" ice, other megalithic monuments in the area glaciated by the "Anglian" and Wolstonian ice may be expected to show similar features. For this reason the stone circles of Stanton Drew and the outlying stones in the Chew valley of north Somerset[32] are an ideal case for investigation. The monument is sited on Triassic marl but local (Triassic) sandstone is present in the vicinity and would have been suitable for quarrying if Neolithic or Bronze age man had been able to do so. Undressed stones up to about 27 tons in weight, but of variable size and shape, have been used at Stanton Drew, none of them of local origin. The monument includes three stone circles, and a group known as The Cove. Some of the outlying stones, including Hautville's Quoit, may or may not have formed part of a structure. Rocks used in the monument or found at the Cove and Hautville's Quoit include silicified and mineralized dolomitic conglomerate from Broadfield Down and the Mendips[33], Old Red Sandstone possibly from Wales or from the Portishead Beds of Blackdown or the Portbury district, Dundry Freestone (Inferior Oolite) and Tertiary sarsen stone. The massive blocks of Dundry Freestone can only have come from the western end of Dundry Hill. The sarsen, which it has not yet been possible to examine petrologically, can be matched by sarsen fragments found on Felton Common [ST 518651] the broad west–east valley crossing Broadfield Down 6.5 miles (10 km) south-west of Bristol. Fragments of sarsen stone at Felton Common were previously mistaken for Rhaetic (which also occurs on Broadfield Down), but the rock contains the characteristic rootholes. Sarsen erratics, presumably introduced by the "Anglian" ice, are not uncommon on high ground south of Bristol. These may not have been over-ridden by the Wolstonian ice sheet.

Tracks of the stones used at Stanton Drew have a strong west–east component and it is noticeable that though Triassic sandstones are present locally and hard Coal Measures sandstones crop out about 1.5 miles (2.4 km) east of Stanton Drew, neither of these rocks has been used in the construction of the megalithic monument. The situation of the stones of Stanton Drew is entirely consistent with the possible site of a moraine upstream of the Chew gorge at Pensford [ST 617637]. The latter may have operated for a time as a spillway from a glacial lake covering the upper part of the Chew valley, including the area of the present reservoir. In this instance the erratics were probably introduced by the "Anglian" ice, final emplacement having taken place in Wolstonian times. Some of these large tabular erratics, notably the dolomitic conglomerate of The Cove, could have been in the form of giant slabs which were later broken up, either by natural processes or by man.

Other large isolated stones or groups of stones formerly existing in otherwise stoneless areas of Wessex have been broken up for hard core or destroyed as pagan monuments. Some of these acts of vandalism have been inscribed but the only record of the existence of other vanished stones is to be found in the local place names or in folklore[34]. The surviving greywethers in the downlands of Wessex and Sussex, the Mendips and west of England, probably give only a faint impression of the former extent of the boulder strewn surfaces.

Many erratics are almost certainly enclosed in chambered long barrows and similar structures and a comprehensive study of these rocks might yield valuable evidence bearing on the direction of "Anglian" ice movements. Grinsell[32] has estimated that there are about 200 long barrows in Wessex alone. Not all of them are chambered, and the average weight of stone per barrow can only be guessed, but if 100 long barrows contain an average of only 50 tons a piece it would mean that 5,000 tons of medium–large erratics could be included within them. This would still leave uncounted the enormous quantities of stone originally used in sites such as Avebury where some of the sarsen erratics weigh up to 40 tons and one of 90 tons formerly existed.

Erratic boulders of up to 350 tons are said to exist in the Carnac area of south Brittany and giant erratics and glacial rafts of huge dimensions are found in Britain and many parts of the world. Nobody knows the original size of the Stonehenge sarsen erratics before they were split and trimmed, but their stratigraphy suggests that a number of them may have formed parts of one or more giant slabs stranded somewhere in the vicinity of the monument. As an example demonstrating the size which can be attained by such glacial erratics we may refer to the immense mass of tabular Silurian limestone found in Warren County, Ohio. This slab, though only 5 feet (1.5 m) thick has a surface area of 20,000 square feet (1,858 m^2)[35]. Even larger erratics consisting solely of quartzite are found in a boulder train near Calgary, Alberta[36]. Beneath such huge tabular masses, till, pebbles and boulders may be preserved even when the surrounding areas have been cleared by erosion. This may well be the explanation of the survival at Stonehenge of lumps of relatively soft volcanic rock such as the ones represented only by stumps in the bluestone circle. Soft rocks, or compact but frost-susceptible erratics partly buried in glacially disturbed Chalk or chalky till and preserved from erosion beneath huge flat-lying frost-resistant sarsen stones, may have been unearthed by Neolithic or Bronze age man when building his monuments. Some of these smaller erratics may have appeared to be hard enough to use for building and may have survived for a time before breaking down under the influence of frost and rain. Without some such explanation it is difficult to see why the rocks should ever have been used at all, much less carted from Pembrokeshire and then trimmed on site with all the attendant risk of wasting stone.

It has also been claimed that the sarsens of Stonehenge were dragged overland from Avebury, but there is no geological evidence to support this. Nor does it seem at all likely that they were moved on frozen ground in late Neolithic or early Bronze age times[37], when the climate may have been warmer than at the present day.

There seems to be a close correlation between the distribution of long barrows in Wessex (ref. 32, Map 1) and the downland areas now thought to have been invaded by the vast but ancient "Anglian" ice sheet. For example, the presence of Jurassic limestone built into the chambered West Kennet Long Barrow [SU 10506775] and other barrows in the Bishop's Cannings valley on Marlborough Downs[32,38] suggests that the Avebury sarsens may have been dumped as part of a moraine at the terminus of a stream of ice moving in an easterly or north-easterly direction (Fig. 1). It is not unreasonable to suppose that the presence of non-local clay found in the centre of some barrows may be the remains of till deposits disturbed during the lifting and erection of erratics.

Much work will be needed on the chambered long barrows before detailed conclusions can be drawn about these movements, and in many areas there is little reliable information as to the nature of the stones used in their construction. It may be no coincidence, however, that the western part of the Marlborough Downs has some of the most characteristically glaciated profiles to be seen in this part of Britain. The moulded outlines, broad U-shaped dry valleys and melt water channels of the downlands are in some respects the most interesting of the glacial features, but their assessment demands more extended treatment than is possible at this stage.

Any calculation of the original quantity of erratic material on Salisbury Plain necessarily ignores the smaller pebbles and fine grained material dissolved, blown, washed away, or moved by solifluction during dissection of the uplands in the two major cold periods following the "Anglian" glaciation. To these later periods belong the valley gravels of the Wylye, Avon, Test and other rivers, and the solifluction streams which concentrated the medium and some of the big sarsens in the dry valleys of the downlands. Only the inertia of the larger erratics and their situation on normally waterless uplands preserved them for the use of Neolithic man.

I thank everybody who has provided information and material, especially Mr Brian Hawkins, Dr Brian Kelk, Mr R. K. Harrison and Mr C. Wood. I also thank Mr H. S. Shortt, Mr C. Bowen and Mr R. S. Newall for help with archaeological problems. The manuscript was edited by Miss E. M. Pyatt.

[1] Hawkins, A. B., and Kellaway, G. A., *Proc. Geol. Assoc.*, **82**, No. 2, 267 (1971).
[2] Harmer, F. W., *Quart. J. Geol. Soc.*, London, **63**, 470 (1907).
[3] John, B. C., *Geol. Mag.*, **107**, 439 (1970).
[4] Bowen, D. Q., in *The Glaciation of Wales and Adjoining Regions* (edit. by Lewis, C. A., London and Colchester, 1970).
[5] Woodland, A. W., and Evans, W. B., *Geology of the South Wales Coalfield, IV, The Country around Pontypridd and Maesteg., Mem. Geol. Surv.* (1964).
[6] Mitchell, F., *Proc. Roy. Geol. Soc., Cornwall*, **20**, No. 1 for 1956–6, 65 (1968).
[7] Edmonds, E. A., *Rep. Inst. Geol. Sci.* (in the press).
[8] Flett, J. S., *The Geology of the Lizard and Meneage, Mem. Geol. Surv.* (1946).
[9] Kellaway, G. A., Horton, A., and Poole, E. G., *The Development of some Pleistocene Structure in the Cotswolds and Upper Thames Basin, Bull. Geol. Surv.* (in the press).
[10] Sandford, K. S., in Pringle, J., *The Geology of the Country around Oxford, Mem. Geol. Surv.* (1926).
[11] Sandford, K. S., *Quart. J. Geol. Soc., Lond.*, **85**, 359 (1929).
[12] Taylor, C. W., *Trans. Devon. Assoc.*, **88**, 52 (1956).
[13] Taylor, C. W., *Trans. Devon. Assoc.*, **90**, 187 (1958).
[14] Reid, C., *The Geology of the Country around Bognor, Mem. Geol. Surv.* (1897).
[15] Calkin, J. B., *Proc. Prehist. Soc. East Anglia*, **7**, No. 3, 333 (1934).
[16] Reid, C., *The Geology of the Country around Salisbury, Mem. Geol. Surv.* (1903).
[17] Buckland, W., and Conybeare, W. D., *Trans. Geol. Soc.*, No. **2**, **1**, 210 (1824).
[18] Griffiths, J. C., *The Glacial Deposits West of the Taff*, thesis, Univ. London (1940).
[19] Bulleid, A., and Jackson, W., *Proc. Somerset Archaeol. Nat. Hist. Soc.* for 1937, **83**, 171 (1938).
[20] Dobson, D. P., *The Archaeology of Somerset* (Methuen, London, 1931).
[21] Chatwin, C. P., *The Hampshire Basin and Adjoining Areas, British Regional Geology, Mem. Geol. Surv.* (1948).
[22] Atkinson, R. J. C., *Stonehenge* (Penguin, London, 1900).
[23] Thomas, H. H., *Antiquaries*, **3**, 240 (1923).
[24] Judd, J. W., in Gowland, W., *Archaeologia*, Second Series, **8**, 106 (1902).
[25] Maw, G., *Quart. J. Geol. Soc. London*, **20**, 445 (1864).
[26] Dewey, H., *South-West England, British Regional Geology, Mem. Geol. Surv.* (1935).
[27] Zeuner, F. E., *The Pleistocene Period* (Hutchinson, London, 1959).
[28] Grimes, W. F., *The Prehistory of Wales* (National Museum of Wales, Cardiff, 1951).
[29] Newall, R. S., *Stonehenge, Wiltshire* (HMSO, London, 1959).
[30] Stone, J. F. S., *Archaeol. J.*, **104**, 7 (1948).
[31] Cox, A. H., and Wells, A. K., *Quart. J. Geol. Soc. London*, **76**, 254 (1921).
[32] Grinsell, L. V., *The Archaeology of Wessex* (Methuen, London, 1958).
[33] Morgan, C. L., *Proc. Somerset Archaeol. Soc.*, 33, No. 2, 37 (1887).
[38] Crawford, O. G. S., *Mounds, Standing Stones and other Objects, The Long Barrows of the Cotswolds* (Gloucester, 1925).
[35] Flint, R. F., *Glacial Geology and the Pleistocene Epoch* (Wiley, New York and London, 1947).
[36] Stalker, A. MacS., *The Erratics Train Foothills of Alberta, Geol. Surv. Canad. Bull.*, 37 (1956).
[37] Hawkins, G. S., *Stonehenge Decoded* (London and Glasgow, 1970).
[38] Piggott, S., *The West Kennet Long Barrow, Excavations 1955–6 Min. of Works Archaeological Reports*, No. 4 (1962).

Was there really a Big Bang?

G. BURBIDGE
University of California, San Diego

The evidence in favour of a big bang cosmology is much less definite than is widely realized, and it is not impossible that we are living in a steady state universe.

In any given field of science, activity has its peaks and its valleys. Many view activity as synonymous with progress. If one judges by the number of papers currently appearing and by the interest of the younger astrophysicists, activity in cosmology is at a peak at present. To the outsider and even to many inside, it looks, at first sight, as though considerable progress has been made in recent years, so that the outline of the way in which the universe has evolved is understood and only the details need to be explained. Is there any justification for this? Those who have read widely in the earlier and more recent cosmological literature, as well as those who rely on North's account[1], are aware that views of cosmology at any epoch are largely determined by the ideas of a few strong individuals, rather than by an objective appraisal of the information available. Today a similar situation prevails, and it is further complicated because we are often trying to use classes of objects to test cosmological theories long before we understand the physics of them.

In my view, modern developments have been influenced by several factors. They include new observational discoveries combined with an extremely simple minded approach to them, a deep seated conviction by many that general relativity is correct in all details, a belief that astronomy has nothing to teach us about fundamental physics, and, last but not least, a deep seated hostility by both observers and theoreticians in the astronomical community toward the steady state theory. Usually this last point is not mentioned in polite society, but it is blatantly obvious in Dingle's remarks[2] while president of the Royal Astronomical Society; it is often apparent in private discussions, and a further manifestation is a complete disregard of the theory when the state of cosmology is reviewed (see, for example, ref. 3).

What are the major scientific events which make up the case for a big bang? They started, of course, with the work of Einstein in 1917. Incidentally, he, as did his contemporaries, took it for granted that the universe in the large is unchanging, that is, he assumed that it is in a steady state. But it was Lemaitre[4,5] and, independently, Friedmann[6] who showed that the Einstein universe is unstable so that, when perturbed in the direction of expansion, it will continue to expand, tending to a de Sitter universe in the limit. Lemaitre's analysis showed that there is an infinity of models satisfying Einstein's equations. Similar solutions are obtained in the scalar-tensor theory. The most interesting model is the one which starts at a finite time in a state of infinite density. It was therefore inferred that, according to the theory of general relativity, the universe must be expanding, and most likely began in a state of high density. Almost immediately following this major prediction of general relativity, Hubble and Humason[7,8] conclusively established the redshift-apparent magnitude relation, which gave observational proof that the universe is expanding. Thus by 1930 Einstein's theory had made what many considered to be the greatest successful prediction in the history of science. For the next twenty years the drive in observational cosmology, almost completely in the hands of Hubble and his colleagues, was to attempt to decide from observation which of the myriad of general relativistic models best represent the universe. Soon, however, there developed an apparent paradox associated with the ages of different systems, because it appeared that the ages of various astronomical objects, notably the Earth, seemed to be greater than the age of the universe derived from the rate of expansion obtained by Hubble. Two possible paths of escape from this dilemma were the Lemaitre model with its arbitrarily long waiting period, and the steady state cosmology requiring the continual creation of matter[9,10].

The present situation with regard to this age difficulty is that we now realize that the uncertainties associated with the determination of H and those associated with obtaining the ages of the oldest stars are great enough that we no longer have any strong evidence that there is any problem. Most recently it has been stated that $H \sim 50$ km/s Mpc^{-1}, or $H^{-1} \simeq 20 \times 10^9$ years[11,12], while the age of an old galactic cluster, NGC 188, is given as 10×10^9 years[13–15], and the age of the elements obtained from the radioactive isotopes is $\sim 10 \times 10^9$ years, with uncertainties depending on assumptions made about the sequence of events leading to the isolation of U and Th in the solar system[16].

The prediction that the universe is expanding, followed by the discovery of the expansion, was the first major success of cosmology based on general relativity. The second came from the comparatively elementary discussion of the physics of the early stage in a dense expanding universe—the primaeval fireball—by Gamow and his colleagues[17,18], and the discovery in 1965, by Penzias and Wilson[19], of background radiation which may well be relict radiation from this fireball. These two predictions, followed by two major discoveries which, at first sight, appeared to confirm the theory, provide the basic scientific case for evolving models of the universe.

If we wish to account for the expansion without invoking an initially dense phase, we are left with either the steady state model in which matter is created or with an oscillating model. The steady state alternative seemed attractive to some, especially "when taken in conjunction with aesthetic objections to the creation of the universe in the remote past. For it is against the spirit of scientific enquiry to regard observable effects as arising from causes unknown (and unknowable) to science, and this in principle is what creation in the past implies"[10]. There is apparently a great gulf between those who find creation in the past acceptable, but continuous creation unacceptable, and those who have no such strong feelings. It is my belief that much of the conflict in cosmology in modern times has arisen because of this division which has little to do with science. More to the point, we must critically evaluate the detailed evidence which has accrued in the past twenty years concerning the question of whether or not we have definitely established that the universe is evolving.

Determination of q_0

In 1956 Humason, Mayall, and Sandage[20] gave a detailed account of the status of the Hubble relation. At that time the largest redshifts which had been measured were $z \simeq 0.2$. In the fifteen years that have elapsed since then, Sandage and his collaborators have worked intensively on the problem of refining the measurements of H and q_0. Many progress reports on this work have been given by Sandage[21–25]; see also Peach[26]. As far as the value of H is concerned there is some reason to believe that we are beginning to converge toward a realistic value some ten times smaller than that obtained by Hubble in 1936. As far as the determination of the deceleration parameter q_0 is concerned, the position is not so good. There are two basic reasons for this. First, very few clusters with redshifts greater than 0.2 have been discovered. The most recent plot given by Sandage[25] contains forty-two first-ranked galaxies in clusters, and only three of these, with $z = 0.29$, 0.36 and 0.46, are in excess of $z = 0.2$. The three clusters were observed by Baum[27] more than ten years ago, and a detailed discussion of his data has never been given. Many people believe that the radio astronomer's ability to detect more distant objects will lead, or has led, to an improvement of the situation. The only major achievement of radio astronomy in this connexion so far was the identification, in 1960, of 3C 295 with a galaxy with a redshift of $z = 0.46$ (ref. 28). This source lies in a cluster, and Baum obtained photometry which, combined with the redshift, gave this last point on the Hubble diagram.

About 100 galaxies associated with radio sources and 200 quasi-stellar objects, most of which are radio sources, have been optically identified, and redshifts have been obtained. None of these, with the exception of the cluster around 3C 295, has given us any information about the classical Hubble diagram beyond $z \sim 0.2$. This is because the radio sources are associated with galaxies with redshifts ≤ 0.2, or they are associated with galaxies which obviously cannot be treated as "standard candles", or they have been identified with QSOs which show a very large dispersion in the redshift-apparent magnitude diagram. Using the radio properties as a guide, we can single out a certain class of objects, but it is not clear at all that they can be used for cosmological investigations of the classical kind. If we make a table of optical types of radio galaxies as a function of increasing redshift[29], we see that, at small redshifts, almost all types of galaxy are being identified. At intermediate redshifts the galaxies are mostly giant ellipticals, while at the largest redshifts, between 0.2 and 0.3, the galaxies with published redshifts have nearly all been classified as N-type, meaning that a large part of the optical energy that they are emitting is due to non-thermal processes and hot gas rather than stars. Thus they cannot yet be used as standard candles, as the data available at present are inadequate.

Redshifts of Faint Sources

So far it has not been possible to make optical identifications and obtain redshifts of radio sources which are fainter than about 18 mag in the case of non-stellar objects (galaxies) or about 19.5 mag in the case of stellar objects. These are limits set by optical astronomy. They are set, first, by the fact that the 48 inch Palomar Sky Survey only goes down to 20.0 mag in the red and 21.1 mag in the blue; objects which are fainter than this cannot be identified unless the large reflectors, which typically have very small fields ($\sim 20'$), are used. Second, the difficulties associated with studying very faint objects are very great, because of the increasing difficulty in detecting photons against the natural and man-made noise, even with the use of image-tube techniques; also, only a very limited fraction of observing time on the large telescopes is presently devoted to this work.

Even if one admits that many of the problems can be overcome so that it will eventually be possible to measure the redshifts and do photometry of galaxies down to magnitude 21–23, we still have a problem in finding suitable objects to observe, because there is no prospect of doing surveys to this level. Sandage and his colleagues[30] are now attempting to make identifications by using very accurate radio source positions for which no optical objects can be seen at the level of the 48 inch Palomar Sky Survey. By using special photographic plates and the 200 inch telescope, they have been able to go as faint as magnitude 22 to 23 and find optical objects in some of these positions. Even when such objects are found the problem of measuring redshifts and energy distributions will be very time consuming, and it is not obvious that it will succeed. Those objects which turn out to be QSOs will be useless for this type of investigation. We can use the redshifts of those that turn out to be galaxies, but even then it will be necessary to observe other galaxies which are associated with them to obtain energy distributions which are likely to be sufficiently normal so that they can be used to derive apparent bolometric magnitudes.

Correction for Evolution

A second problem concerns the corrections to the observed energy distribution, based on the view that we are looking at standard galaxies, if they exist. These, when seen at large redshifts, are much younger than the galaxies comparatively close to us, from the viewpoint of stellar evolution. To make such corrections it is necessary to know what types of stars give rise to the bulk of the radiation from elliptical galaxies. A limited amount of work has now been done on the nuclear regions of nearby spirals and on one or two ellipticals in the Virgo cluster. This has led Sandage to conclude that the evolutionary corrections may amount to no more than 0.03 mag per 10^9 years, a value considerably smaller than earlier estimates. Tinsley has argued that theoretical models of galaxies which show a greater degree of evolution, so that the colour correction may be considerably larger, are also compatible with the observations. Most observers are doubtful of her conclusions, but it is far from clear that the present results are conclusive.

At a redshift of $z = 0.2$, the difference in bolometric magnitude of the standard galaxies, m_{bol}, to be expected between $q_0 = 0$ and $q_0 = +1$ is only 0.2 mag, while at $z \simeq 0.5$ the difference in m_{bol} between $q_0 = +1$ and $q_0 = -1$ (steady state) is 0.9 mag.

Because the three clusters with redshifts greater than 0.2 appear to lie closest to the $q_0 = +1$ relation, it has been stated for the past 10 years, with different degrees of emphasis, that $q_0 \simeq +1$. At the same time it also has sometimes been implied that q_0 is not really known at all. And, in terms of hard data, this latter statement is true. The fact that Baum's measurements have neither been published in detail nor repeated, that we have such a small amount of data anyway, and that the local anisotropy in the velocity field may affect the determination of q_0 all suggest that the numerical value of q_0 is only known to lie between wide limits; so both the exploding models and the steady state models are compatible with the measurements made so far.

Stebbins–Whitford Effect

In 1948 Stebbins and Whitford[32] published the first results of their photo-electric measurements of the colours of faint clusters of galaxies. They concluded in this preliminary investigation that after they had put in the usual corrections for the effect of the redshift there appeared to be an excess reddening which increased with increasing redshift. This meant that the distribution of energy radiated by stars in a galaxy is a function of epoch so that an evolutionary effect is present. In the early 1950s, this result was used as observational evidence against the steady state cosmology and there was some discussion in the literature about its significance[33,34]. But the result was only preliminary and by 1956 Whitford[35] no longer believed that it was correct. It was not until 1968 that Oke and Sandage[36] put this result to rest. Unfortunately, for many years after the realization by many observers that the result was spurious, theoreticians were using it as direct evidence against the steady state theory.

Counts of Radio Sources

If any class of extragalactic object is distributed uniformly in space, counts to successively increasing metric distance, and hence to fainter apparent brightnesses, will give numbers which are proportional to the volumes searched. The volumes in Riemannian space depart from the volumes calculated on the Euclidean approximation by factors which are determined by the space curvature. Hubble[37] first attempted to count optical galaxies to apply this test but he abandoned the attempt, and more recently Sandage[22] has shown that, unless we go to much fainter galaxies than can be observed from the Earth, attempts to distinguish between different cosmological models will not succeed.

The method has, however, been applied extensively to extragalactic radio sources by several groups, who have counted sources at a number of frequencies down to different minimum flux levels. The early work by Ryle and his colleagues[38,39] gave a log N–log S slope for the brighter sources of -3, far steeper than the Euclidean value of -1.5. (If N is the number of sources per steradian brighter than S, and R is the distance to a source, then $N \propto V \propto R^3$, $S \propto R^{-2}$ so that in Euclidean space $N \propto S^{-3/2}$. For distant sources in Riemannian space, $N \propto S^{-3/2} + \mu(S)$ where $\mu(S) \geq 0$ is a function depending on the maximum source distance and the cosmological model.) Quite early on it became clear that the first log N–log S slope obtained in Cambridge was far too steep, and other surveys[40–42] all led to the conclusion that at the bright end of the curve the slope is steeper than -1.5, and has a value somewhere between -1.75 and -1.85. Following Ryle and his associates it has commonly been argued that this steep slope can only be explained as being due to an excess of sources at great distances. This requires evolution of the source numbers with epoch, and is then evidence against the simple steady state theory. Observations to fainter flux levels show that the slope of the log N–log S plot gradually flattens to a slope of only ~ -0.8 at $S \simeq 0.01$ flux units (1 f.u. $= 10^{-26}$ W m^{-2} Hz^{-1}). All of these results have been obtained by making surveys at comparatively low frequencies, typical values being 408 MHz and 178 MHz. The work which has been done at higher radio frequencies (2,700 MHz) leads to a log N–log S slope of -1.4 ± 0.1 (ref. 43). If this were the only radio survey no one would have argued that significant departures from a homogenous universe exist. But it has been argued that because of the wide dispersion in spectral indices of the radio sources and their variation with flux density, the slope of the log N–log S curve should decrease with decreasing frequency of observation. The lower frequency survey containing a more homogenous source sample would then be the one most representative of the gross features of the universe. This situation naturally leads to the following unsolved problem: at what observing frequency (radio, microwave, infrared, optical, X-ray) is an unbiased sample most likely to be selected? It would really be preferable to integrate the flux over a suitably large bandwidth and then construct a log N–log S_t curve.

How can we interpret the total slope for the log N–log S curve for sources measured at low frequencies? Ryle and his associates[44,45] have argued that the data indicate that the universe must be evolving and that there was an excess of sources in an epoch defined by $z \lesssim 2$, but that at even earlier epochs the source density was much lower. The alternative proposal is that either the slope for the bright sources does not depart significantly from the Euclidean value or that its steepness is due to a statistical fluctuation in the number of very bright sources, requiring a deficit of from 5–7 sources per steradian. Hoyle[46] has argued that the remainder of the curve is directly compatible with the prediction of the steady state model. Ryle and his associates disagree with this, and argue that the whole curve can only be explained by using Friedmann models with source evolution[44,45]. The arguments depend on the details of the radio luminosity function and the exact slope of the log N–log S curve for faint sources.

Volume Effect or Luminosity Evolution?

We have optical identifications and redshifts for only a very small fraction of the extragalactic radio sources. But if we restrict ourselves to the 3C (revised) catalogue[47] containing about 250 of the brightest radio sources ($S \geq 9$ f.u.) a total of about 40 per cent of the sources including about forty quasi-stellar objects and sixty-eight galaxies have been identified and redshifts have been obtained. The identification of the QSOs seems to be complete, so the remainder of the sources are thought to be associated with galaxies for which identifications have been made but for which no redshifts have been obtained, or, in a minority of cases, for which there are no optical objects visible on the Palomar Sky Atlas at the radio positions. Plots of radio flux S against redshift have been made both for the QSOs and the galaxies[48–51] and in each case it has been found that there is no correlation between the apparent flux and the redshift. Thus it seems that for the identified sources the shapes of the log N–log S curves are attributable to a luminosity effect and not to a distance–volume effect. The key to the interpretation of the log N–log S curve for the 3C sources lies in the remaining radio galaxies. If they turn out to have systematically larger redshifts than those already measured, as has been generally assumed because they are optically fainter, then their log N–log S curve is of cosmological significance. If, however, they do not have systematically greater redshifts it must be concluded that the log N–log S curve is dominated by the effects of the radio luminosity function in a comparatively local region. The QSOs do have large redshifts. If these are of cosmological origin then their log N–log S curve and the luminosity volume test[52] do demonstrate a powerful evolutionary effect. But if the redshifts are not of cosmological origin these analyses give us no evidence of cosmological importance.

A more detailed discussion of the analyses of the counts of sources has been given elsewhere. The limitations set by the source counts on various cosmological models are summarized in Table 1 which is taken from the work of Brecher, Burbidge and Strittmatter[79]. This shows that contrary to popular opinion the counts of radio sources do not rule out a steady state universe.

Quasi-stellar Objects

The QSOs are the only extragalactic objects so far discovered which have very large redshifts.[1] Therefore they are of the greatest importance for cosmology provided that their redshifts are largely due to the expansion of the universe and do not have any other explanation. If the redshifts are of cosmological origin then the luminosity–volume test previously mentioned for the QSOs in the 3CR catalogue, or a similar analysis also carried out by Schmidt[53] for a sample of QSOs which are not powerful radio sources, leads to the conclusion that they are not distributed uniformly in space, but that the space density is very strongly dependent on z and hence on epoch. This means that they provide powerful evidence against a simple steady state universe, and if one wishes to preserve the steady state model it must be argued that it is a fluctuating steady state universe in which evolution can occur within an epoch corresponding to redshifts up to ~ 2. In any case the cut-off of redshifts beyond 2 is of great cosmological significance and some have attempted to relate this to the appropriate epoch in a universe of Lemaitre type[54,55].

None of these arguments has any validity unless it can be demonstrated conclusively that the redshifts of the QSOs are of cosmological origin, and this has simply not been achieved. There are a number of arguments that can be adduced for and against the cosmological hypothesis but there is no certain answer. Until this is reached, use of QSOs for cosmological investigation is premature.

Table 1 Limitations Set by Source Counts on Various Cosmological Models

Source	Strict steady state	Fluctuating steady state	Friedmann	Friedmann with source evolution
QSOs				
$z \rightarrow$Distance	Excluded	Allowed *	Excluded	Allowed †
$z \nleftrightarrow$Distance	Allowed	Allowed	Allowed	Allowed
Radio galaxies				
z Known $z \rightarrow$Distance	Allowed	Allowed	Allowed	Allowed
z Unknown $m_v \rightarrow$Distance	Excluded	Allowed‡	Excluded	Allowed ‡
z Unknown $m_v \nleftrightarrow$Distance	Excluded§	Allowed	Excluded§	Allowed

* Only if fluctuation to $z \sim 2$ is permitted.

† Background limits evolution to $z_{\mathrm{QSO}}^{\max}$ where $2 < z_{\mathrm{QSO}}^{\max} < 4$.

‡ Background limits evolution to $z_{\mathrm{RG}}^{\max} \lesssim 0.6$.

§ This case would require a special rather implausible luminosity function to avoid exclusion.

Microwave Background Radiation

As mentioned above, the discovery of microwave background radiation, following the prediction that primaeval fireball radiation generated in a big bang would still be present, was one of the strongest pieces of evidence for such a beginning. Is the information that we now have on the microwave background still compatible with this interpretation? Observations made at wavelengths between a few millimetres and about 20 cm fit quite well on a black-body curve with a temperature of about 2.7 K. The energy density of the radiation is then about 4×10^{-13} erg cm^{-3}. If the black-body form is confirmed, this will be the strongest evidence in favour of an evolving universe. But it is not yet certain whether or not the spectrum is really of black-body form, because the wavelength range directly observed, and not in dispute, is the Rayleigh–Jeans part of the curve where $I(\nu) \propto \nu^2$.

The observations which have been made either directly or indirectly close to and just beyond the peak of the black-body curve are in conflict unless it is argued that some very strong line radiations are present. The indirect observation of the radiation field in the galactic plane which is exciting the CN, CH and CH$^+$ interstellar molecules suggests that the temperature is less than ~ 8 K at 9.36 mm, less than 5 K at 0.56 mm, less than ~ 4 K at 1.3 mm, and it appears to be about 2.8 K at 2.64 mm. Direct observation from rockets and a balloon above the Earth's atmosphere at wavelengths in the range 0.4–1.3 mm gives a flux which corresponds to a black-body temperature of about 6 K (refs. 56–58). It is possible that the radiation detected by these observers is concentrated in a line which could be produced in a geocorona, in the Galaxy, or have a cosmological origin. The intepretation of the results is unclear at present.

If it turns out that the microwave radiation field departs strongly from the black-body form, then either extra components are present, or the radiation has a quite different origin. In both cases the answer must be that the excess radiation, or the total flux, ultimately arises in discrete sources. A number of investigations have been made in which these possibilities have been explored. The sources are most likely to be the nuclei of galaxies which radiate largely at infrared and microwave frequencies.

It was the discovery of the microwave background radiation that led to a large number of theoretical investigations concerned with the early history of the universe. What should be made clear, however, is that the existence of this radiation is very powerful evidence for an initially dense state, provided that the radiation has a black-body spectrum as is predicted in the simple theory. If it turns out that it departs strongly from the black-body shape, many attempts will undoubtedly be made to explain it in terms of a primordial radiation field plus large contributions from discrete sources, or even a more complex big bang. But this must not obscure the important point that the absence of a black-body form would mean that the prediction has not been fulfilled and the strong and direct evidence for an initial dense state will have disappeared.

This concludes my discussion of direct observational evidence bearing on whether or not the universe is evolving and began in a dense state. I believe that if one attempts to evaluate this evidence objectively there is still no really conclusive evidence in favour of such a universe.

Nucleosynthesis and Origin of Galaxies

Another approach can be taken in looking for evidence which bears on the question of whether or not the universe began in a dense state. The bulk of the mass that we see is condensed in the form of stars and galaxies with a wide range of masses and angular momenta. This matter is not distributed uniformly in space but is condensed into dense aggregates. Moreover, it has a complicated chemical composition. The major problems of cosmogony are therefore to understand the origin of the elements, and the formation of discrete objects—galaxies and other compact massive objects.

Does the existence of these features of the universe suggest that their origin is closely connected with a big bang?

I shall consider first the problem of nucleosynthesis. Early work on this problem made little progress because knowledge of nuclear physics was still comparatively primitive. It was Gamow, Alpher and Herman[59–63] who first made a serious attempt to explain the synthesis of the heavier elements from an initial dense cloud of baryons, leptons and radiation in a hot big bang. It is well known that this attempt failed due to the difficulty associated with building beyond mass 4. In the 1950s a serious attempt was made to understand the relative abundances of all of the isotopes on the assumption that they have nearly all been synthesized in the interior of stars[64]. This theory has been, in large part, highly successful and is generally accepted. It was originally thought that the fact that heavy elements are synthesized from lighter ones in stars meant that there is a slow build-up of heavy elements as a galaxy grows older, and that the relative abundance would be correlated with the ages of the stars. It is now clear that while this effect is operating, a large amount of nucleosynthesis takes place early in the life of a galaxy and the processes going on steadily throughout its life are in some ways of lesser importance.

From the point of view of cosmology the most important result is that very detailed calculations made by Wagoner, Fowler and Hoyle[65] have demonstrated conclusively that the bulk of the elements cannot have been made in a big bang. The only important element, from the point of view of nuclear physics, that could have been synthesized in a big bang is helium. Thus soon after the discovery of the microwave background radiation there was a serious attempt to argue that the helium that is detected in stars and gaseous nebulae in our own galaxy and in other galaxies is primordial helium made in a big bang. The argument appeared plausible because, on the one hand, it was suggested that the helium/hydrogen ratio is the same in different celestial objects (stars, gaseous nebulae in our own galaxy, and a few gaseous nebulae in one or two nearby galaxies) and is 25–30 per cent by mass, while, on the other hand, the fractional amount of helium relative to hydrogen that is made in a big bang is close to about 27 per cent. This simple argument broke down, however, as soon as detailed studies of different aspects of the problem were made. First of all, it was pointed out that there appear to be stars which contain very little helium so that perhaps there is no universal helium abundance. While this argument is still open, detailed studies of big bang models led to the conclusion that there are ways of making very little helium in such an initial process[66–68]. Fowler has recently emphasized that the helium–hydrogen ratio is determined completely by the choice of baryon and lepton numbers in a big bang[69], which has nothing to do with the theory of general relativity, but is tied only to initial conditions which lie outside the known laws of physics. Thus, while the helium abundance in the universe might be related to the occurrence of a big bang, there is no direct evidence that the two are related, and there are a number of alternative ways of understanding the large helium abundance found in some stars and galaxies. For example, it appears that helium could have been synthesized in massive objects evolving in the nuclear regions of galaxies.

Finally, we come to the vexed question of the origin of galaxies. As the big bang bandwagon has gained momentum, an increasing number of investigations have been carried out in which attempts are made to explain the condensation of dense objects from an initial cloud of matter and radiation which is expanding. It has been known for many years that this is very difficult to understand[70–72], and the investigations have now reached the point where it is generally accepted that the existence of dense objects cannot be understood unless very large density fluctuations in a highly turbulent medium, or otherwise, are invoked in the first place[73–75]. There is again no physical understanding of the situation; it is a condition which is put in, in a hypothetical state, to explain a major property of the universe. Thus these "theories" amount to nothing more than the statement that protogalaxies have a cosmological origin, and their origin cannot be understood any better than can the original baryons and leptons in an evolving universe. At this level therefore these theories are in no better shape than the apparently much more radical views of Ambartsumian[76,77] or Hoyle and Narlikar[78] who believe that the origin of galaxies is tied closely to the properties of the dense active nuclei and that perhaps the initial states of galaxies were high density states. Hoyle and Narlikar have even dared to suggest that perhaps "new" physics is involved. What the debate largely reduces to is that on one side there is an apparent belief by the majority that creation in the distant past is acceptable, but that creation at recent epochs is unthinkable, while on the other it is thought that there is little to choose between the two points of view, and that only with much more original work can we hope to resolve the issue. It is clear that the evolving universe concept gains no support from attempts to understand either the origin of the elements or the origin of galaxies.

Was there really a big bang? I believe that the answer clearly must be that we do not know, and that if we are ever to find an answer much more effort must be devoted to cosmological tests, with a much more openminded approach, and that much more original thinking must be done to attempt to explain the large amount of observational material, and not only that material that can be used in a narrow sense to fit preconceived ideas. Probably the best argument in favour of a beginning is the general result that the ages of many stars in our galaxy are approximately equal to H^{-1}. Probably the strongest argument against a big bang is that when we come to the universe in total and the large number of complex condensed objects in it, the theory is able to explain so little.

[1] North, J. D., *The Measure of the Universe* (Clarendon Press, Oxford, 1965).
[2] Dingle, H., *Observatory*, **73**, 42 (1953).
[3] Novikov, I. D., and Zeldovich, Y. B., *Ann. Rev. Astron. Astrophys.*, **5**, 627 (1967).
[4] Lemaitre, G., *Ann. Soc. Sci. Bruxelles*, **47**, A49 (1927).
[5] Lemaitre, G., *Mon. Not. Roy. Astron. Soc.*, **91**, 483 (1931).
[6] Friedmann, A., *Z. Physik.*, **10**, 377 (1922).
[7] Humason, M. L., *Proc. US Nat. Acad. Sci.*, **15**, 167 (1929).
[8] Hubble, E. P., *Proc. US Nat. Acad. Sci.*, **15**, 168 (1929).
[9] Bondi, H., and Gold, T., *Mon. Not. Roy. Astron. Soc.*, **108**, 252 (1948).
[10] Hoyle, F., *Mon Not. Roy. Astron. Soc.*, **108**, 372 (1948).
[11] Peach, J. V., *Intern. Astron. Union Symp.*, No. 44, Uppsala (1970).
[12] Sandage, A. R., *Mayall Symp.*, Tucson, May (1971).
[13] Iben, I., and Faulkner, J., *Astrophys. J.*, **153**, 101 (1968).
[14] Iben, I., and Rood, R., *Astrophys. J.*, **161**, 587 (1970).
[15] Demarque, P., Mengel, J. G., and Aizenman, M., *Astrophys. J.*, **163**, 37 (1971).
[16] Fowler, W. A., *Rutherford Symposium Volume* (1968).
[17] Alpher, R. A., and Herman, R., *Phys. Rev.*, **75**, 1089 (1949).
[18] Gamow, G., *Kgl. Danske Videnskab Selskab Mat. Fys. Medd.*, **27**, No. 10 (1953).
[19] Penzias, A., and Wilson, R., *Astrophys. J.*, **142**, 419 (1965).
[20] Humason, M. L., Mayall, N. U., and Sandage, A. R., *Astron. J.*, **61**, 97 (1956).
[21] Sandage, A. R., *Astrophys. J.*, **127**, 513 (1958).
[22] Sandage, A. R., *Astrophys. J.*, **133**, 355 (1961).
[23] Sandage, A. R., *Proc. Course 35, International School of Physics Enrico Fermi*, 10 (Academic Press, New York, 1966).
[24] Sandage, A. R., *Observatory*, **92**, 91 (1968).
[25] Sandage, A. R., *Phys. Today*, **23**, 34 (1970).
[26] Peach, J. V., *Astrophys. J.*, **159**, 753 (1970).
[27] Baum, W. A., *Astron. J.*, **62**, 6 (1957); Baum, W. A., *Intern. Astron. Union Symp.*, No. 15 (edit. by McVittie, G. C.), 390 (Macmillan, New York, 1961).
[28] Minkowski, R., *Astrophys. J.*, **132**, 908 (1960).
[29] Burbidge, G. R., *Ann. Rev. Astron. Astrophys.*, **8**, 369 (1970).
[30] Sandage, A. R., *Pont. Acad. Scient. Scripta. Varia. 35, Nuclei of Galaxies*, Vatican, 601, (1971).
[31] Kristian, J., and Sandage, A. R., *Astrophys. J.*, **162**, 391 (1970).
[32] Stebbins, J., and Whitford, A. E., *Astrophys. J.*, **108**, 413 (1948).
[33] Bondi, H., Gold, T., and Sciama, D., *Astrophys. J.*, **120**, 597 (1954).
[34] Whitford, A. E., *Astrophys. J.*, **120**, 599 (1954).
[35] Whitford, A. E., *Astron. J.*, **61**, 353 (1956).
[36] Oke, J. B., and Sandage, A. R., *Astrophys. J.*, **154**, 21 (1968).
[37] Hubble, E., *The Realm of the Nebulae* (Oxford University Press, 1936).
[38] Ryle, M., *Observatory*, **75**, 137 (1955).
[39] Ryle, M., and Scheuer, P. A. G., *Proc. Roy. Soc.*, A, **230**, 448 (1955).
[40] Mills, B. Y., *Paris Symp. on Radio Astronomy* (edit. by Bracewell, R. N.) (Stanford, 1959).
[41] Day, G. A., Shimmins, A. T., Ekers, R. J., and Cole, D. J., *Austral. J. Phys.*, **9**, 35 (1966).
[42] Ryle, M., and Scott, P. F., *Mon. Not. Roy. Astron. Soc.*, **122**, 389 (1961).
[43] Shimmins, A. T., Bolton, J., and Wall, J. V., *Nature*, **217**, 818 (1968).
[44] Ryle, M., *Ann. Rev. Astron. Astrophys.*, **6**, 249 (1968).
[45] Longair, M., *Mon. Not. Roy. Astron. Soc.*, **133**, 421 (1966).
[46] Hoyle, F., *Proc. Roy. Soc.*, A, **308**, 1 (1969).
[47] Bennett, A. S., *Mem. Roy. Astron. Soc.*, **68**, 163 (1962).
[48] Hoyle, F., and Burbidge, G. R., *Nature*, **210**, 1346 (1966).
[49] Longair, M., and Scheuer, P. A. G., *Nature*, **215**, 919 (1967).
[50] Hoyle, F., and Burbidge, G. R., *Nature*, **227**, 359 (1970).
[51] Bolton, J. G., *Astron. J.*, **74**, 131 (1969).
[52] Schmidt, M., *Astrophys. J.*, **151**, 393 (1968).
[53] Schmidt, M., *Astron. J.*, **162**, 371 (1970).
[54] Petrosian, V., Salpeter, E. E., and Szekeres, P., *Astrophys. J.*, **147**, 1222 (1967).
[55] Shklovsky, I. S., *Astrophys. J. Lett.*, **150**, L1 (1967).
[56] Shivanandan, K., Houck, J. R., and Harwit, M., *Phys. Rev. Lett.*, **21**, 1460 (1968).
[57] Houck, J. R., and Harwit, M., *Astrophys. J.*, **157**, L45 (1969).
[58] Muelhner, D., and Weiss, R., *Phys. Rev. Lett.*, **24**, 742 (1970).
[59] Gamow, G., *Phys. Rev.*, **70**, 572 (1946).
[60] Gamow, G., *Phys. Rev.*, **74**, 505 (1948).
[61] Alpher, R. A., *Phys. Rev.*, **74**, 1577 (1948).
[62] Alpher, R. A., and Herman, R. C., *Phys. Rev.*, **74**, 1737 (1948).
[63] Alpher, R. A., and Herman, R. C., *Rev. Mod. Phys.*, **22**, 153 (1950).
[64] Burbidge, E. M., Burbidge, G. R., Fowler, W. A., and Hoyle, F., *Rev. Mod. Phys.*, **29**, 547 (1957).
[65] Wagoner, R., Fowler, W. A., and Hoyle, F., *Astrophys. J.*, **148**, 3 (1967).
[66] Hawking, S. W., and Tayler, R. J., *Nature*, **209**, 1278 (1966).
[67] Thorne, K., *Astrophys. J.*, **148**, 51 (1967).
[68] Dicke, R., *Astrophys. J.*, **152**, 1 (1968).
[69] Fowler, W. A., *Comments on Astrophys. and Space Phys.*, **2**, 134 (1970).
[70] Lifshitz, E., *J. Phys.* (USSR), **10**, 116 (1946).
[71] Bonnor, W. B., *Z. Astrophys.*, **39**, 143 (1956).
[72] Lifshitz, E., and Khalatnikov, I. M., *Adv. Phys.*, **12**, 185 (1963).
[73] Harrison, E. R., *Mon. Not. Roy. Astron. Soc.*, **148**, 119 (1970).
[74] Ozernoy, L. M., and Chernin, A. D., *Astron. Zh.*, **44**, 1131 (*Sov. Astron.*, **11**, 907) (1967).
[75] Ozernoy, L. M., and Chernin, A. D., *Astron. Zh.*, **45**, 1137 (*Sov. Astron.*, **12**, 901) (1968).
[76] Ambartsumian, V. A., *Solvay Conf. on Structure and Evolution of the Universe*, Brussels (edit. by Stoops, R.), 241 (Univ. of Brussels, 1958).
[77] Ambartsumian, V. A., *Structure and Evolution of Galaxies, Proc. Thirteenth Conf. Phys.*, Univ. Brussels, 1 (Wiley, Interscience, 1965).
[78] Hoyle, F., and Narlikar, J. V., *Proc. Roy. Soc.*, A, **290**, 177 (1966).
[79] Brecher, K., Burbidge, G., and Strittmatter, P., *Comments Astrophys and Space Physics* (in the press).

On the Nature of Mass

F. HOYLE & J. V. NARLIKAR
Institute of Theoretical Astronomy, University of Cambridge

The increasing number of observations of discrepant redshifts means that no longer can these be passed off as chance juxtapositions. A possible explanation of the data is given here in terms of a theory that incorporates a gravitational "constant" that is decreasing with time.

EVIDENCE concerning "discrepant redshifts" has accumulated fairly rapidly in recent months. The case where it is hardest to deny the evidence was reported at the beginning of the present year by Arp[1]. The galaxy NGC 7603 was found to be connected to an appendage by two arms which intersect at the appendage $z=0.056$. In the customary Doppler interpretation of the redshift this difference exceeds 8,000 km s^{-1}. A redshift difference of the same order was found many years ago[2] by Minkowski, Humason and Zwicky in "Zwicky's triple system". The galaxies in this system are also connected by a bridge.

If it is accepted that a discrepancy of $\sim$8,000 km s^{-1} exists for NGC 7603, then there seems no good reason for denying the two cases with discrepancies $\sim$20,000 km s^{-1} obtained by Sargent (ref. 3, and W. L. W. Sargent's contribution to the Vatican Conference, 1970). Both the latter cases are compact groups of galaxies in which one member has the discrepant redshift. Less than ten such groups have been examined. The probability of a more distant galaxy being projected against a nearer cluster by chance is small. Arp (private communication) has obtained what appears to be a jet connecting the quasi-stellar object Makarian 205, $z \simeq 0.07$, to the galaxy NGC 4319. The discrepancy is again of order 20,000 km s^{-1}.

Three cases have been observed of a quasi-stellar object apparently associated with a small compact cluster of faint galaxies. In one case Gunn has reported[4] the same redshift for the QSO as for one of the galaxies of the cluster. In the other two cases there appear to be large redshift differences (Hazard, Jauncey, Sargent and Gunn, private communication).

In a recent preprint Burbidge, Burbidge, Solomon and Strittmatter have drawn attention to a bridge which appears on the Palomar sky prints to connect the quasi-stellar object PHL 1226 to the bright galaxy IC 1746. The redshift of PGL 1226 is $z=0.404$, whereas that of the galaxy has not yet been measured, but must be small because of its brightness.

Because all objects are projected on the sky there must be some apparently peculiar juxtapositions of objects that are really at very different distances. This has hitherto led to a situation in which all but a very few astronomers have dismissed apparent redshift discrepancies as simply unusual projection effects. Yet there has to be a point of balance in one's judgment as to the extent of the chance juxtapositions one is willing to accept. One of us was aware at an early stage of the case of Zwicky's system (Zwicky, private communication) but it seemed that, in spite of the bridge, there might be a very peculiar chance juxtaposition. But the case of VV 172[3] formed the point of balance and NGC 7603 has turned the balance.

If a highly convincing theory of discrepant redshifts were available then we think there is little doubt that the data would today be considered reasonably clear-cut. It has been the absence of such a theory that has caused most astronomers to prefer to believe in unusual projection effects. If, as seems very possible, the accumulation of data forces us over a watershed (not only in our thinking but in the history of astronomy) it will clearly become necessary to arrive at a theory of discrepant redshifts. We wish to emphasize the need for a thoroughly radical assessment of the problem, considering it unlikely that a satisfactory theory will be achieved by a small change in our concepts. Explicitly, we do not think discrepant redshifts will be explained adequately either as simple Doppler peculiar motions or as excess reddening due to gravitation.

In the following discussion we shall present the usual Friedmann cosmological models and the Einstein de Sitter case in particular, from an unusual point of view. Mathematically, this point of view is at first entirely equivalent to the usual formulation. Then at a later stage we shall arrive at a possible major shift from the usual theory. The balance between the usual theory and an entirely different view both of physics and astrophysics hinges on a question raised by Dirac[5] in 1937.

It is well known that when c and $\hbar$ are set equal to unity, only a single dimensionality is needed for the whole of physics. We take this to be length and we denote the unit by L. Every quantity has a dimensional form L^n, for example, mass $\sim L^{-1}$, frequency $\sim L^{-1}$, charge $\sim L^0$, magnetic field $\sim L^{-2}$ and the gravitational 'constant' has dimensionality L^2.

Every observation is concerned with a dimensionless number, so that every observation is concerned with a product of quantities such that the dimensional dependencies on L cancel to zero. So far as experimental physics and engineering are concerned it is possible to convert a quantity of dimensionality L^n into a quantity of dimensionality L^m by means of a linear device provided $n=m$. Non-linear devices are needed if $n \neq m$. This property makes it comparatively easy to see what kind of physical device is needed to relate one quantity to another. There is no doubt that physics and engineering are made quite unnecessarily complicated by the current practice of using multiunit systems.

Because observed quantities are dimensionless they are unaffected by a scale change in the length unit. But what if we elect to change the length unit in accordance with a well behaved function $\Omega(x)$ (no zeros of Ω as well as no infinities) which varies with the position of the space-time point X? Nothing should be changed provided that first, we always combine physical quantities at the same X, and, second, every physical quantity is scaled according to its dimensionality—a quantity of dimensionality L^n being scaled by Ω^n.

Physical theories with these properties are said to be conformally invariant. This is a different kind of invariance from the coordinate invariance of relativity. Not all physical theories, however, are conformally invariant. Maxwell's theory is conformally invariant. The derivative terms of Dirac's equation are conformally invariant, but the mass term is not. Einstein's theory is not conformally invariant and physical theories cease to be conformally invariant whenever "mass" is involved. This is because the second property is then not satisfied. Clearly, we cannot expect classical dynamics to be conformally invariant unless the classical action

$$S = -\int m\,\mathrm{d}a \qquad (1)$$

of particle a is invariant. Since the element $\mathrm{d}a$ of the path of the particle becomes multiplied by Ω we would require the mass m to be multiplied by Ω^{-1}, which indeed would accord with the dimensionality of mass, $\sim L^{-1}$. But Ω can now be different at different space–time points, so that in general the mass of the particle will not be the same for all points on its path. Plainly then, we cannot expect to arrive at a conformally invariant system of dynamics so long as the mass of a particle is considered a fixed quantity belonging autonomously to the particle.

We have been concerned[6,7] since 1964 in developing a conformally invariant theory of dynamics and of gravitation. The central idea of this work is that the mass of a particle m say, is given by

$$m(X) = \lambda\, M(X) \qquad (2)$$

where λ is a coupling constant belonging to the particle itself and $M(X)$ is a "mass field" generated by all the particles in the universe. In a very general way we can write

$$M(X) = \sum_b \int P(X, B)\,\mathrm{d}b \qquad (3)$$

the summation being over all particles b, the line integral being over the world line of b, and $P(X, B)$ being a propagation function from point B at $\mathrm{d}b$ to the field point X. To avoid self-action problems in the classical theory it is convenient to add the refinement that in determining the mass of any particular particle a this particle is omitted in the summation with respect to b. A comparable situation occurs in classical electrodynamics.

Mathematically we require that when all lengths on the right hand side of (3) are changed by the function Ω the resulting mass field is multiplied by $\Omega^{-1}(X)$. This requirement is met by putting

$$P(X, B) = \lambda\, \tilde{G}(X, B) \qquad (4)$$

where $\tilde{G}(X, B)$ is the time-symmetric elementary solution of

$$\Box_x \tilde{G}(X, B) + 1/6\, R(X)\tilde{G}(X, B) = [-g(B)]^{-\frac{1}{2}}\, \delta_4(X, B) \qquad (5)$$

in which $\Box_x$ is the d'Alembertian operator, R the scalar Riemannian curvature and δ_4 the 4-dimensional Dirac delta-function. So far as we are aware the wave-equation (5) was first considered with respect to its conformal properties by Penrose.

The action of particle a is now

$$-\lambda^2 \int \mathrm{d}a \,.\, \sum_{b \neq a} \int \tilde{G}(A, B)\,\mathrm{d}b \qquad (6)$$

and the total action of all particles is given by

$$S = -\lambda^2 \sum_{a \neq b}\sum \iint \tilde{G}(A, B)\,\mathrm{d}a\,\mathrm{d}b \qquad (7)$$

Subtleties arise for a universe with infinitely many particles concerning the finiteness of (7), but the situation in this respect is not really different from the usual theory.

Enough has already been done to make the Dirac equation conformally invariant, including the mass term. To obtain conformally invariant gravitational equations we work with the usual condition that S be stationary for small changes of the metric tensor, $\delta S = 0$ for $g_{ik} \rightarrow g_{ik} + \delta g_{ik}$. The resulting equations are different and more complicated than those of Einstein. For cosmology however, and for all problems concerned with weak gravitational fields, the equations can be reduced to the Einstein form with an exceedingly high degree of approximation. The situation in regard to the classical tests of general relativity—the bending of light, the perihelion of Mercury—is therefore exactly the same as with Einstein. To effect the reduction to Einstein's equations one chooses the conformal frame so that $M(X)$ becomes independent of X. If, to begin with, $M(X)$ varies with X we simply choose $\Omega \propto M$. Particle masses are independent of X in this particular conformal frame, just as we normally take them to be.

The gravitational 'constant' comes out to be given by

$$G = \frac{3}{4\pi\, M^2} \qquad (8)$$

Again in the special conformal frame in which M is constant, G is indeed a constant. It is interesting that G is necessarily positive, so that weak gravitational fields are necessarily attractive.

It should be added that the gravitational equations do not reduce to the Einstein form in certain cases where the field is strong. The discussion of "black holes" is greatly changed, for example.

Because the gravitational theory is conformally invariant we can now effect a major simplification in discussing the isotropic homogeneous models usually employed in cosmology. These models are usually discussed with respect to the Robertson–Walker form of the line element

$$\mathrm{d}s^2 = \mathrm{d}\tau^2 - Q^2(\tau)\left[\frac{\mathrm{d}\rho^2}{1 - k\rho^2} + \rho^2\,(\mathrm{d}\theta^2 + \sin^2\theta\,\mathrm{d}\varphi^2)\right] \qquad (9)$$

Here τ is cosmic time and $Q(\tau)$ is the expansion function. The coordinates ρ, θ, φ are spherical polars for an observer at the origin. The constant k can be zero or ± 1. $k = 0$ gives the Einstein de Sitter model, $k = +1$ is closed, $k = -1$ hyperbolic. It is well known that a conformal transformation function Ω can be found that changes the line element (9) into flat Minkowski space.

It is not usually possible to take advantage of this geometrical simplification because the physical theory is not conformally invariant. Here, however, the physics is conformally invariant and we can transform to Minkowski space. When we have done so the theory is as good as it was in the Robertson–Walker representation. It turns out that while the $k = 0$ case is still homogeneous in the Minkowski space

$$\mathrm{d}t^2 - \mathrm{d}r^2 - r^2\,(\mathrm{d}\theta^2 + \sin^2\theta\,\mathrm{d}\varphi^2) \qquad (10)$$

the cases $k = \pm 1$ are simply localized clouds of no special interest.

The full Minkowski space, $t < 0$ as well as $t > 0$, can be used. There is, however, nothing corresponding to both halves of Minkowski space in the Robertson–Walker representation. The reason for this emerges when we make the inverse transformation from (10) to (9). The inverse transformation has a singularity at $t = 0$, and this introduces a singularity at $\tau = 0$ in (9). This singularity, which now emerges as a mathematical construct, is usually interpreted as the 'origin' of the universe. There need be no 'origin' in the Minkowski representation.

Taking the particle masses to be constant in the Robertson–Walker frame, and applying Ω^{-1} to the mass, Ω being chosen for transformation from (9) with $k = 0$ to (10), we obtain

$$m = (\text{constant}).\, t^2 \qquad (11)$$

The particle masses are a quadratic function of cosmic time t in the Minkowski representation. Even though space is now flat this leads to a redshift effect. Remembering that atomic sources emit frequencies proportional to m, we see that such a source at distance r from an observer at $r=0$ would be judged to have a redshift given by

$$1 + z = \left(\frac{t}{t - r}\right)^2 \tag{12}$$

the time of observation being t and the time of emission $t-r$.

A standard galaxy may be considered to have a specified number of stars each containing a specified number of particles arranged in the same way with respect to chemical composition. The luminosity of a star has dimensionality L^{-2}. This is proportional to m^2, and therefore by (11) proportional to the fourth power of the cosmic time. The bolometric flux from such a standard galaxy observed at time t is therefore

$$\frac{L(t-r)}{4\pi r^2} \propto \frac{(t-r)^4}{r^2} \tag{13}$$

where $L(t-r)$ is the luminosity of the galaxy at the time $t-r$ of emission of the observed radiation. Eliminating r from (13) with the aid of (12) we find that the bolometric flux depends on z according to

$$\frac{1}{1+z} \cdot \frac{1}{[\sqrt{1+z} - 1]^2} \tag{14}$$

which is the classic Hubble redshift-magnitude relation for the Einstein de Sitter model. The reader will note how much more easily we have arrived at (14) than in the usual treatment.

The present treatment is still deficient in one important respect. The form of equation (11) for the particle mass was obtained for the Minkowski representation by assuming constant mass in the Robertson–Walker representation. To make the argument complete it is essential to show that (11) follows from an explicit evaluation of (2), (3), (4), (5). This evaluation turns out to involve interesting problems which will not be considered here, since details have been given elsewhere[8]. It is convenient to choose the length unit L so that the particle density is everywhere unity. We then obtain from (2), (3), (4), (5),

$$m = \tfrac{1}{2}\lambda^2 t^2 L^{-3} \tag{15}$$

which has the required quadratic dependence on t.

Expanding (12) for $r/t \ll 1$ we obtain

$$z \simeq 2r/t \tag{16}$$

Since the Hubble constant H is defined by $z = Hr$ for small r, we have

$$t = 2H^{-1} \tag{17}$$

for the present epoch. Empirically $H^{-1} \simeq 2 \times 10^{28}$ cm. Empirically also, if we consider the particles to be hydrogen atoms, the average particle density n is given by

$$n = L^{-3} \simeq 2.5 \times 10^{-6}\ \text{cm}^{-3} \tag{18}$$

From (17), (18), and $H^{-1} = 2 \times 10^{28}$ cm,

$$nt^3 \simeq 10^{80} \tag{19}$$

This cosmological number is dimensionless, and is the number of particles contributing to the mass field $M(X)$ at the present epoch.

The dimensionless number λ^2 has no effect on gravitation, since it multiplies the action (7), and therefore disappears entirely from $\delta S = 0$, $g_{ik} \to g_{ik} + \delta g_{ik}$. But the value of λ^2 has a profound effect on the behaviour of individual particles when electromagnetic terms are added to the action. The value of λ^2 can be obtained empirically in the following way. Applying (15) to protons,

$$m_p = \tfrac{1}{2}\lambda^2 t^2 L^{-3} = 2\lambda^2 H^{-2} L^{-3} \tag{20}$$

for the present epoch. The empirical values (18) for L^{-3} and $H^{-1} \simeq 2 \times 10^{28}$ cm then lead to

$$m_p \simeq 2 \times 10^{51}\ \lambda^2\ \text{cm}^{-1} \tag{21}$$

The laboratory value of the Compton wavelength of the proton m_p^{-1} is $\sim 10^{-14}$ cm, so that empirically $m_p \simeq 10^{14}\ \text{cm}^{-1}$, and

$$\lambda^2 \simeq 5 \times 10^{-38} \tag{22}$$

is therefore required by atomicity.

The remarkable fact now emerges that

$$\lambda^2 (nt^3)^{\frac{1}{2}} = O(1) \tag{23}$$

On the left λ^2 apparently belongs autonomously to the particle, whereas $(nt^3)^{\frac{1}{2}}$ is a cosmological number. Apparently also λ^2 and n are independent of t, so that (23) would seem only a coincidence of the present epoch. This is the usual view.

At this point we reach our watershed. How if we require that (23) shall apply at all t? Then λ^2 or n, or both, must depend on t. This was the proposal of Dirac[5]. It is clear that (23) can be maintained by $n\lambda^4 \propto t^{-3}$, which allows many possible combinations of n, λ^2. Here we discuss a possibility which involves no change in the mass field $M(X)$. This possibility is similar in some respects to the case discussed by Dirac but is different in other respects. According to (2), (3), (4),

$$M(X) = \lambda \sum_b \int \tilde{G}(X, B)\, \mathrm{d}b \tag{24}$$

from which it is not hard to see that provided the product λn is independent of t there will be no change in $M(X)$. From

$$\lambda \propto n^{-1},\ n\lambda^4 \propto t^{-3} \tag{25}$$

we then get

$$\lambda \propto t^{-1},\ n \propto t \tag{26}$$

Moreover, the total action (7) is the same as before. It is as if more particles are being created by arranging that the masses of individual particles, $m = \lambda M$, shall increase linearly with t, instead of as t^2. In so far as we discuss the gravitational aspects of cosmology in terms of a smooth fluid, instead of in terms of discrete particles, nothing is changed. The interaction of each particle with the mass field M weakens as t^{-1}, but there are more particles because of $n \propto t$, and the total interaction of all particles remains the same.

An important by-product of these considerations is that the universe now becomes a perfect absorber in the sense of Wheeler and Feynman. The original Einstein de Sitter model did not meet the perfect absorber condition, which in our view is a fatal flaw of this model. The present model is not the steady state model, but by moving some way towards the steady state model, by permitting $n \propto t$, we have managed to meet an essential electrodynamic requirement.

It is possible to make a conformal transformation $\Omega \propto t$ from the Minkowski representation to a representation in which individual particle masses are constant, as we consider them to be in all terrestrial and astrophysical problems. The line

$$\mathrm{d}s^2 = \mathrm{d}T^2 - 2T[\mathrm{d}r^2 + r^2(\mathrm{d}\theta^2 + \sin^2\theta\, \mathrm{d}\varphi^2)] \tag{27}$$

which is the same as the line element of a radiation-dominated Friedmann model. The present model, however, is not radiation-dominated and it has a gravitational 'constant' that varies with time. In this representation the mass field M is proportional to $T^{\frac{1}{2}}$ and G, given by (8), is proportional to T^{-1}. Such a variation would have a profound effect on astrophysics and geophysics.

Stars would be much brighter at early T than we usually suppose them to be. There are advantages to be gained from such behaviour, but $G \propto T^{-1}$ may be too drastic[9], for example, in its effect on the past luminosity of the Sun. On the other hand, there are aspects of geophysics that seem as if they would be greatly helped by this kind of dependence. Steadily weakening gravity would gradually release the interior of the Earth from compression. It can be calculated[8] that the radius of the Earth would be required to increase at about 10 km per 10^8 yr. There is no possibility of this expansion being resisted by the crust, which must be cracked open repeatedly to make way for new surface material. At all times there would be an excess upward force on the crust at the limit of its strength. The possibility of large horizontal pressure differences, of order 10^9 dyne cm^{-2}, also exists, provided in particular regions that excess pressure is conveyed to the immediate subsurface by fluid material.

We are reminded in this connexion of the old controversy concerning continental drift. Our impression is that, while modern evidence shows unequivocally that drift actually takes place, the early calculations demonstrating the need for exceedingly large forces, really remain valid. If this is so, we would be inclined to think that some such behaviour of G as is given by our model becomes essential for an understanding of the geophysical evidence.

It may be added that Shapiro, Smith, Ash, Ingalls and Pettengill[10] have recently placed an observational upper limit of 4×10^{-10} yr^{-1} on $\dot{G}/G$. The variation expected here, with a Hubble constant of 5×10^{-11} yr^{-1}, would be 10^{-10} yr^{-1}.

We turn finally to the problem of how we should interpret $\lambda \propto t^{-1}$ in relation to the redshift problems discussed at the outset. The proportionalities $n \propto t$, $\lambda \propto t^{-1}$ maintain cosmological homogeneity. We must contemplate, however, that these proportionalities represent the smoothed-out effect of changes that could possess spatial irregularities. There could be spatially adjacent variations of λ. This we feel to be the kind of concept needed to come to grips with the problem of redshift anomalies. The smoothed-out homogeneous behaviour of the particle masses gives the normal cosmological redshift, obeying the usual Hubble relation. Local variations give anomalies. A more rapid decrease locally of λ is required in order that the anomalies be in the sense of an increased redshift. One can speculate that such variations occur as a consequence of the physical conditions in regions of strong gravitational fields. These are characterized by the condition that local particles make a contribution to the total mass field M that is comparable with the contribution of particles at cosmological distances. Such localities may be able to produce their own environmental conditions, leading to the local variations of λ.

Received July 27, 1971.

1 Arp, H. C., *Astrophys. Lett.*, **7**, 221 (1971).
2 Zwicky, F., *Naturwissenshaften*, **29**, 344 (1956).
3 Sargent, W. L. W., *Astrophys. J. Lett.*, **153**, L135 (1968).
4 Gunn, J., *Astrophys. J. Lett.*, **164**, L113 (1971).
5 Dirac, P. A. M., *Nature*, **139**, 323 (1937).
6 Hoyle, F., and Narlikar, J. V., *Proc. Roy. Soc.*, A, **294**, 138 (1966).
7 Hoyle, F., and Narlikar, J. V., *Ann. Phys.*, **62**, 44 (1971).
8 Hoyle, F., and Narlikar, J. V., *Mon. Not. Roy. Astron. Soc.* (in the press).
9 Pochoda, P., and Schwarzschild, M., *Astr. J.*, **139**, 587 (1964).
10 Shapiro, I. I., Smith, W. B., Ash, M. B., Ingalls, R. P., and Pettengill, G. H., *Phys. Rev. Lett.*, **26**, 27 (1971).

Origin of the Alps and Western Mediterranean

K. J. HSÜ

Geological Institute, ETH, Zürich

The origin of the Alpine geosyncline may have been related to the eastward motion of Africa relative to Europe during the Mesozoic, and orogenesis in western Europe could have been caused by a westward and then a northward movement of Africa.

IN contrast to mountains such as the Himalayas and the Andes, the Alpine chain snakes its way through Europe with numerous bends, convergences and divergences[1,2]. Compared with the Atlantic, the opposite shores of which fit together almost perfectly, the Mediterranean is bounded by three peninsulas on the north, which run at right angles to the trend of the sea. To make matters worse the Mediterranean Ridge has proved to be a compressional feature[3], quite distinct from the normally extensional mid-ocean ridges.

The complex state of the Mediterranean reflects its past history and conventional interpretations have related the origin of the Alps and the Mediterranean to the interaction between the European and African plates. In many of these interpretations a broad, east-west sea (the Tethys Sea) was placed between the two continents (Fig. 1), and the Alpine folding was postulated as a consequence of the northward march of Africa[4,5]. Such a broad new picture left many of the intricacies unexplained and the important geological fact that a deep oceanic seaway did not exist in western Europe (the Alps, Apennines, and Betic Cordilleras) before the Jurassic was ignored.

Geometrical Analysis

Staub[4] considered the winding Alpine trend as a trait inherited from the European Hercynian system and thereby evaded the issue. Argand[6], however, presented a brilliant geometrical analysis and invoked continental drift and rotation of microcontinents to account for the puzzling geography of the western Mediterranean. But his kinematical analyses were based on geological data alone and have been proved untenable by recent geophysical and oceanographical discoveries. This article is an attempt to modify Argand's scheme; if the drifting chronology is revised, the geometrical displacements postulated by Argand acquire a different geological significance.

Argand[6] postulated that Africa was pushed over Eurasia in the early Tertiary, and formed the Alpine and Carpathian mountains while Eurasia was still attached to North America. After the climax of the Alpine deformation in the Oligocene,

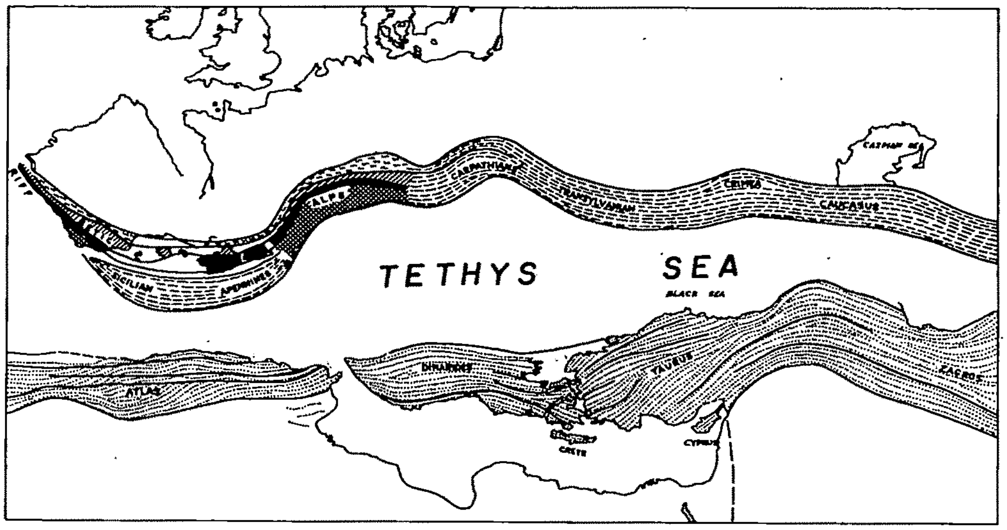


Fig. 1 A current interpretation of the Mesozoic palaeogeography of the Tethyan geosyncline (after ref. 5).

the northern margin of the African plate was bounded by the ophiolite zone of the Rif, the Betic Cordilleras, Corsica, Liguria, and the Pennine Alps in the west, and by the Carpathian Flysch in the north-east (Fig. 2). The Mediterranean was supposedly reduced into a network of epicontinental seas and Italy, Corsica, Sardinia, the Balearic Islands, and Spain were all crowded to one corner in South-Western Europe and joined to France and the stable Europe. He then postulated the start of an important phase of extension during the Late Oligocene or Early Miocene, when Africa moved south-eastward relative to Europe, inducing the rift of the Alboran Sea, causing the rotation of Spain, and creating thereby the Bay of Biscay. Meanwhile Corso-Sardinia and Italy somehow drifted counter-clockwise and left behind the Balearic and Tyrrhenian Basins. The collision of the Italian Peninsula and the Balkan block also accounted for the genesis of the Apennines.

Sphenochasm

Argand's scheme was slightly modified by Carey[5], who spoke of a Ligurian sphenochasm. The closing of such a sphenochasm should place the Corso-Sardinia Block next to the Gulf of Lyon but Carey put this block east rather than north of the Balearic Island. His reconstruction would make it almost impossible to correlate the Subbetic Zone of the Balearic Islands with the Pennine Zone of Corsica and Elba. Carey was also obliged to postulate an unwieldy set of shear motions, which could not easily be related to a rational driving mechanism. Such a scheme seems to me to be a backward step and does not represent any improvement.

But Argand's geometrical postulates do have considerable merit. The idea that the genesis of the Bay of Biscay was related to the rotation of Spain can be considered proved[7]. Several lines of evidence have supported the notion of the counter-clockwise rotations of Corso-Sardinia[8,9] and of Italy[10]. Most of Argand's chronological postulates have, however, been contradicted by new evidence; the opening of the Bay of Biscay, for example, could not have started as late as the Oligocene[11]. All the evidence indicates a Mesozoic date probably during the Late Jurassic and certainly not later than the Early Cretaceous[12,13]. There is likewise no compelling reason for assuming a Middle Tertiary rotation of Italy away from Corso-Sardinia.

The rotations postulated by Argand were supposedly caused by a displacement of the African plate south-eastwards relative to stable Europe, yet such a relative motion could not have taken place during the Late Tertiary when Europe was moving away from Greenland at such a rate that a north–south compression

Pure Che :stry Library
SCIENCE COLLEGE
92 Upper Circular Road
CALCUTTA

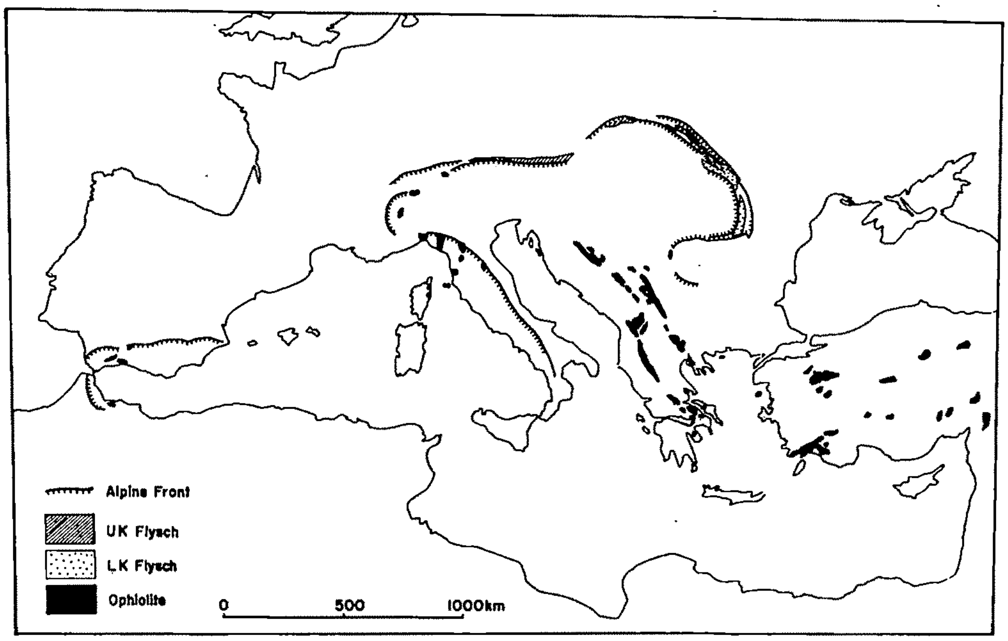


Fig. 2 Distribution of Mesozoic ophiolites and Flysch in Europe.

between Europe and Africa resulted[14]. (Pittman's important work is being completed in collaboration with Ryan, Dewey, and Bonin. Their kinematical analysis of the North Atlantic opening defined the succession of relative movements between Africa and Europe and thus paved the way for my attempt to revise Argand's chronology on the genesis of the western Mediterranean.) In fact this motion still seems to be continuing today[3].

Relative Motion

The magnetic anomaly patterns of the North Atlantic and the results of the JOIDES drilling there indicate that the history of the relative motion between Africa and Europe can be subdivided into three stages. These are (1) the Middle Jurassic to the Middle Cretaceous (165 to 80 m.y.) when the Middle Atlantic was being opened as Africa pulled away from North America[3,16]. The motion of Africa relative to Europe was eastward, because Europe was still joined to North America. (2) The Middle Cretaceous to the Late Eocene (80 to 40 m.y.) when Greenland and Europe were also being separated from North America[11]. The motion of Africa relative to Europe was then westward, as Europe was separating from North America at a greater rate[14]. (3) The Late Eocene to the Present (40 m.y. to now) when Europe was separating from Greenland. The motion of Africa relative to Europe was east and then northward.

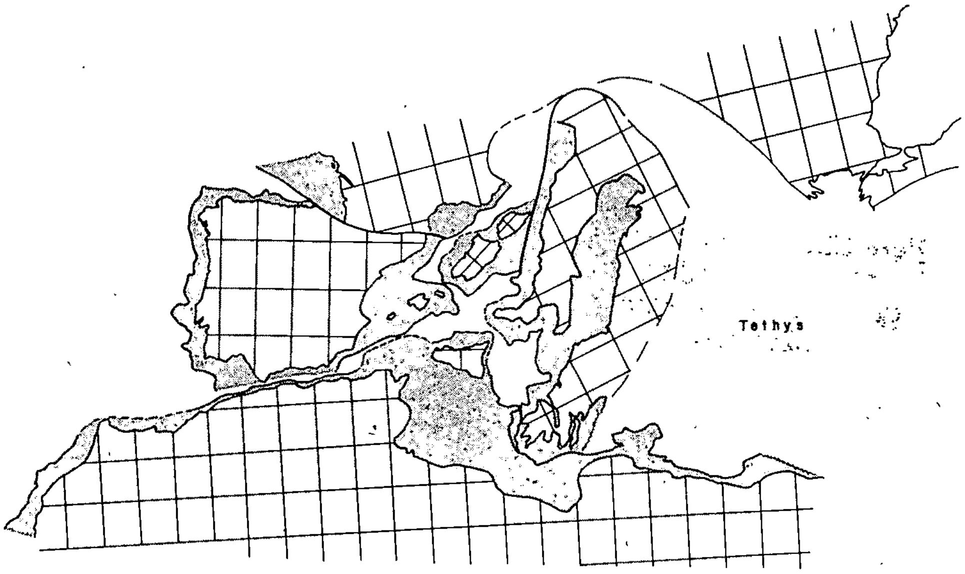


Fig. 3 Early Jurassic palaeogeography of Europe before the start of the motion of Africa eastward relative to stable Europe.

This kinematical analysis agrees well with the geological information. Stratigraphical evidence suggests unequivocally that western Europe was covered by a shelf-sea during the Triassic, when there was no oceanic seaway separating Spain from Corso-Sardinia, or Italy from stable Europe[15]. All those future microcontinents were still tucked in between Europe and Africa west of the open Tethyan Sea (Fig. 3). Thus the stage was set for Argand's postulated extensional phase in the Early Jurassic, not in the Late Oligocene[16]. Evidence of the start of crustal extension in the Jurassic is given by the first deposition of continental margin sediments in the Alps[15]. By the Middle Jurassic the oldest ophiolites of western Europe were emplaced and the microcontinents were split up, thus creating the Alpine, Apennine, Betic, and Pyrenees geosynclines (Fig. 4). The ophiolites in those deep-sea troughs were overlain by Upper Jurassic and Lower Cretaceous radiolarian and nannofossil oozes[15,17], forming the typical sequences of the Steinmann's Trinity[18].

One of the most significant drilling results of the JOIDES Legs X and XIII was the discovery of a Lower Cretaceous and Upper Jurassic pelagic sequence above an ophiolitic oceanic basement, almost identical to the coeval Alpine sequences of western Europe[3,19]. The eastward march of Africa which rotated the various microcontinents also permitted the opening of the Atlantic. The geological history of the Atlantic and the Alpine regions ran a parallel course as long as this movement continued, but the early Atlantic could not have been very broad, nor was the circulation quite normal as suggested by the abundant pyritized fossils and other Red Sea type of metallic deposits in the JOIDES cores[3,19]. Slump blocks from the nearby steep walls have also been observed[3]. But the Mesozoic Alpine geosynclines—those inland seas, a few hundred kilometres wide, which might be considered as the ancestral western Mediterranean (Fig. 4)—did not reach oceanic proportions[15].

Different Ophiolites

Alpine geologists have long recognized two different sets of ophiolites in the European Tethyan system[4] (Fig. 2). The Penninic ophiolites of the Subbetic Cordilleras, the Apennines, and the Alps could not be traced beyond the central Carpathians but a second set of Bosnian ophiolites marks the approximate position of a suture zone in the Balkans. Whereas the Penninic ophiolites are not older than Jurassic, the Bosnian ophiolites could be Triassic or even older, and Palaeozoic ophiolites from Anatolia are known[20]. These palaeozoic ophiolites were obviously the remnants of the once extensive Tethyan Sea between Eurasia and Africa. As Africa pushed the Greco-Italian Block eastward and caused it to rotate counterclockwise, the Tethyan Ocean north-east of the Block was consumed as is evidenced by the Jurassic and Lower Cretaceous Flysch of the eastern Carpathians[21,22] (Fig. 2).

The Greco-Italian microcontinent was joined onto the African plate during the Middle Cretaceous, and has moved with it ever since. (In ref. 23, D. P. McKenzie did not define precisely the present plate boundary between Africa and Europe. The possibility that Italy and western Balkan are a part of the African plate was discussed with him after the Royal Society Symposium on Plate Tectonics in February 1971. He found that my idea is not contradicted by available data on the seismicity of these regions.) Geological evidence also suggests a Late Cretaceous westward motion of the Italo-African plate;

the Austroalpine nappes of the Austrian and Swiss Alps represented the original northern margin of this Cretaceous Italo-African plate and these rigid basement blocks were thrust westward onto the Pennine ophiolites during orogenic movements which started during the Middle Cretaceous and culminated in the Late Eocene[24]. The Pennine ophiolites were almost completely consumed by the beginning of the Oligocene, at which time this northern margin of the Italo-Africa plate had a sutured contact with Europe; the debris eroded from the Austroalpine nappes were then being deposited in nearly Molasse troughs on the southern margin of the European plate.

The western margin of the Italo-African plate overrode the ophiolites of the Apennine geosyncline, giving rise to the Late Eocene deformation of the ophiolitic mélanges of Liguria and on the islands of Elba and Corsica[25-27]. The first deformation of the Betic must also have taken place during this westward motion of Africa, when the Betic ophiolitic geosyncline was almost eliminated. Unfortunately, geological evidence from this relatively poorly investigated orogenic belt is meagre.

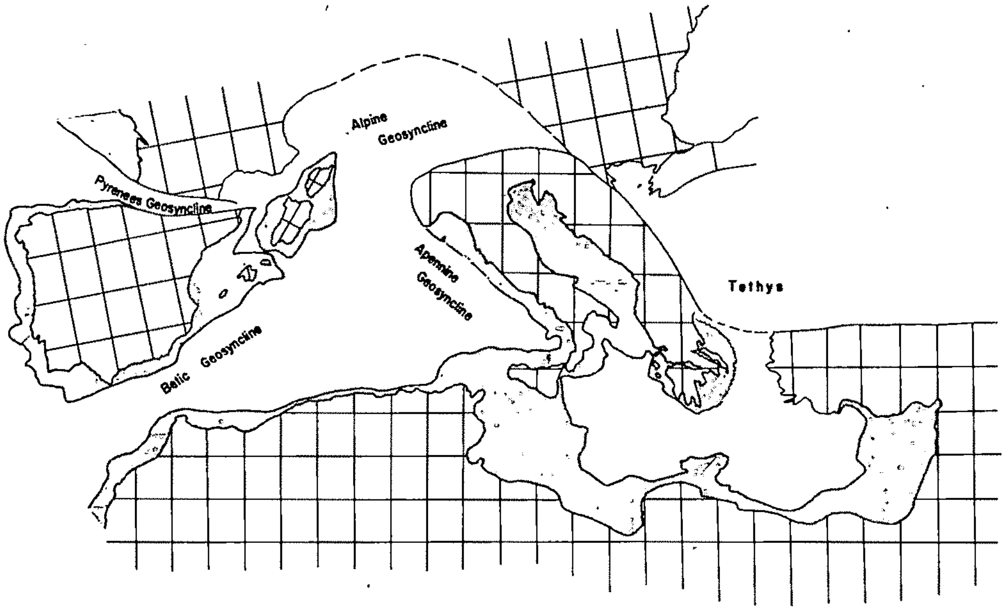


Fig. 4 Middle Cretaceous palaeogeography of Europe when the westward motion of Africa relative to stable Europe began.

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA

Westward Movement

When the Iberian Block was joined to the African Plate during the Eocene and began to share its westward movement, the deformation of the Pyrenees commenced and may have continued during the subsequent northward movement of Africa. The present plate boundary between Europe and Africa seems to be located near the Strait of Gibraltar[23] and may represent a later development.

The kinematics of the Corso-Sardinia motion is very uncertain. Sedimentological evidence indicated that this block was still partly connected to southern France during the Early Oligocene[9], and palaeomagnetic evidence suggested a post-Oligocene rotation of Sardinia[25]. The JOIDES drilling results are also not inconsistent with a young age for the northern Balearic Basin[3]. Furthermore no Alpine ophiolite zone exists between this block and the stable Europe. Thus we tended to agree with Argand and modern Italian geologists[25] that the Corso-Sardinia block was not separated until the culmination of ophiolite deformation in the Late Eocene.

Our understanding of the origin of the Alps and the Mediterranean has been made difficult because of the last phases of the Alpine deformation which involved a northward motion of Africa relative to Europe[15]. Geological evidence of this movement is clear in the well investigated Alps, Apennines and Carpathians, but the geology is less well known elsewhere. The northward motion of Africa has continued to this day and has resulted in the underthrusting of the Hellenic Trench[3,23] and the elevation of the Mediterranean Ridge[3]. We are, however, baffled by the latest history of the Alboran Basin, where geological evidence seemed to indicate a late Caenozoic rifting[3,6] in contrast to the present state of compression[23].

We are presenting a very speculative idea in an attempt to rationalize some of the very puzzling aspects of the Mediterranean geography and geology. We are in general agreement with some of the geometrical postulates of Argand, but disagree with him on the timing of events. We postulate that the origin of the Alpine geosyncline was related to the Mesozoic eastward motion of Africa relative to Europe, and that the orogenic movements in western Europe were caused by a westward and later northward motion of Africa relative to Europe. This hypothesis is not offered as a final solution; the proposal of some definite ideas will, however, focus attention on several areas where geological and geophysical data could be synthesized and freshly gathered to check, improve, or disprove this working hypothesis.

Received March 19, 1971.

[1] Suess, E., *Das Antlitz der Erde* (Freytag, Leipzig, 1885).
[2] Bucher, W., *The Deformation of the Earth's Crust* (Princeton University Press, 1933).
[3] Ryan, W. B. F., Hsü, K. J., Nesteroff, V., Pautot, G., Wezel, F., Lort, J., Cita, M., Maync, W., Stradner, H., and Dumitrica, P., *Geotimes*, **15**, 12 (December 1970).
[4] Staub, R., *Der Bewegungsmechanismus der Erde* (Bornträger, Berlin, 1928).
[5] Carey, S. W., *Symp. Continental Drift*, 177 (Tasmania, 1958).
[6] Argand, E., *Cong. Geol. Intern., XIIIe, Belgique, C.R.*, 171 (1924).
[7] *Proc. Symp. sur l'Histoire Structurale du Golfe de Gascogne, December* 1970 (Rueil-Malmaison, France) (in the press).
[8] Zijderveld, J. D. A., De Jong, K. A., and van der Voo, R., *Nature*, **226**, 933 (1970).
[9] Stanley, D., and Mutti, E., *Nature*, **218**, 6 (1968).
[10] Zijderveld, J. D. A., and De Jong, K. A., *Geologie en Mijnbouw*, **48**, 559 (1969).
[11] Laughton, A. S., Berggren, W. A., Bensen, R., Davis, T. A., Franz, U., Musich, L., Perch-Nielsen, K., Ruffman, A., van Hinte, J. E., and Whitmarch, R. B., *Geotimes*, **17**, 10 (November 1970).

[12] Zijderveld, J. D. A., and De Jong, K. A., *Symp. Golfe de Gascogne*, (Rueil-Malmaison, 1970).

[13] Stauffer, K. W., and Tarling, D. J., *Symp. Golfe de Gascogne*, (Rueil-Malmaison, 1970).

[14] Pittman, W., *Geol. Soc. Amer. Ann. Meetings Program*, 2,752 (1970).

[15] Trümpy, R., *Geol. Soc. Amer. Bull.*, 71, 843 (1960).

[16] Smith, A. G., *Proc. Roy. Soc. Symp.* Plate Tectonics.

[17] Maxwell, J. C., in *The Megatectonics of Continents and Oceans* (edit. by Johnson, H., and Smith, B. L.) (Rutgers University Press, 1970).

[18] Bailey, E. B., and McCallien, W. J., *Trans. Roy. Soc. Edinburgh*, **65**, 315 (1963).

[19] Ewing, J., Hollister, C., Hathaway, J., Paulus, F., Lancelot, Y., Habib, D., Poag, C. W., Luterbach, H. P., Worstell, P., and Wilcoxon, J. A., *Geotimes*, **15**, 14 (1970).

[20] Arni, P., *Z. Inst. Lagerstättenforschung Türkei*, 3, 472 (1942).

[21] Birkenmajer, K., *Bull. Acad. Polonaise Sci.*, 4, 451 (1957).

[22] De Raaf, J. F. M., *The Science of Petroleum*, **6**, 10 (Oxford University Press, 1953).

[23] McKenzie, D. P., *Nature*, **226**, 239 (1970).

[24] Trümpy, R., Manuscript in press for van Bemmelen Festschrift volume.

[25] Boccaletti, M., and Guazzone, G., *Mem. Soc. Geol. Italiana*, **9**, 177 (1970).

[26] Hsü, K. J., *Bull. Amer. Assoc. Petrol. Geol.*, **51**, 65 (1967).

[27] Sestini, G., *Sediment. Geol.*, 4, 203 (1970).

Molecular Mechanisms of Chromosome Pairing, Folding and Function

DAVID E. COMINGS & ARTHUR D. RIGGS

Departments of Medical Genetics and Biology, City of Hope National Medical Center, Duarte, California 91010

Allosteric proteins are proposed which, on binding to specific DNA sites, undergo conformational changes that enable them to bind to each other and to control pairing, condensation, and genetic function of chromosomes.

In this article we wish to show that allosteric DNA binding regulatory proteins such as the *lac* repressor are admirably suited to direct eukaryotic chromosome folding and homologous pairing in addition to controlling gene expression. The significant properties include tight and specific binding to DNA at a unique base sequence, tight and specific interaction between their subunits, their sensitivity to inducers or effector ligands and the conformational changes induced by DNA binding. Thus these proteins would bind to specific DNA sites and undergo allosteric changes that would enable them to bind to each other or to change enzymatic activity. They could be called "nucleosteric proteins". On this basis, it is possible to propose mechanisms to account for a number of biological phenomena, such as homologous chromosome pairing, that have previously been extremely difficult to explain.

Pre-meiotic and Somatic Pairing

Chromosome pairing during meiosis in eukaryotes probably occurs in three stages[1,2]. These are pre-meiotic pairing or alignment of homologous chromosomes, chromosome synapsis mediated by the synaptonemal complex, and intimate molecular pairing between short stretches of DNA containing similar base sequences. The last stage may be initiated by the formation of heteroduplex DNA molecules[3,4] and may lead to recombination. The synaptonemal complex seems to enhance the efficiency of molecular pairing, because recombination associated with meiotic pairing is much more frequent than that associated with somatic pairing[5,6]. Yet chromosome synapsis mediated by the synaptonemal complex seems to be non-specific, for it can also pull together non-homologous chromosomes[1,2,7,8]. It seems to act by zippering together chromosomes which have their homologous telomeres attached next to each other at the nuclear membrane[1,9,10]. Thus one of the most important aspects of meiotic chromosome pairing concerns the mechanism by which homologous chromosomes become partially aligned before the formation of the synaptonemal complex[11-17]. This may be the same mechanism that is responsible for the precise somatic pairing that is seen clearly in diptera species. It must be capable of acting throughout the length of the chromosome, since complex inversions in *Drosophila* show perfect homologous somatic pairing in spite of the absence of the synaptonemal complex.

Fig. 1 illustrates a scheme by which this chromosomal pairing might be accomplished. It requires no new phenomena or forces, only the three properties already mentioned. We suggest that genes exist which code for "pairing proteins", each capable of binding specifically to a unique base sequence. On binding to DNA, these protein pairs undergo allosteric changes in surface configuration which enhance their complementarity. Their subsequent association enables homologous pairing between specific segments of the chromosomes. The allosteric modification after DNA binding prevents association of the unbound pairing proteins and ensures that the only proteins in the nucleus that can associate are those that are bound to the two homologous DNA sites. It is suggested that a series of these DNA sites and their specific pairing proteins occur throughout each chromosome. The intriguing aspect of this model is that it provides a mechanism for the pairing of native DNA molecules that is as specific and thermodynamically stable as the Watson–Crick pairing of half DNA helices.

The alternative mechanisms of homologous pairing are less appealing. For example, it could be assumed that this type of chromosome pairing is mediated by the presence of specific nucleases that keep homologous regions of DNA partially unpaired and that pairing would be mediated by heteroduplex formation between these single stranded whiskers. The difficulty with this mechanism is that it would lead to a high frequency of recombination when in fact recombination between somatically paired chromosomes is rare. Furthermore, if pre-meiotic pairing is accomplished in this manner, why should a synaptonemal complex evolve to facilitate essentially the same thing at a later stage in meiosis? A second alternative could involve the formation of a bivalent pairing protein with two identical DNA recognition sites. A fusion gene formed by unequal crossing over could produce such a protein. The bivalent pairing protein would attach first to one homologous site, then to the other. The difficulty with this scheme is that the efficiency of such a pairing protein would be reduced by competition between partially bound and unbound molecules. Furthermore, it seems unlikely that a whole series of such genes could be produced by the hazardous route of unequal crossing over.

Chromosome Folding

The proposed mechanism of chromosome pairing involves recognition sites that exist as single copies per haploid set, but it is equally plausible to propose a repetitious sequence as the recognition site, thus providing a mechanism for chromosome folding or condensation (Fig. 2). A DNA sequence repeated many times throughout the genome could recognize a specific folding protein synthesized before mitosis. This protein would be capable of self-association only after binding DNA. The net-like arrangement of chromatin that would be formed would closely resemble that seen in whole mount electron microscopy of mitotic chromosomes[18–20]. Such a mechanism could provide an explanation for the condensation of interphase chromatin when the nucleus is fused with a cell in mitosis[21]. The aggregation could be destroyed at the end of mitosis by the synthesis of new proteins that interfere with the binding of the folding protein to DNA, releasing them for destruction before the next mitosis. Shifts in cation concentration or low molecular weight ligands could also accomplish this release. Similar mechanisms could be evoked to account for the binding and release of chromatin to the nuclear membrane during the cell cycle[22–24]. The attachment of the chromosomes to localized portions of the nuclear membrane would help to ensure that they condensed on themselves rather than fusing to each other.

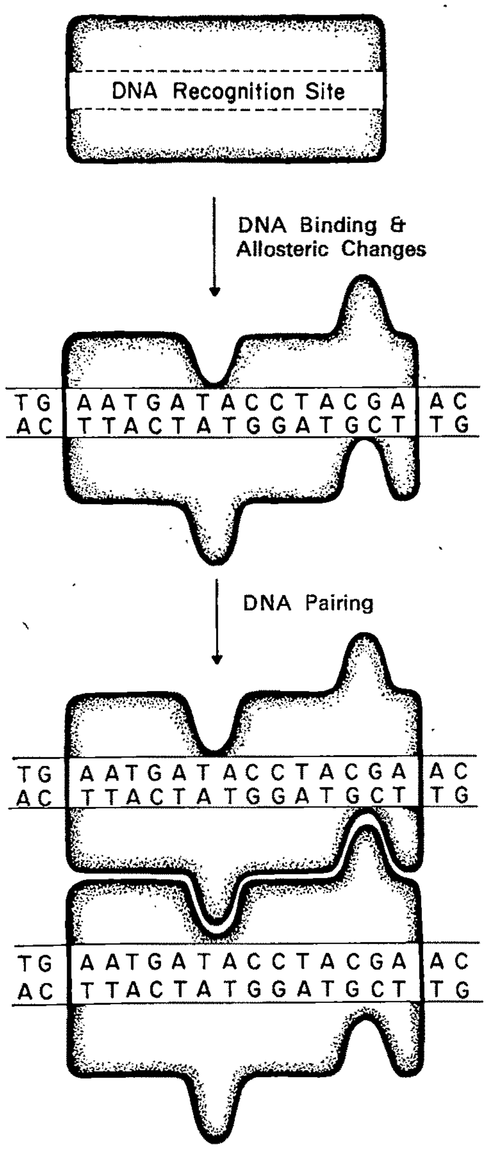


Fig. 1 A proposed mechanism of homologous chromosome pairing in somatic and premeiotic cells. Pairing proteins are synthesized which possess a DNA recognition site capable of firm binding to a specific base sequence. After binding, allosteric changes occur which allow the protein to bind itself, thus initiating the homologous pairing of that site. It is suggested that a series of such regions with their specific pairing proteins are located throughout the length of each chromosome.

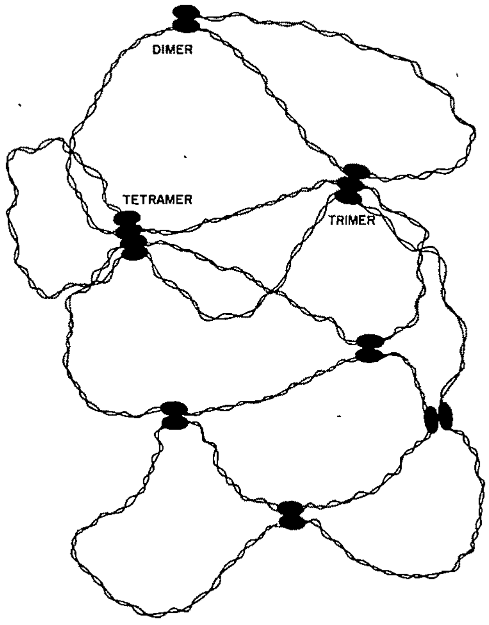


Fig. 2 Chromosome condensation or folding during mitosis could be accomplished by a repetitious DNA sequence that is recognized by a "folding protein". After binding to DNA this protein would undergo allosteric changes that allow it to bind to itself (see text).

There may be at least two categories of folding proteins, heterochromatin specific and euchromatin specific. Heterochromatin is chromatin that remains condensed and densely staining throughout interphase[25]. Many lines of evidence suggest that specific types of DNA occur within heterochromatin[26–29]. Thus, it is reasonable to propose that it may contain specific DNA recognition sites not found in euchromatin and that the folding proteins that bind to these sites are synthesized throughout the cell cycle. This could provide a simple mechanism for keeping this chromatin permanently condensed. It is also consistent with the observation that heterochromatin may be condensed less during mitosis than euchromatin[30] suggesting that its folding sites have already been taken up and that those utilized for the rest of the chromosome are different and more efficient.

Properties of Allosteric Proteins

We have proposed mechanisms that involve proteins with the properties of binding and specificity that are, as we shall show here, typical of repressor proteins. It has now been clearly shown that regulatory proteins such as the *E. coli lac* repressor can bind tightly and specifically to a unique base sequence in double stranded DNA[31–34] and similar results have been obtained for the λ and 434 phage repressor[35–37]. The *lac* repressor can identify the sequence of perhaps twenty base pairs corresponding to the *lac* operator. Twenty base pairs would be more than adequate to provide unique sequences even in genomes 1,000 times as large as *E. coli*. The *lac* repressor binds very tightly to the *lac* operator. At low salt concentrations, the dissociation constant can be less than 10^{-13} M, corresponding to a free energy change of 18 kcalories. In aqueous solution many covalent bonds are not nearly as strong. At higher salt concentrations the binding is weakened but remains very tight; in 0.2 M KCl the dissociation constant is still 10^{-11} M[32]. The binding of sequence specific proteins to DNA is certainly strong enough to hold homologous chromosomes in register, especially if there is cooperative binding at many distinct sites.

The bonding forces involved in the association of identical protein subunits to form oligomers are also sufficiently specific and strong. That the specificity and speed of association is adequate is made clear by the fact that even though there are only about ten copies of tetrameric repressor per haploid *E. coli* cell, and the concentration of subunits (which bind IPTG and would be detected) is even less[38,39] the repressor subunits assemble quickly to form active tetramers[40]. The presence of all other *E. coli* proteins does not interfere. The tightness with which repressor subunits bind to each other has not been ascertained, but since assembly takes place in such dilute solutions the bonding forces must be strong. The assembly of β-galactosidase is also rapid in dilute solution[41]. For other proteins, the binding between subunits can be as high as 13 kcalories/mol of dimer formed[42].

Allosteric proteins are by definition sensitive to effector ligands[43]. These ligands can be substrates or compounds unrelated to the substrate. Effector ligands cause or stabilize allosteric transitions which result in a changed conformation of the polypeptide chains[44,45]. Very often these conformational changes affect the subunit interaction and can promote either association or dissociation[43,44,46]. The *lac* repressor is clearly an allosteric protein in that it has a galactoside binding site distinct from the DNA binding site[33]. Furthermore, these effector ligands can change considerably the stability of repressor–operator complex. One inducing ligand, IPTG, is known to increase more than 100 times the rate of repressor–operator dissociation, while another, ONPF, stabilizes the complex[33].

There is only limited evidence at present that the conformation of the operator-bound repressor is different from that of the free repressor, but it has been shown that the apparent affinity of IPTG for DNA bound repressor is considerably less than for repressor free in solution. This strongly suggests differences in conformational state (A. D. R., unpublished data). That DNA should act as an allosteric effector, changing the conformation of the bound protein, is very plausible. Substrates often behave as allosteric effectors and when such strong binding forces are involved, it would, we feel, be more surprising if changes were not observed.

Chromosome Function

The above postulate that DNA may act as an allosteric effector leads us to suggest a novel mechanism that can account for positive control of gene activation: there may be enzymes which alter histones and which are active only when associated with their DNA binding site; these would be known, therefore, as DNA-dependent histone inactivating enzymes. The alteration of histone binding could be produced by a number of reactions including methylation, acetylation and phosphorylation[47,48]. In the micro-environment around the specific DNA binding site, the concentration of histone inactivating enzyme would be very high and the non-specific repression by histones would be greatly reduced, permitting gene expression. DNA would then serve the purpose of both activating and fixing the spatial location of regulatory enzymes.

In conclusion, we propose the existence of a class of nucleosteric proteins which bind to specific DNA sites and, on binding, undergo allosteric changes that allow them to bind to each other or to change enzymatic activity. These proteins could play a role in initiating homologous chromosome pairing, provide for the condensation of chromosomes at mitosis, condensation of heterochromatin during interphase, and they could be involved in both positive and negative control of gene function. Techniques are available to test for the existence of proteins with these unique properties.

This work was supported by grants from the National Institutes of Health.

Received April 26, 1971.

[1] Comings, D. E., and Okada, T. A., *Nature*, **227**, 451 (1970).
[2] Comings, D. E., and Okada, T. A., in *Adv. Cell Mol. Biology* (edit. by DuPraw, E. J.) (Academic Press, New York, in the press).
[3] Whitehouse, H. L. K., and Hastings, P. J., *Genet. Res.*, **61**, 27 (1965).
[4] Holliday, R., in *Replication and Recombination in Genetic Material* (edit. by Peacock, W. J., and Brock, R. D.), p. 157 (Australian Acad. Sci., Canberra, 1968).
[5] Pontecorvo, G., *Trends in Genetic Analysis* (Oxford University Press, 1959).
[6] Stern, C., *Genetics*, **21**, 625 (1936).
[7] Menzel, M. Y., and Price, J. M., *Amer. J. Bot.*, **53**, 1079 (1966).
[8] Ting, Y. C., *Genetics*, **61**, s58 (1969).
[9] Comings, D. E., and Okada, T. A., *Chromosoma*, **30**, 269 (1970).
[10] Moens, P. B., *Chromosoma*, **28**, 1 (1969).
[11] Smith, S. G., *Canad. J. Res.*, **20**, 221 (SD) (1942).
[12] Moens, P. B., *Chromosoma*, **15**, 231 (1964).
[13] Feldman, M., *Proc. US Nat. Acad. Sci.*, **55**, 1447 (1966).
[14] Maguire, M., *Chromosoma*, **21**, 221 (1967).
[15] Brown, W. V., and Stack, S. M., *Bull. Torrey Bot. Club.*, **95**, 369 (1968).
[16] Driscoll, C. J., and Darvey, N. L., *Science*, **169**, 290 (1970).
[17] Lu, B. C., and Raju, N. B., *Chromosoma*, **29**, 305 (1970).
[18] DuPraw, E. J., *Nature*, **206**, 338 (1965).
[19] Comings, D. E., and Okada, T. A., *Cytogenetics*, **9**, 436 (1970).
[20] Comings, D. E., and Okada, T. A., *Cytogenetics*, **9**, 450 (1970).
[21] Johnson, R. T., and Rao, P. N., *Nature*, **226**, 717 (1970).
[22] Comings, D. E., *Amer. J. Human Genet.*, **20**, 440 (1968).
[23] Comings, D. E., and Okada, T. A., *Exp. Cell Res.*, **62**, 293 (1970).
[24] Comings, D. E., and Okada, T. A., *Exp. Cell Res.*, **63**, 62 (1970).
[25] Heitz, E., *Jahrb. F. Wissensch. Bot.*, **69**, 762 (1928).
[26] Yasmineh, W. G., and Yunis, J. J., *Exp. Cell Res.*, **59**, 69 (1970).
[27] Pardue, M. L., and Gall, J. G., *Science*, **168**, 1356 (1970).
[28] Jones, K. W., *Nature*, **225**, 912 (1970).
[29] Mattoccia, E., and Comings, D. E., *Nature New Biology*, **229**, 175 (1971).
[30] Comings, D. E., *Exp. Cell Res.*, (in the press).
[31] Gilbert, W., and Muller-Hill, B., *Proc. US Nat. Acad. Sci.*, **56**, 1891 (1966).
[32] Riggs, A. D., Suzuki, H., and Bourgeois, S., *J. Mol. Biol.*, **48**, 67 (1970).
[33] Riggs, A. D., Newby, R. F., and Bourgeois, S., *J. Mol. Biol.*, **51**, 303 (1970).
[34] Riggs, A. D., Bourgeois, S., and Cohn, M., *J. Mol. Biol.*, **53**, 401 (1970).
[35] Ptashne, M., *Proc. US Nat. Acad. Sci.*, **57**, 306 (1967).
[36] Ptashne, M., *Nature*, **214**, 232 (1967).
[37] Pirrotta, B., and Ptashne, M., *Nature*, **222**, 541 (1969).
[38] Muller-Hill, B., Crapo, L., and Gilbert, W., *Proc. US Nat. Acad. Sci.*, **59**, 1259 (1968).
[39] Jobe, A., Riggs, A. D., and Bourgeois, S., *J. Mol. Biol.* (in the press).
[40] Branscombe, E. W., and Stuart, R. N., *Biochem. Biophys. Res. Commun.*, **32**, 731 (1968).
[41] Adamson, L., Gross, C., and Novick, A., in *The Lactose Operon* (edit. by Beckwith, J. R., and Zipser, D.), 317 (Cold Spring Harbor Laboratory, New York, 1970).
[42] Klotz, I. M., Langerman, N. R., and Darnall, D. W., *Ann. Rev. Biochem.*, **39**, 25 (1970).
[43] Monod, J., Changeux, J. P., and Jacob, F., *J. Mol. Biol.*, **6**, 306 (1963).
[44] Koshland, D. E., and Neet, K. E., *Ann. Rev. Biochem.*, **37**, 359 (1968).
[45] Perutz, M. F., *Nature*, **228**, 726 (1970).
[46] Tao, M., Salas, M. L., and Lipmann, F., *Proc. US Nat. Acad. Sci.*, **67**, 408 (1970).
[47] Allfrey, V. G., Faulkner, R., and Mirsky, A. E., *Proc. US Nat. Acad. Sci.*, **51**, 786 (1964).
[48] Kleinsmith, L. J., Allfrey, V. G., and Mirsky, A. E., *Proc. US Nat. Acad. Sci.*, **55**, 1182 (1966).

Pure Che stry Library
SCIENCE COLLEGE.
Upper Circular Road
CALCUTTA

LETTERS TO NATURE

PHYSICAL SCIENCES

Discrimination of Earthquakes and Explosions using Short Period Seismic Array Data

THE problem of identification of earthquakes and underground explosions on a global basis, for seismic events with body wave magnitudes (m_b) below 4.7 has not been solved[1,2], although many partially successful discriminants have been proposed. A positive identification parameter based solely on the short period seismic data is interesting as the equivalent long period energy detection threshold is often difficult to achieve, particularly at teleseismic distances.

Using data recorded at the Yellowknife short period seismic array in northern Canada, a simple two dimensional identification parameter has been found that completely separates a population of underground explosions ($4.2 \leq m_b \leq 6.2$) at the Urals, Caspian, East Kazak, Sinkiang and Novaya Zemlya test sites from a population of shallow ($h \leq 50$ km) earthquakes ($4.3 \leq m_b \leq 6.0$) within about 15° of these test sites. The data were examined in both the time and frequency domain for optimum identification parameters that could be used either singly or in a multivariant discriminant. Short period multivariant discriminants have been published in various reports of the Lincoln Laboratory of MIT[3] and have provided positive identification for events of m_b 4.7.

For the Yellowknife data, the optimum discriminant in the time domain was found to be a complexity parameter C defined as

$$C = \sum_{t=2}^{t=35} |x(t)| \qquad \sum_{t=0}^{t=2} |x(t)|$$

where $|x(t)|$ is the rectified sum of the nineteen signal channels of the array, phased to that beam that maximizes the correlogram of the P phase[4]. The algorithm, which is used to define $t=0$, is based on the correlogram and $t=0$ is assumed where the value of the correlogram first rises above a given bias value. Using this automated procedure for defining $t=0$, results in a starting time that is always fractionally later than the actual onset time of the P phase, the difference is of the order of 0.2 s. This complexity parameter is the same as one used at the Lincoln Laboratory[5] except for the definition of $t=0$.

Using the same data set, the optimum discriminant in the frequency domain was found to be the third moment of frequency (TMF), defined as

$$\mathrm{TMF} = \sum_{n=1}^{n=19} \left(\sqrt[3]{\sum_{f=0.32}^{f=5.0} (A_n(f) - B_n(f)) \cdot f^3 \Big/ \sum_{f=0.32}^{f=5.0} (A_n(f) - B(f))} \right) \Big/ 19$$

where $A_n(f)$ is the spectral amplitude at frequency f(Hz) of the first 128 samples of the signal (twenty samples per second), of the data channel n, and $B_n(f)$ is the mean spectral amplitude, at the same frequency and on the same data channel, of two noise samples taken in the 20 s before the onset of the event. If $A_n(f) < B_n(f), A_n(f) - B_n(f)$ is set to zero. The spectral amplitudes were obtained using the Cooley–Tukey algorithm, the data windows are cosine tapered by 12.5% at each end. Weichert[6], without using the $B_n(f)$ term has also found the third moment of frequency to be the optimum discriminant in the frequency domain.

The earthquake population of fifty-one shallow events was selected from the 1969 USCGS PDE lists using all those events available from the Yellowknife array digital tape library. The explosion population of twenty-seven covers the years 1968 to 1970 and likewise was obtained from all Eurasian events on the digital tape library. For some events, not all of the nineteen array channels were operational or free from system noise, but these events were still retained in the populations in order to give a realistic data sample; however, inoperative or excessively noisy array channels were omitted from the calculations of C and TMF.

Using the complexity alone results in about 20% of the explosions overlapping 40% of the earthquakes, compared with the third moment of frequency discriminant for which 20% of the explosions overlap about 15% of the earthquakes. Taking the data as a two dimensional discriminant (Fig. 1), however, a complete separation of the explosion population from the earthquake population is obtained when referenced to the arbitrary decision line $\mathrm{TMF} = 13 + 0.25 \times C$. For one

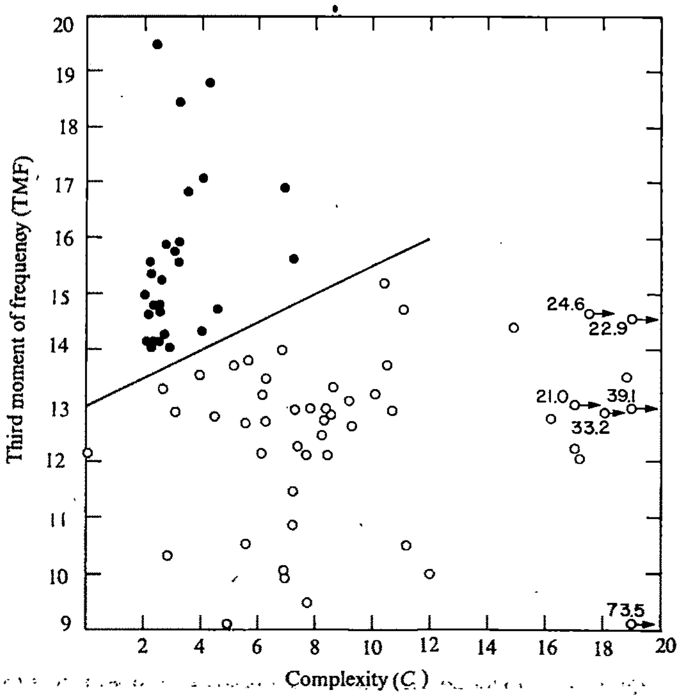


Fig. 1 Average third moment of frequency against phased beam complexity for a population of twenty-seven Eurasian explosions (●) and fifty-one shallow Eurasian earthquakes (○).

earthquake, C was set to zero as the data file was too short for the calculation of C. The orthogonal distances D of the data points from this line as a function of magnitude m_b (Fig. 2) shows D to be independent of m_b with the possible exception that the smaller explosions appear to be further from the decision line than the larger explosions. The value of D in this population has not been found to be a function of the seismic region or test area and for the earthquakes ($h < 50$ km) D has not been found to be a function of depth.

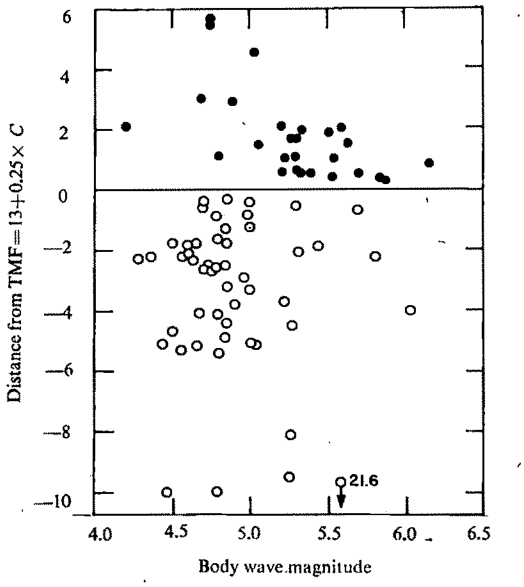


Fig. 2 Distance from the arbitrary decision line TMF=13 +0.25×C as a function of body wave magnitude for a population of twenty-seven Eurasian explosions (●) and fifty-one shallow Eurasian earthquakes (○).

The empirical conclusion is that the earthquakes, which contain the higher frequencies, are sufficiently more complex than the corresponding explosions, so that complete separation is maintained using the array data from the regions of the five test sites. Similarly the more complex explosions such as those at Novaya Zemblya maintain a sufficiently high frequency character in the first 6.4 s.

As this study has not shown any need to regionalize the data within Asia, data are being collected from other test sites and seismic areas within the third seismic zone from Yellowknife. Extensive testing will be necessary to show whether this short period discriminant will continue to hold on a worldwide basis, or for larger event populations. If it does, it will provide a positive discriminant for explosions with yields smaller than 10 kT in hard rock.

I thank P. W. Basham, K. Whitham and E. Manchee for discussions.

F. M. Anglin

Seismology Division,
Earth Physics Branch,
Department Energy, Mines and Resources,
Ottawa K1A 0E4

Received July 16, 1971

[1] Evernden, J. F., *J. Geophys. Res.*, 74, 15, 3828 (1969).
[2] SIPRI, *Seismic Methods of Monitoring Underground Explosions* (Almqvist and Wiksell, Stockholm, 1969).
[3] Lincoln Laboratory, Massachusetts Institute of Technology; Technical Note, 1968-8, 1969-24; Semiannual Technical Summary (June 30, 1968), (December 31, 1968).
[4] Weichert, D. H., Manchee, E. B., Whitham, K., *Geophys. J. Roy. Astron. Soc.* 13, 277 (1967).
[5] Lincoln Laboratory, Massachusetts Institute of Technology, Semiannual Technical Summary (December 31, 1969).
[6] Weichert, D. H., *Z. Geophysik* (in the press).

Black Holes as Agents of Magnetic Fields

THE recent discovery of pulsating X-ray sources[1] has led to considerable speculation that these objects might be black holes. So far no mechanisms have been seriously proposed to explain how pulsed X-rays could be produced by black holes, but it is likely that any such mechanism would require the presence of a magnetic field. I wish to point out that it is highly unlikely that the magnetic field of a black hole could ever be large enough to produce observable effects.

It is well established that sufficiently massive stars must undergo complete gravitational collapse after they have exhausted their thermonuclear energy. All available theoretical evidence[2] suggests that the final state of this collapse is a Kerr-Newman black hole. In particular, there is considerable evidence (refs. 2–5 and unpublished results of R. Price and myself) that the only electromagnetic field a black hole can have is the Kerr–Newman electromagnetic field. The Kerr–Newman electromagnetic field differs substantially from an ordinary magnetic dipole field, but its magnetic dipole moment, given by the formula[6],

$$\mu_K = |q|J/Mc$$

is a convenient indicator of the strength of the field. Here q is the charge of the black hole, J is its angular momentum, M is its mass, and c is the speed of light. One can obtain a firm upper limit on the value of μ_K from the following simple considerations: for a proton, $q_p/(\sqrt{G}\ M_p) \approx 10^{18}$ and for an electron $|q_e|/(\sqrt{G}\ M_e) \approx 10^{21}$, where G is the gravitational constant. If an uncollapsed star had a net positive charge $q > 10^{-18}\sqrt{G}\,M$ or a net negative charge $|q| > 10^{-21}\sqrt{G}\,M$, the electrostatic repulsive force on the excess protons or electrons would be greater than the gravitational attraction, and thus the excess charges would be driven away. Thus an uncollapsed star must have $|q|/(\sqrt{G}\ M) < 10^{-18}$. (On the other hand, a net positive charge somewhat less than this upper limit can be expected to occur since the less massive and faster moving electrons escape from the star more readily than protons.) No mechanism is known by which significant charge separation could occur during gravitational collapse; the above argument about the driving away of excess charge applies here also. Thus, the maximum value of $|q|/(\sqrt{G}\ M)$ that a Kerr-Newman black hole resulting from gravitational collapse could reasonably be expected to have is 10^{-18}. In fact, even if a black hole could be produced with $|q|/(\sqrt{G}\,M)$ somewhat greater than 10^{-18}, selective accretion of charged particles would be likely to lower the value of $|q|/(\sqrt{G}\,M)$ to less than 10^{-18} in a short time. In addition, for a Kerr–Newman black hole, one must have

$$\left(\frac{GM}{c^2}\right)^2 \geq \left(\frac{\sqrt{G}q}{c^2}\right)^2 + \left(\frac{J}{Mc}\right)^2$$

(This relation is necessary for the existence of an event horizon.) Thus one obtains

$$\mu_K < 10^{-18} G^{3/2} M^2/c^2$$

or, in a more readily recognizable form,

$$\mu_K < 10^{-8} (M/M_\odot)^2 \mu_E$$

where μ_E is the magnetic dipole moment of the Earth. Even this small an electromagnetic field could still have an effect on the motion of charged particles near the event horizon. But it seems highly unlikely that this magnetic field could be large enough to produce any astrophysically observable effects.

ROBERT M. WALD

Joseph Henry Laboratories,
Princeton University,
Princeton, New Jersey 08540

Received June 22, 1971.

[1] Oda, M., Gorenstein, P., Gursky, H., Kellogg, E., Schreier, E., Tanabaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **166**, L1 (1971).

[2] Wald, R. M., *Phys. Rev. Lett.* (in the press).

[3] Anderson, J. L., and Cohen, J. M., *Astrophys. Space Sci.*, **9**, 146 (1970).

[4] Cohen, J. M., and Wald, R. M., *J. Math. Phys.* (in the press).

[5] de la Cruz, V., Chase, J. E., and Israel, W., *Phys. Rev. Lett.*, **24**, 423 (1970).

[6] Carter, B., *Phys. Rev.*, **174**, 1559 (1968).

Map of the Galactic Nucleus at 10 μm

We have scanned the centre of our galaxy at a wavelength of 10 μm with a beam 5.5 arcsec in diameter. The scans have been combined to produce the map shown in Fig. 1. At 10 kpc, commonly assumed to be the distance to this region, 5.5 arcsec corresponds to 0.3 pc.

These observations were taken during the day on February 15, 1971, with the NASA 61 inch telescope at the Catalina Observatory. Because of the imprecision in the drive of the telescope, the scans could not be located accurately in declination; the telescope also drifted slowly in right ascension. These problems were overcome by comparing the scans with some made earlier by one of us (F. J. L.) and D. E. Kleinmann with the Steward Observatory 90 inch telescope. Minor distortions may persist in the map. All the features described below stand out clearly above the noise, which has a peak-to-peak value of about 6×10^{-17} W/m^2 Hz ster.

Four sources can be distinguished and are numbered in Fig. 1. An additional broad zone of emission can be seen extending to the north. Sources 2 and 4 are partially resolved and have diameters of about 0.5 pc. There are indications on the individual scans that source 2 would break up into two or three sources at higher resolution. Source 1 is unresolved and must be less than 0.2 pc in diameter. Source 3 may also be unresolved with our beam, although the ratio of signal to noise is inadequate to permit a strong statement.

We have also made observations at 2.2, 5 and 22 μm. A detailed description of all this work will be published after we have analysed it more fully. We have located the "point-like" source discovered at 2.2 μm by Becklin and Neugebauer[1] relative to our map; to within the accuracy of the measurements, its position agrees with that of Source 3. At 5 μm, scans across the complex of sources have been made at five declinations. Although these scans have a relatively low ratio of signal to noise and are insufficient in number to construct a map, they do show the same general features as the 10 μm map.

At 22 μm, we have constructed a map with the same resolution and nearly the same ratio of signal to noise as the 10 μm map. The noise for this map has a peak to peak value of about 1.8×10^{-16} W/m^2 Hz ster; at this level, sources 2 and 3 were not detected. Source 1 stands out prominently. It lies near the centre of an extended source which has a radius of about 20 arcsec.

Two field stars are plotted in Fig. 1 and labelled A and B. Additional scans at 10 μm enabled us to locate our map relative to field star A. The coordinates of this star were measured from a Palomar Sky Atlas plate, allowing us to determine the absolute position of the 10 μm sources to within about 2 arcsec. (A finding chart for the galactic centre has been published by Spinrad *et al.*[2] (their Fig. 1.) Star A is 2.5 mm south and 2.0 mm east of the indicated position of the galactic centre on this chart and star B is 1.5 mm north and 1.0 mm west. It should be noted that the position of the 10 μm sources differs somewhat from the one indicated on the published finding chart.)

From our map we estimate the total flux from the galactic centre to be $480\pm50\times10^{-26}$ W/m^2 Hz at 10 μm. The 22 μm map indicates that the flux at this wavelength is $2{,}300\pm250\times10^{-26}$ W/m^2 Hz, of which about 60% or $1{,}400\pm150\times10^{-26}$ Hz would fall within a field of view 25 arcsec in diameter. These results are in reasonably good agreement with earlier measurements made with a 25 arcsec field of view of $550\pm60\times10^{-26}$ W/m^2 Hz at 10 μm and $1{,}700\pm200\times10^{-26}$ W/m^2 Hz at 22 μm (ref. 3).

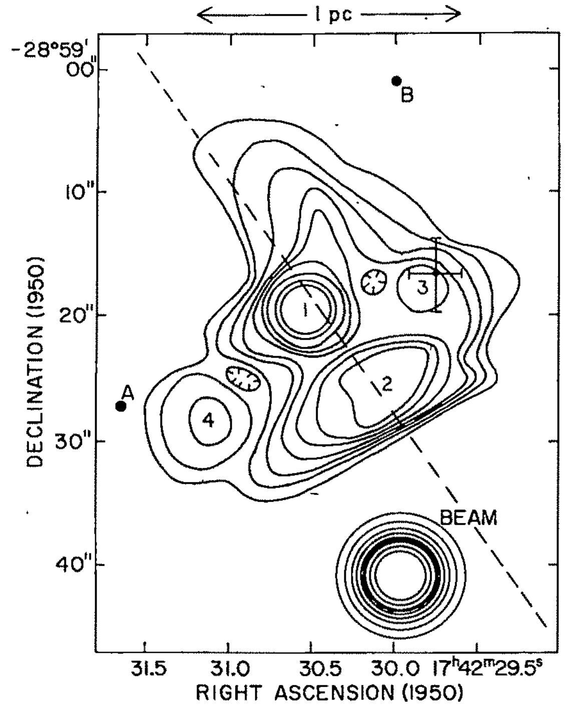


Fig. 1 Map of the galactic centre at 10 μm. The peak-to-peak noise of the observations is 6×10^{-17} W/m^2 Hz ster. The contours are at levels of 1, 2, 4, 6, 8, 10, 12, 16 and 20 times this value. The brightest 10 μm sources are numbered in order of decreasing maximum flux. The beam used for these observations has a full width at half maximum of 5.5 arcsec. It is shown with the same contour levels as are used for the map. The location of the 2.2 μm "point-like" source is indicated by a set of error bars which correspond to the uncertainty in our measurement of its position. The two most prominent field stars have been plotted and labelled A and B. The direction of the galactic plane is indicated by the dashed line.

Maps of the galactic centre at 2.2 μm have already been published[1]. At this wavelength, the detected flux arises almost entirely from unresolved stars. Except for the "point-like" source, the brightest features at 2.2 μm would produce a flux of 0.2×10^{-26} W/m^2 Hz in a field of view the size of ours. At 10 μm, the flux from these stars should be an order of magnitude lower. Our peak to peak noise is about 3.5×10^{-26} W/m^2 Hz; thus, the general stellar background detected at 2.2 μm makes virtually no contribution to the 10 μm map. Becklin and Neugebauer[4] have published the results of a scan across the galactic centre at 10 μm. Their scan seems to have passed near the centre of source 2.

The 10 μm sources fall within the region of maximum intensity of the extended 2.2 μm source, which is centred[1] at $\alpha(1950)=17$ h 42 m 30 s±1 s and $\delta(1950)=-28°\ 59'.4\pm0'.1$. Their position also agrees with that of the radio source Sgr A[5], $\alpha(1950)=17$ h 42 m 30.6 s±1 s and $\delta(1950)=-28°\ 59'\ 14''\pm15''$.

In spite of the consistency of these positions, significant ambiguities remain if one looks closely at the data. Our 22 μm map suggests that source 1 lies at the galactic centre. On the other hand, a scan across the maximum of the 2.2 μm extended source, published by Becklin and Neugebauer[4], places the greatest density of stars near source 2. The 2.2 μm "point-like" object can be identified with source 3; if it is, the 2.2 μm scan must be shifted in a direction which improves the correspondence with source 2. The existence of source 2 indicates that objects considerably cooler than normal stars lie very near the maximum 2.2 μm intensity. The contribution of these objects to the 2.2 μm flux is not known, leaving the position of the point of maximum stellar density uncertain.

Resolution of 6 arcsec at radio frequencies (D. Downes, personal communication) reveals that Sgr A consists of two objects, one to the east and one to the west of our position for the infrared sources. The published position reflects the contribution of both sources; its agreement with the infrared positions seems to be coincidental.

The first measurements of the discrete sources in the galactic nucleus were made by Low and D. E. Kleinmann[6]. These data were essential to our present efforts. We thank A. A. Hoag for giving us a plate from the Palomar Atlas and E. Roemer for assistance in carrying out the measurements.

G. H. RIEKE
F. J. LOW

University of Arizona,
Tucson, Arizona

Received July 29, 1971.

[1] Becklin, E. E., and Neugebauer, G., *Astrophys. J.*, **151**, 145 (1968).

[2] Spinrad, H., Liebert, J., Smith, H. E., Schweizer, F., and Kuhi, L. V., *Astrophys. J.*, **165**, 17 (1971).

[3] Low, F. J., Kleinmann, D. E., Forbes, F. F., and Aumann, H. H., *Astrophys. J. Lett.*, **157**, L97 (1969).

[4] Becklin, E. E., and Neugebauer, G., *Astrophys. J. Lett.*, **157**, L3 (1969).

[5] Maxwell, A., and Taylor, J. H., *Astrophys. Lett.*, **2**, 191 (1968).

[6] Low, F. J., Semaine d'Etude on the Nuclei of Galaxies, Vatican City, April, 1970.

BIOLOGICAL SCIENCES

Adaptive Significance of a Biased Sex Ratio in *Orchestia*

IN ecological studies of the marine supralittoral genus *Orchestia* (Crustacea, Amphipoda) many populations have been found that have biased sex ratios (ref. 1 and unpublished results of D. J. W.). Thelygeny has also been found in some of the terrestrial species of *Orchestia*[2], and in the sublittoral, species, *Orchestia gammarella* (Pallas), both amphogenous and thelygenous populations have been found[3]. I have investigated the possibility that biased sex ratio populations have an adaptive advantage in altering reproductive potential and the phylogenetic mechanism controlling its origin.

The annual reproductive potential (R) of an amphipod population may be expressed as:

$$R = X(bnp)$$

where X is the absolute number of adults, b the mean brood number, n the estimated number of broods in a season and p the percentage of females in the adult population. A theoretical consideration of the effect of sex ratio on R, ignoring physical and biological environmental factors and assuming b and n to be constants, is shown in Fig. 1. Unequal distribution of the sexes within a population would increase or decrease R by nearly 100%, provided each female could mate successfully. There are three categories of reproductive potential. First, there are the predominantly arrhenogenous populations in which R depends on the actual proportion of females and the intensity of male competition for mates. Second, there are those with a high or low proportion of females where mating competition is absent and the actual proportion of females controls R. And third, there are predominantly thelygenous populations where R depends on the actual proportion of males available to mate with females and possibly on the intensity of female competition for mates.

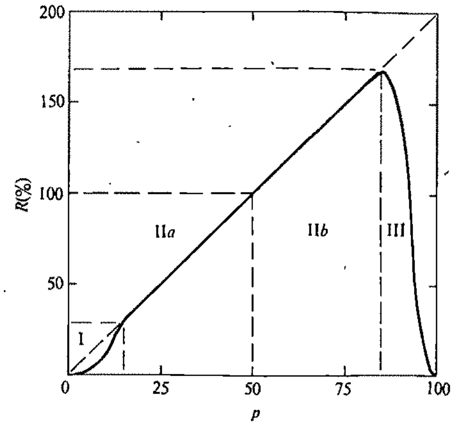


Fig. 1 Theoretical relationship between sex ratio and reproductive potential in *Orchestia*.

In *Orchestia*, a biased sex ratio may occur when the population exhibits genetically controlled monogeny, when cross fertilization is the rule, parthenogenesis is absent, males are polygamous and when only one male can inseminate a female. Although these conditions occur in many of the known species of *Orchestia*, the exact genetic control mechanisms of sex determination and monogeny remain unknown. It has been suggested that in *O. gammarella* a polygenic system is involved[4]. There are three selective mechanisms, each operated by a different physical or biological environmental condition, which may determine the degree of sex bias in a given population. These are selective mortality, unequal fertility potential of monogenous parents and selective mating. Laboratory experiments have demonstrated selective female mortality and that the reproductive potential of *O. mediterranea* A. Costa, morph *mediterranea* is reduced when substrate and food salinity is low[5]. *O. mediterranea* is a polymorphic species that, near the limit of its estuarine habitat, is replaced by morph *aestuarensis* which differs from morph *mediterranea* in dorsal pigment patterns, the incidence of intersexes and in having a high proportion of reproductive females[1]. The reproductive strategy of morph *aestuarensis* may be to maximize R (Type IIb) in the stringent estuarine conditions. It has also been shown[1] that crosses between *mediterranea* and *aestuarensis* have low fertility potential, although the reciprocal cross is fertile. In *O. gammarella*, discreet amphogenous, thelygenous or arrhenogenous colonies, apparently unrelated to physical environmental conditions, are known (ref. 3 and unpublished results of D. J. W.), although irregular fluctuations in the latter do affect optimum density conditions. In *O. gammarella* population density stresses could select biased sex ratio producing parents in isolated colonies. The mechanism would supplement the effects of density dependent regulation and provide a population homeorheostat that would maximize (Type IIb) or minimize (Type IIa) R (Fig. 2) according to the optimum permitted by the environment. An optimum R value would enable a wider range of niches to be occupied without stressing individual females. But whether natural or sexual selective processes are involved in this process is not known.

In relatively static populations which are part of a large inter-breeding group and integrated by sophisticated behavioural homeostatic mechanisms, as with many vertebrates, the stricture of Haldane[6], that natural selection cannot influence population density, applies and the increased or decreased production is wasted. The situation in *Orchestia* populations was thought to deviate from this theory, the phenomenon of sex ratio bias being limited to demes where the population is integrated by primitive social behaviour and population regula-

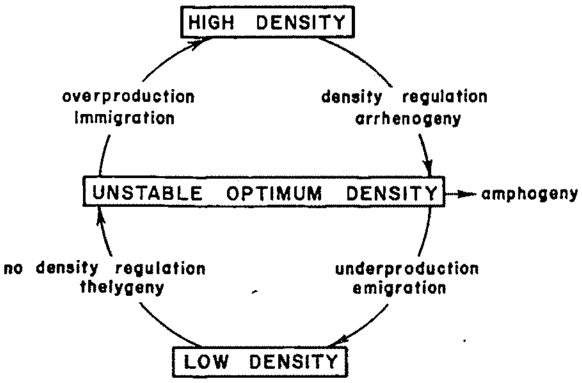


Fig. 2 Theoretical population homeorheostat in *Orchestia gammarella.*

tion methods, and/or where populations occur in discreet inbreeding colonies and are subject to irregular environmental fluctuations affecting optimum density. Sex bias would also cause a loss of genetic diversity[7] and it was expected that where environmental conditions were stable there would be a tendency for the population to maintain an unbiased sex ratio.

The gene controlling fitness to survive or breed may either be linked to the one that controls sex expression or it may be the same one. If a polymorphic supergene of this type does control sex then the possibility of group selection as an explanation of biased sex ratio is unnecessary in *Orchestia.* It has been supposed that natural selective or sexual selective forces operating at the individual level are involved in sex ratio bias and thus monogeny need have no selective value for the individual, although as has been demonstrated, it may have considerable adaptive value in regulating reproductive strategy at the population level.

I thank A. M. Gower, N. J. Poole, V. Zitko and colleagues at St Andrews for helpful discussions.

D. J. Wildish

Fisheries Research Board of Canada,
Biological Station, St Andrews,
New Brunswick

Received March 11, 1971.

[1] Wildish, D. J., *Crustaceana,* **19,** 113 (1970).
[2] Hurley, D. E., *Pacif. Sci.,* **13,** 107 (1959).
[3] Charniaux-Cotton, H., *Ann. Sci. Nat. Zool. Biol.,* **19,** 411 (1957).
[4] Ginsberger-Vogel, T., *Ann. Biol.,* **9,** 201 (1970).
[5] Wildish, D. J., *J. Exp. Mar. Biol. Ecol.,* **5,** 276 (1970).
[6] Haldane, J. B. S., *New Biol.* **15,** 9 (1953).
[7] Kalmus, H., and Smith, C. A. B., *Nature,* **186,** 1004 (1960).

Stereoscopic Vision in the Cat

Stereopsis is the perception of relative depth by means of the disparate retinal stimulation which occurs when horizontally separated eyes are fixed on a common point. The stimulus conditions which produce stereopsis in humans have been studied thoroughly, but only recently has it been possible to study the neural mechanisms[1-4]. We have been able to demonstrate stereoscopic vision in the cat, a result which complements neurophysiological data demonstrating the presence of cells in cat visual cortex with receptive field geometry which is specialized for the detection of retinal disparity[1,2].

To test for stereopsis we used conditioned suppression[5-12], whereby an animal trained to emit a frequent response will inhibit or suppress that response on perception of a stimulus which signals a forthcoming noxious event. First, the cat was trained to lick a water-filled tube continuously for a small food reward. During these training sessions the cat viewed three real vertical rods, arrayed in the same frontal plane, each subtending 18′ and separated by 9° 33′. The rods, enclosed in a viewing tunnel, appeared as black lines seen against a back-illuminated homogeneous white projection screen, whose luminance was 0.32 foot Lamberts. Next, a conditioned suppression response was established, based on a discrimination between two real-depth positions of the centre rod. Near depth was introduced by moving the centre rod toward the cat, resulting in an angular disparity of 1° 2′ relative to the stationary side rods; 5 s later a 1 s shock was delivered through the grid floor of the restraint box and then the rod returned to its original position. Far depth was introduced by moving the centre rod away from the cat, resulting in an angular disparity of 48′, and returned to its original position 5 s later; no shock occurred during this kind of trial. Five near depth and five far depth counterbalanced trials were presented daily. Formation of the differential discrimination was clearly shown by the suppression of licking in the presence of near depth and the continuation of licking in the presence of far depth.

Once the discrimination between the near and far depth positions of the centre rod had been established, the cat was required to make the same discrimination for a rod-like shadow that appeared to move to near and far positions in stereoscopic depth. To produce this effect, a stereoscopic shadow caster was used, in which two point sources slightly separated horizontally cast the shadows of three vertical rods on a rear-projection screen. The shadows stimulated the eyes separately by means of chromatic filters (Wratten No. 58 green and No. 25A red) at the point sources and matching filters laminated to contact lenses placed in the cat's eyes. The geometric principles of shadow casting and the use of chromatic filters for dichoptic stimulation are established techniques, the theory and practical applications of which are well known[13,14]. When the point source filters and eye filters are matched left and right and the centre rod is closer to the point sources, the resulting virtual image of the centre rod appears closer to the observer than the images of the side rods (crossed disparity). When the point source filters are interchanged, the virtual images are transposed and the centre rod appears farther from the observer (uncrossed disparity).

Stereoscopic depth discrimination was tested for 7 successive days, with four near depth trials (accompanied by shock) and four far depth trials each day. The centre rod was moved a distance which produced an angular disparity of 1° 9′ (adjusted for the cat's interpupillary distance) between the centre shadow and the side shadows. Numerous blank trials, consisting of an interchange of the point source filters without movement of the centre rod, were included in the schedule; these trials produced no changes in the cat's behaviour. On all trials the eyes of the cat were observed closely, but neither eye closure nor head movement was observed.

In Fig. 1, performance on the last 3 days of real depth discrimination and on the 7 days of stereoscopic depth discrimination is expressed in terms of a suppression ratio ($W/S+W$, where W refers to the number of licks during the 5 s between rod movement and shock, and S refers to the number of licks during the 5 s preceding rod movement). There is little overlap between the distributions of near and far stereoscopic depth suppression ratios during the 7 days, a significant difference ($P<0.01$) as measured by the non-parametric matched-pairs signed-ranks test.

It is not likely that the cat based its discrimination on a cue other than stereopsis. The intrinsic nature of the shadow caster precludes most cues. Control trials eliminated the operation of a potential compound cue based on filter interchange and lateral movement of the shadows. It is logically possible that the discrimination could be made if the cat could discern which shadow stimulated each eye (that is, possessed

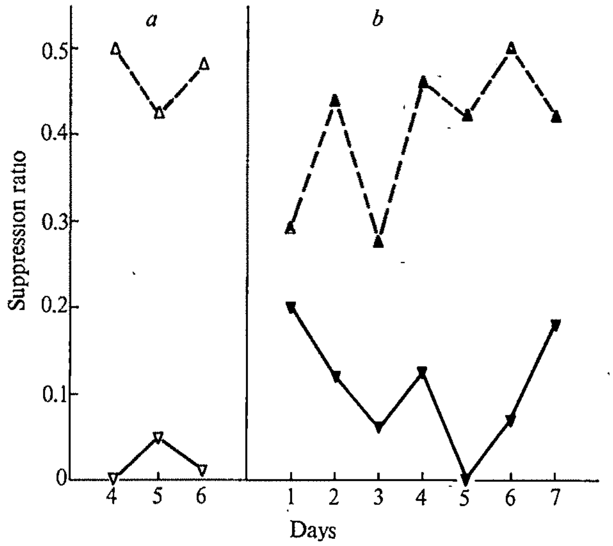


Fig. 1 Mean suppression ratios for (*a*) the last 3 days of real depth training and (*b*) 7 days of stereoscopic depth presentations. △, Backward; ▽, forward; ▲, uncrossed disparity; ▼, crossed disparity. Crossed disparity equals near stereoscopic depth; uncrossed disparity equals far stereoscopic depth. A ratio of 0.5 indicates no suppression; as ratios approach 0 suppression increases.

eye-of-origin information). Yet considerable research on humans indicates that eye-of-origin discriminations are virtually impossible[15]; humans who viewed our display could not make such discriminations. We think it unlikely that cats could readily do it when humans cannot.

Taken together, all considerations suggest that the cat possesses stereopsis and used it in making the conditioned suppression discrimination, an interpretation consistent with the neurophysiological data.

In this experiment a moving stimulus of large disparity was deliberately chosen to attract the cat's attention and, in general, to maximize the likelihood of demonstrating stereopsis. Now that the evidence suggests that the cat has some capacity for stereopsis, it should be possible to measure both the minimum and maximum limits of stereoscopic vision. Further, it may be possible to determine the ease with which cats can utilize the disparity information in random element stereograms[16,17].

We thank Drs R. Stroud, R. Althiasar and J. Greene for fitting, designing and constructing contact lenses. This study was supported in part by a US Public Health Service grant and a National Institute of Mental Health predoctoral fellowship.

ROBERT FOX
R. RANDOLPH BLAKE

Department of Psychology,
Vanderbilt University,
Nashville,
Tennessee 37203

[1] Barlow, H. B., Blakemore, C., and Pettigrew, J. D., *J. Physiol.*, **193**, 327 (1967).
[2] Joshua, O. E., and Bishop, P. O., *Exp. Brain Res.*, **10**, 389 (1970).
[3] Hubel, D. H., and Wiesel, T. N., *Nature*, **255**, 41 (1970).
[4] Bough, E. W., *Nature*, **255**, 42 (1970).
[5] Estes, W. K., and Skinner, B. F., *J. Exp. Psychol.*, **29**, 390 (1941).
[6] Brady, J. V., and Conrad, D. G., *J. Comp. Physiol. Psychol.*, **54**, 149 (1961).
[7] Kamin, L. J., *J. Comp. Physiol. Psychol.*, **53**, 128 (1960).
[8] Hoffman, H. S., Selekman, W. L., and Fleshler, M., *J. Exp. Anal. Behav.*, **9**, 659 (1966).
[9] Sidman, M., Ray, B., Sidman, R., and Klinger, J., *Exp. Neurol.*, **16**, 377 (1966).
[10] Hendricks, J., *J. Exp. Anal. Behav.*, **9**, 501 (1966).
[11] Shumake, S. A., Smith, J. C., and Tucker, D., *J. Comp. Physiol. Psychol.*, **67**, 64 (1969).
[12] Hefner, H., and Masterton, R. B., *J. Comp. Physiol. Psychol.*, **71**, 175 (1970).
[13] Gregory, R. L., *Nature*, **203**, 1407 (1964).
[14] Lee, D. N., *Vision Res.*, **9**, 145 (1969).
[15] Templeton, W. B., and Green, F. A., *Quart. J. Exp. Psychol.*, **20**, 200 (1968).
[16] Julesz, B., *Science*, **145**, 356 (1964).
[17] Anonymous neurophysiological correspondent, *Nature*, **216**, 1067 (1967).

Relation between Prostaglandin E_2 and Adrenaline Reversal in the Rat Uterus

THE inhibitory response of the rat uterus to adrenaline in the presence of isoprenaline inhibition can be reversed in induced oestrus but not in the progestational state, and it has been postulated that a motor substance accumulates during the isoprenaline inhibition and is released on addition of adrenaline[1]. The same sequence of events has also been observed when adrenaline was used as the initial inhibitory agent. The motor response to adrenaline is even more readily obtained in the late pregnant uterus but not in dioestrus[2].

Attempts have now been made to detect and identify the postulated motor substance. Many motor substances can be found in extracts of tissues, and to avoid confusion with these it was decided to look for the substance in the bath fluid, on the assumption that it was being released. The late pregnant uterus was chosen as a possible source because it apparently releases a greater quantity of the substance than when it is in induced oestrus, and adrenaline was used as the initial inhibitory agent. The dioestrous uterus was used to detect the substance, because reversal of adrenaline does not occur in this state. Mature female Sprague–Dawley rats weighing about 200 g were used. The stage of cycle was assessed by vaginal smearing. Pregnancies were dated and the day of finding sperm was denoted day 1. The animals were used on days 19–22. Uterine horns or strips were set up in organ baths according to a method described before[1,2]. Adrenaline (100 ng/ml.) was added to a normally contracting strip from a late pregnant uterus; immediate inhibition occurred, but after about 2 min reversal occurred and the preparation began to contract. The bath fluid was then removed and transferred to a bath containing a dioestrous horn which had been inhibited with adrenaline for the same time. Two minutes after addition of the fluid the preparation began to contract. The response was small at first, increased and then died away, after which the preparation remained inhibited (Fig. 1*a*). The bath fluid contained adrenaline, but this had no motor effect on the dioestrous uterus, and the result therefore shows that a motor substance was being released into the bath fluid from the pregnant strip.

The results of experiments with the appropriate blocking agents eliminated the possibility that the motor substance might be acetylcholine, histamine or 5-hydroxytryptamine. Among the remaining possibilities, it was decided to test for the presence of a prostaglandin. The bath fluid was removed and adjusted to *p*H 3 with N HCl and extracted twice with ethyl acetate. The extract was then evaporated to dryness on a rotary evaporator; the residue was dissolved in 1 ml. of distilled water and applied to the rat fundal strip[3]. When the active substance was present it was examined by thin-layer chromatography with the AI and AII solvent systems of Green and Samuelsson[4], using unequilibrated tanks. In ten determinations all biological activity was associated with a substance with a mean R_F of 0.66 in the AI solvent system. This behaved in a manner identical to that of a reference sample of authentic prostaglandin E_2 (R_F value 0.66), and the AII system showed it to be E_2. For confirmation parallel bioassays of the separated substance were done on guinea-pig ileum and rabbit jejunum. Prostaglandin E_2 was found in amounts varying from 30 ng to 1.5 μg in ten extracts. Prostaglandins

E_1, $F_1\alpha$ and $F_2\alpha$ were also looked for but were not found. No prostaglandin was found in bath fluids from ten control strips incubated in the absence of adrenaline. This eliminated the possibility that prostaglandin E_2 was liberated as a result of the manipulation to which the uterine tissue had been subjected.

The possibility remained that liberation of prostaglandin E_2 was purely incidental. Further experiments were therefore carried out with a reference sample of authentic prostaglandin E_2. Addition in a concentration of 20 ng/ml. to the bath fluid containing a dioestrous uterus was followed by a response which closely resembled in its course that produced by the motor substance liberated into the bath fluid (Fig. 1*b*). From this it seems that the liberation of prostaglandin E_2 was directly connected with the reversal of the inhibitory action of adrenaline.

From these results we conclude that the principal adrenergic action of the catecholamines on the rat uterus is stimulation of β-receptors resulting in inhibition. This supports the assertions of Levy and Tozzi[5]. In the presence of catecholamines prostaglandin E_2 is liberated and exerts a motor effect previously ascribed to stimulation of α-receptors in the rat uterus. Previous work has revealed anomalies in the behaviour of these receptors, and it has been suggested that they should be referred to as E-receptors pending further clarification[2]. It now seems likely that they are receptors for prostaglandin E_2. Adrenaline reversal in the rat uterus can therefore be explained by an initial β effect which is an intrinsic property of adrenaline, followed by a liberation of prostaglandin E_2 which then gives the opposite effect which has hitherto been interpreted as reversal.

Liberation of prostaglandin by catecholamines has also been observed in other organs (spleen[6], rat epididymal fat pad[7] and diaphragm[8]) and reversal of adrenaline has been documented for one of these[6]. Table 1 shows that there is a correlation between the reversal response and the effect of prostaglandin E_2 in organs other than the uterus. These responses are clearly identical, and there is therefore good reason for supposing that adrenaline reversal, wherever it occurs, is brought about by liberation of prostaglandin.

Table 1 Correlation Between the Adrenaline Reversal and Prostaglandin E_2 Responses in Various Preparations

Preparation	Adrenaline response	Adrenaline reversal response	Prostaglandin E_2 response
Rat uterus oestrogen dominated	Relaxation	Contraction	Contraction
Rat fundal strip	Relaxation	Contraction	Contraction
Gut	Relaxation	Contraction	Contraction[9]
Splenic vascular smooth muscle	Contraction[6]	Relaxation[6]	Relaxation[6]
Blood pressure	Rise	Fall[11]	Fall[10]

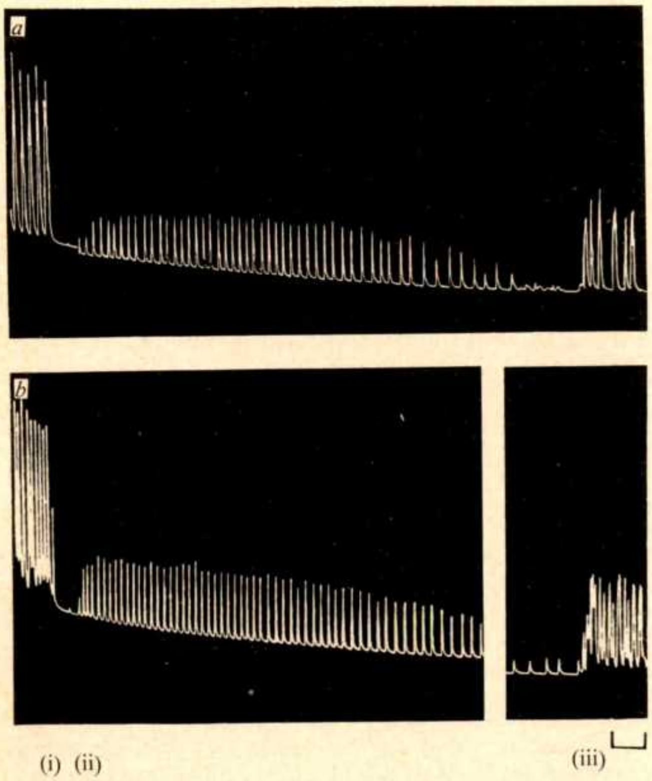


Fig. 1 Uterine horns from a rat in dioestrus. (i), Adrenaline was added to a final concentration of 0.1 μg/ml. (ii), *a*, addition of bath fluid in which a strip of uterus from a pregnant horn had been treated with the same concentration of adrenaline; *b*, prostaglandin E_2 added to a final concentration of 0.02 μg/ml. (iii) Wash. The break in *b* represents 52 min. Time marker, 5 min.

The results also indicate that the liberation of prostaglandin E_2 in the rat uterus is hormone-dependent, and this aspect of the problem and the site of liberation will be the subject of a further communication. We wish to thank Dr John Pike, of the Upjohn Co., Kalamazoo, for prostaglandin E_2.

ANNE TOTHILL

Department of Pharmacology,
The London Hospital Medical College,
*London E*1

LEON RATHBONE

Department of Zoology,
Birkbeck College

EVE WILLMAN

Department of Pharmacology,
School of Pharmacy,
*London WC*1

Received March 25; revised June 3, 1971.

[1] Tothill, A., *Nature*, **213**, 1230 (1967).
[2] Tothill, A., *Brit. J. Pharmacol.*, **29**, 291 (1967).
[3] Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344 (1957).
[4] Green, K., and Samuelsson, B., *J. Lipid Res.*, **5**, 117 (1964).
[5] Levy, B., and Tozzi, S., *J. Pharmac. Exp. Ther.*, **142**, 178 (1963).
[6] Davies, B. H., Horton, E. W., and Withrington, P. G., *Brit. J. Pharmacol.*, **32**, 127 (1968).
[7] Shaw, J. E., *Fed. Proc.*, **25**, 770 (1966).
[8] Ramwell, P. W., Shaw, J. E., and Kucharski, J., *Science*, **149**, 1390 (1965).
[9] Bergstrom, S., Eliasson, R., Von Euler, U. S., and Sjövall, J., *Acta. Physiol. Scand.*, **45**, 133 (1959).
[10] Du Charme, D. W., and Weeks, J. R., *Nobel Symp.* 2, *Prostaglandins*, 173 (Almqvist and Wiksell, Stockholm 1966).
[11] Butterworth, K. R., *Brit. J. Pharmacol.*, **21**, 378 (1963).

Intrinsic Limitations in the Use of Electroantennograms to Bioassay Male Pheromones in Lepidoptera

BEHAVIOURAL assays for the male pheromone of *Trichoplusia ni* (Lepidoptera: Noctuidae) are difficult to carry out because the female apparently lacks an overt behavioural response and mating only occurs at night in very dim illumination. Grant used the electroantennogram (EAG) as an assay[1], but although this technique has developed into a useful tool to bioassay female sex pheromones in Lepidoptera, it does not seem to be a biologically meaningful approach to this receptor system.

The EAG technique is based on the fact that the high sensitivity of many male Lepidoptera to female sex pheromone is achieved by a large proportion, up to 50%, of "odour specialist" receptors which respond at high sensitivity solely to the female sex pheromone[2]. The summated response of these receptors over the length of the antenna gives the measured EAG d.c. potential change. Potential differences induced in neighbouring dendrites also summate over the length of the antenna to reinforce the effect. Only 200 pheromone molecules are needed to signal a behavioural response in males of *Bombyx mori*[3] and a single molecule is sufficient to elicit firing in one of the specialist cells.

On the other hand, the male pheromones of Noctuidae are released in large quantity, about 0.1 mg in *Leucania pallens*[4], very close to the female[5] and so to the target receptors. With so much pheromone present at the receptors, there is little relevance, in relation to natural behaviour, to behavioural or EAG measurements of threshold responses. Grant[1] shows that the excized hairpencils of one male *T. ni* cause a depolarization of 1.0 mV in both male and female antennae, whereas tufts of hair from elsewhere on the male body do not evoke a response. The EAG, however, does not show that the male can distinguish the stimulus as a pheromone from the general olfactory spectrum of the species.

I have recorded EAGs in response to male scent in a number of Noctuidae. Standard procedures were followed using KCl filled glass pipette electrodes and immobilized whole moths rather than isolated heads or antennae. EAG responses to stimuli of conspecific male brushes, to cross-specific brushes and to stimulus equivalent extracts did not differ significantly for each species regardless of whether recording from male or female antennae. Levels of EAG depolarization varied between species, but EAGs within a species were consistent in response to the stimulus of brushes from all other species used. The values obtained were: *Leucania pallens* (L.), −3.4 (±0.3) mV; *Leucania conigera* (Schiffermueller), −3.3 (±0.3) mV; *Apamea monoglypha* (Hufnagel), −2.0 (±0.2) mV; *Phlogophora meticulosa* (L.), −2.6 (±0.2) mV; and *Mamestra persicariae* (L.), −2.1 (±0.3) mV. Stimulus equivalent quantities of compounds isolated from the male brushes and various related standard laboratory compounds elicited EAGs indistinguishable from those produced by the male brushes for a range of dilutions. The isolated compounds are low molecular weight aromatics, monoterpenes and simple carboxylic acids[6]. Positive responses to such a range of compounds are not considered to be artefacts since many other related compounds do not evoke EAG responses.

Grant shows that the female sex pheromone of *T. ni* gives an EAG in the female antenna of about the same size (1 mV) as that produced in both sexes by the male brushes, whereas the female sex pheromone evokes a larger (3 mV) response in the male antenna. Grant uses Callahan's evidence of sexual dimorphism in the antennae of *Heliothis zea*[7] to suggest that perception by morphologically distinct receptors on male and female antennae explains the difference. Jefferson *et al.*[8], however, have shown that there are negligible differences in the number and distribution of sensillae between the sexes in *T. ni* There is greater variation between individuals and between antennae on the same moth. This does not support Grant's hypothesis and it seems more likely that one type of sensilla has become functionally differentiated and very narrowly specialized in the female alone.

The male releases pheromones so close to the female that there has been no selective pressure to develop a receptor which responds preferentially to minute levels of one substance. The receptors which do respond are the "odour generalists"[2], each of which has a different odour spectrum signalled, for example, by latency of rate of firing, or by speed of adaptation to a given odour. Signals from the odour generalists are deciphered by the central nervous system, and it would require an analogous detailed analysis of nerve spikes, or single cell recordings, to separate clearly the response to one odour or component thereof from that to another. EAG responses can be obtained from these receptors but, as a similar number of receptors respond to similar concentrations of many odorous compounds within the olfactory spectrum of the species, the summated EAG recordings are also similar.

EAGs show only that the male Noctuid pheromones can be perceived peripherally. For a more detailed knowledge of the way in which these chemicals stimulate or modify specific behaviour, there is no present alternative to using a behavioural assay. It is dangerous to use techniques based on response at low threshold levels by odour specialist cells to monitor processes based on neither. We still do not know what information is carried by nerve impulses, how it is integrated by the central nervous system, or even the difference between excitatory and inhibitory impulses. Where EAGs are used to investigate the behaviour of insects to olfactory stimuli, it is essential that they are closely correlated with at least laboratory behavioural studies and preferably field investigations.

I thank Drs D. Schneider and E. Priesner for very valuable discussions at Seewiesen and Drs R. T. Aplin, G. G. Grant, T. L. Payne and D. L. Wood for much helpful advice and criticism. The Anti-Locust Research Centre, London, allowed me initial use of a recording apparatus and Mr R. Yeadon gave help in its use.

MARTIN C. BIRCH

Department of Entomology,
University of California,
Berkeley, California 94720

Received April 25; revised June 8, 1971.

[1] Grant, G. G., *Nature*, **227**, 1345 (1970).
[2] Schneider, D., *Science*, **163**, 1031 (1969).
[3] Kaissling, K.-E., and Priesner, E., *Naturwissenshaften*, **57**, 23 (1970).
[4] Birch, M. C., *Trans. R. Entomol. Soc. Lond.*, **122**, 277 (1970).
[5] Birch, M. C., *Anim. Behav.*, **18**, 310 (1970).
[6] Aplin, R. T., and Birch, M. C., *Experientia*, **26**, 1193 (1970).
[7] Callahan, P. S., *Res. Bull. Univ. Georgia, Coll. Agric. Exp. Stn.*, 54 (1969).
[8] Jefferson, R. N., Rubin, R. E., McFarland, S. U., and Shorey, H. H., *Ann. Entomol. Soc. Amer.*, **63**, 1227 (1970).

Acute Effect of Oral and Subcutaneous Administration of Monosodium Glutamate on the Arcuate Nucleus of the Hypothalamus in Mice and Rats

RECENT reports by Olney that monosodium glutamate (MSG) can cause acute neuronal necrosis in the developing brain of mice[1-3], rats[1] and monkeys[4], have been the subject of intensive debate[5-11]. Some people[9,11] deny that a single subcutaneous dose of monosodium glutamate can produce any brain damage in infant rats. Although Arees and Mayer[10] confirm the existence of a necrotic lesion in both mouse and rat brain they restrict the pathology to "microglial" cells. None of these investigators attempted actually to replicate Olney's methods but used entirely different methods of preservation and staining.

Because any reports of this nature demand independent confirmation, a blind study was undertaken to determine both the presence or absence of and the consistency of an MSG effect on the developing hypothalamus of mice using the exact methods of administration of MSG, fixation and staining outlined by Olney[2] (Table 1). Further studies were completed in a similar experimental situation on Wistar rats using Olney's techniques as well as those of Adamo and Ratner[9] (Table 1).

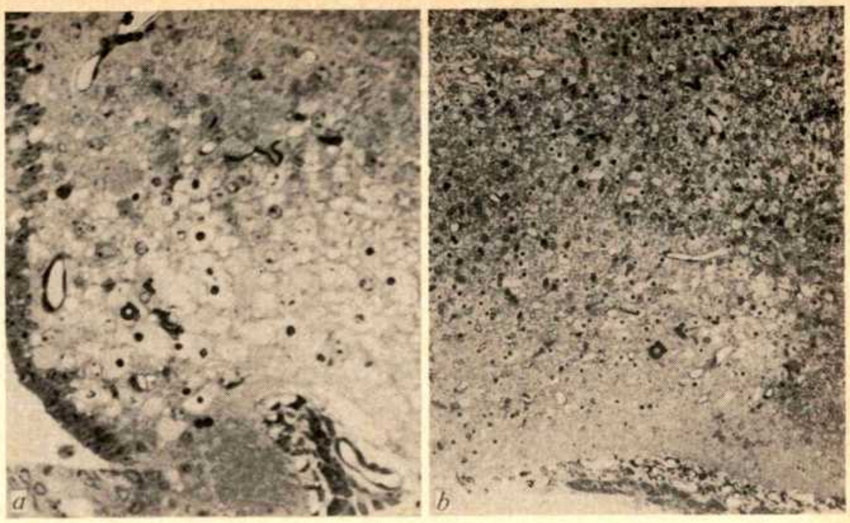


Fig. 1 *a*, Section through the hypothalamus of 10 day old Swiss albino mouse 5 h after oral administration of 1 mg/g of MSG, illustrating a distinct lesion in the region of the arcuate nuclei (AR). The neuronal elements are necrotic with a bloated cytoplasm and pyknotic nuclei (~ × 650). *b*, Section through the hypothalamus of 4 day old Wistar rat 5 h after oral administration of 4 mg/g of MSG, demonstrating a massive lesion involving the arcuate (AR) and ventromedial (VM) nuclei (~ × 450).

Monosodium glutamate was administered either orally or by subcutaneous injection. Sections for light microscopy were read independently by two of us who were unaware of the treatment schedule. Sections for electron microscopy were collected on slot grids and stained with uranyl acetate and lead citrate.

Table 1 Treatment Schedule and Fixation

Animal	Method of administration	Treatment MSG	NACl/saline	Perfusion method
Mouse	Oral	9 (1 mg/g)	9	Olney
Rat	Oral	5 (4 mg/g)	4	Olney
Rat	Oral	4 (4 mg/g)	4	Adamo and Ratner
Rat	Subcutaneous	6 (4 mg/g)	4	Adamo and Ratner
Rat	Subcutaneous	2 (4 mg/g) 2 (2 mg/g)	2	Olney

Light microscopic examination of the brains of infant mice treated orally with monosodium glutamate at a dose of 1 mg/g revealed that all the animals developed lesions in the arcuate area of the hypothalamus and median eminence (Fig. 1*a*), with complete correlation between independent examiners. No evidence of cellular pathology was noted in the controls. Light microscopic examination of the brains of infant rats treated by either route of administration with either 2 mg/g or 4 mg/g of monosodium glutamate similarly demonstrated arcuate lesions in all treated animals (Fig. 1*b*) with an absence of cellular pathology in control animals. Lesions were much more extensive in rats than mice, probably reflecting either the higher dosage used or possibly the younger age of the rats.

The infant rat brains perfused with formalin were extremely fragile. Tissue preservation was not satisfactory for photography, but the affected areas could not be identified and were in the periventricular arcuate area. The lesion could be seen better when haematoxylin and eosin were used instead of cresyl violet.

Examination of both rat and mouse material with the electron microscope revealed necrosis of the neuronal components of the arcuate nucleus. Perikaryon swelling, loss of cytoplasmic density and organelles and nuclear pyknosis are evident. The neuronal nature of these cells, at least in the mouse, is demonstrated by the presence of synaptic contacts both on cell bodies and dendrites (Fig. 2). The presynaptic components, that is, vesicles and membrane density, remain intact, while there is dissolution of the postsynaptic structure in the involved cells.

A more recent report by Olney[3] of studies using both light and electron microscopy, outlines the development of monosodium glutamate-induced lesions from acute neuronal necrosis through biological degradation and phagocytosis to an apparently normal residual cytology except for a markedly decreased population of neurones in the arcuate area. Thus, the photomicrographs of Adamo and Ratner[9], who examined only adult brains by electron microscopy and found no evidence

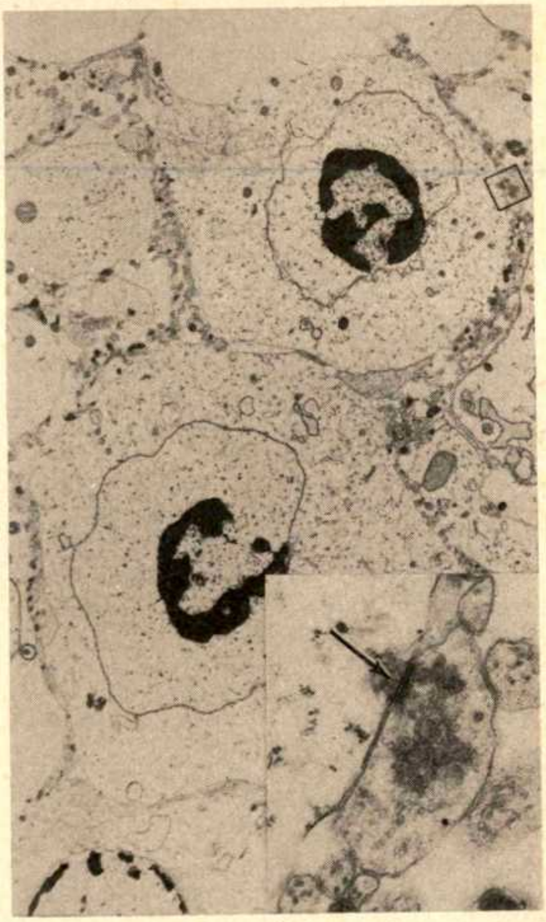


Fig. 2 Electron micrograph of degenerating neurones illustrating a dissolution of cytoplasmic components and alteration of the nuclear chromatin pattern. All the cytoplasmic structures have degenerated beyond recognition (× 12,000). The inset on the lower right is a higher magnification of the area within the box. Apparently normal axon terminal with presynaptic vesicles and increased membrane density (arrow) in synaptic contact with the soma of a degenerating cell. A remnant of postsynaptic structure is evident (× 50,000).

of cellular pathology at the ultrastructural level, are entirely consistent with Olney's[1].

The question remains why neither Adamo and Ratner[9] nor Oser *et al.*[11] found any MSG effect in their light microscopic studies of infant brains of animals. One can only speculate that the method of fixation and staining used by Adamo and Ratner obscured the existence of the lesion. On the other hand, Oser *et al.*[11] used a minimal effective dose and did not examine the rats and mice until 24 h after insult. Olney[3] has demonstrated that by 24 h after insult, all signs of oedema have disappeared and that necrotic cells are already being phagocytized. Oser *et al.*[11] reported finding occasional areas of small pyknotic neurones, large cytoplasmic vacuoles and scattered inflammatory cells. This is entirely compatible with a lesion late in evolution which would be expected if the insult was minimal.

The failure of Adamo and Ratner to find evidence of endocrine dysfunction is not surprising in face of their dose schedule. Olney[1] states that mice treated with a single dose of MSG are obese compared with control litter mates, but he does not allude to any reproductive dysfunction in this group. Reproductive dysfunction has been reported only in the group of animals treated with consecutive doses of MSG during infancy[12,13].

The interpretation by Arees and Mayer[10] that the lesion produced by MSG is limited to "microglia" but totally spares hypothalamic neurones is puzzling. Most cells normally present in the arcuate nucleus are small neurones[14]. Olney[3] has demonstrated, and we have confirmed, that most of the cells of the arcuate nucleus manifest extreme degenerative changes 5 h after MSG treatment even at the light microscopic level. The demonstration of synaptic involvement by Olney[3] and in this article, as well as the previously documented nature of the effect of MSG in the retinae[15–17], clearly points to a lesion involving neurones. These synapses were documented both on the soma and dendrites of damaged cells. Although there is considerable confusion surrounding the concept of "microglia", it is reasonably clear that axon terminals do not synapse on either the processes or the cell bodies of "microglia".

The purpose of this article is not to enter the debate on the safety of monosodium glutamate but merely to confirm or deny previous results in a scientifically valid manner. Using the exact experimental protocol described by Olney and Ho[2], and blind analysis of experimental and control specimens, we have confirmed their claim that 100% of infant mice treated orally with 1 mg/g of MSG develop hypothalamic lesions but that animals subjected to an equivalent oral dose of sodium chloride do not. The similar effect of MSG on the infant rat brain has been demonstrated by both the oral and subcutaneous route of administration. Finally, we believe that the exact duplication of experimental protocols, including age of animals, route of administration, doses and method of fixation, and careful interpretation of obtained results, in the light of carefully read previous reports, is necessary before valid comparisons can be made.

This work was supported in part by grants from the National Eye Institute. Animals were supplied by National Laboratory Animals, Missouri, and MSG was supplied by Sigma Chemical Company.

R. M. Burde
B. Schainker
J. Kayes

Department of Ophthalmology
and Oscar Johnson Institute,
Washington University School of Medicine,
Saint Louis,
Missouri 63110

Received March 5; revised May 11, 1971.

[1] Olney, J. W., *Science*, **164**, 719 (1969).
[2] Olney, J. W., and Ho, O.-L., *Nature*, **227**, 609 (1970).
[3] Olney, J. W., *J. Neuropathol. Exp. Neurol.*, **30**, 75 (1971).
[4] Olney, J. W., and Sharpe, L. G., *Science*, **166**, 386 (1969).
[5] Lowe, C. U., *Science*, **167**, 1016 (1970).
[6] Zavon, M. R., *Science*, **167**, 1017 (1970).
[7] Olney, J. W., and Sharpe, L. G., *Science*, **167**, 1017 (1970).
[8] *Subcommittee on Safety and Suitability of MSG and other Substances in Baby Foods* (National Academy of Sciences—National Research Council, 1970).
[9] Adamo, N. J., and Ratner, A., *Science*, **169**, 673 (1970).
[10] Arees, E. A., and Mayer, J., *Science*, **170**, 549 (1970).
[11] Oser, B. L., Carson, S., Vogen, E. E., and Cox, G. E., *Nature*, **229**, 411 (1971).
[12] Knittle, J. J., and Ginsberg-Follner, F., *Amer. Pediat. Soc. Bull.* (program abstract, April 29, 1970).
[13] Redding, R. W., and Schally, A. V., *Fed. Proc.*, **29** (1970).
[14] Christ, J. F., in *The Hypothalamus* (edit. by Haymaker, W., Anderson, E., and Nanta, W.), 36 (Thomas, Springfield, Illinois, 1969).
[15] Lucas, D. R., and Newhouse, J. P., *Amer. Med. Assoc. Arch. Ophthal.*, **58**, 193 (1957).
[16] Cohen, A. I., *Amer. J. Anat.*, **120**, 319 (1967).
[17] Olney, J. W., *J. Neuropathol. Exp. Neurol.*, **28**, 455 (1969).

Comments on "Neuromythology"

The comments by Comfort on Dawkins's[1] memory theory struck a sympathetic chord, late though they were received here. We failed to detect any of the changes claimed[2] for the nucleus dentatus and found but a fraction[3] of the neurones estimated. Counts covering newborn to presenescent age produced no age correlation in the human olivary nucleus[4]. The need for the provision of more cell counts stressed by Comfort was emphasized too by Blinkow[5] at a recent meeting here. Neuronal population studies done by count are certainly more tedious than those based on general estimates. They render figures more like those of bankers than of economists. Delorenzo[6] found no evidence for the loss of Purkinje cells in man reported by Ellis[7]. Thus not only for rodents, but in man too, the more recent studies based on larger material, fail to demonstrate loss of neurones with advancing age. Available information about the regular Purkinje cell layer is so contradictory, the multilayered cerebral cortex with cell number estimates ranging[8,9] from 2.6×10^9 to 16.5×10^9, that it is even less likely to provide unequivocal data easily. The billion range is not of variability but one of uncertainty. Side by side illustrations demonstrating age changes suffer from what may be called the Kara Mustapha fallacy *. Recent work by Dayan[10] raises doubts about some more tenets of the neuropathology of ageing. Dawkins's model of memory helps to bring to light the narrow base supporting neurone loss as an uncontestable axiom.

Joseph Tomasch

Department of Anatomical Sciences,
Pahlavi University,
Shiraz,
Iran

Received May 17, 1971.

---

* Unsuccessful in his siege of Vienna, Kara Mustapha was presented with a silken rope by the sultan's emissary. Museum guides maintain that the skull on display in Budapest is of an earlier period than the one in the Vienna museum.

[1] Dawkins, R., *Nature*, **229**, 118 (1971).
[2] Hoepker, W., *Z. Alternsforsch.*, **5**, 256 (1951).
[3] Heidary, H., and Tomasch, J., *Acta. Anat.*, **74**, 290 (1969).
[4] Moatamed, F., *J. Comp. Neurol.*, **128**, 109 (1966).
[5] Blinkow, S. M., *Proc. Pahlavi Med. Congr.*, **2**, 181 (1971).
[6] Delorenzo, E., *Z. Zellforsch. Mikr. Anat.*, **14**, 310 (1932).
[7] Ellis, R. S., *J. Comp. Neurol.*, **32**, 1 (1920).
[8] Pakkenberg, H., *J. Comp. Neurol.*, **128**, 17 (1966).
[9] Haug, H., and Rebhahn, I., *Acta. Anat.*, **28**, 551 (1956).
[10] Dayan, A. D., *Acta Neuropathol.*, **16**, 85 (1970).

Sensitivity of Fish ATPases to Polychlorinated Biphenyls

In spite of widespread concern about the possible hazards of polychlorinated biphenyls (PCBs)[1–11], little is known about the physiological action of these materials. We have therefore examined the effect of PCBs on the ATPase enzyme system, which is known to be sensitive to chemically similar compounds such as DDT[12–18].

It is known that PCB ('Aroclor 1242') will cause oedema and depress growth when fed to chicks in large amounts (200 and 400 p.p.m. in diet), the rate of steroid metabolism is increased in liver homogenates from birds dosed with PCBs and that the materials stimulate oestrogenic activity in the rat uterus[10,11]. We have examined the activity of the ATPase enzyme system in homogenized and fractionated tissues of the blue gill fish *Lepomis machrochirus*, because this system is sensitive to DDT and related compounds and because distinctive differences in effect have been found among cyclodiene-type chlorinated hydrocarbons.

Tissues were dissected, homogenized and fractionated according to the procedure reported by Koch[18]. The fraction used (B) contained mitochondria plus nerve endings. Each preparation was diluted appropriately, quick frozen in liquid nitrogen and stored at −20° C. ATPase activities were determined essentially according to the enzymatic procedure described before[19–21]. Proteins were determined by the method of Lowry *et al.*[22]. Reaction mixtures used are given in Table 1.

Table 1 Percentage Inhibiton of Mg^{2+} ATPase from Muscle of Blue Gill Fish by PCB Preparations *

Concentration (p.p.m.)	Percentage inhibition 'Aroclor 1221'	'Aroclor 1254'	'Aroclor 1268'	'Aroclor 5460'
0.03	18.7	20.6	37.2	31.9
0.33	29.9	30.6	47.1	42.9
3.33	65.4	73.1	60.3	—
10.00	80.4	84.3	67.5	—

A 3 ml. reaction mixture contained compounds at the following concentrations: 4.3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 135 mM imidazole buffer (*p*H 7.5), 0.19 mM NADH, 0.5 mM PEP, 0.02% BSA, approximately 9 U pyruvate kinase and 12 U lactic dehydrogenase, and 100 µl. homogenate fraction. Absorbance changes were measured at 340 nm for 15 min using a Beckman DU spectrophotometer with temperature controlled at 27° C in reaction mixture. When used, ouabain was present at 0.85 mM. Total ATPase activity was determined in the presence of Na^+, K^+ and Mg^{2+}. Mg^{2+} ATPase activity was measured using same mixture plus ouabain. Na^+–K^+ ATPase activity is total activity minus Mg^{2+} ATPase activity[20,21]. Ethanol was used as solvent for the 'Aroclor' except 'Aroclor 1268', where acetone was used. Between 1 and 5 µl. of the 'Aroclor' solutions was added to the enzyme reaction mixture by slowly releasing the solution from a Hamilton microsyringe into a rapidly stirred reaction mixture.

* Each value represents the mean from three separate preparations. Specific activities (µmol Pi mg^{-1} protein h^{-1}; mean ±s.e.) for untreated muscle Mg^{2+} ATPase and Na^+–K^+ ATPase were 57.1±2.2 and 2.3±0.8 respectively. Low specific activity of Na^+–K^+ ATPase in muscle made accurate determinations of inhibition very difficult.

Three PCB preparations, 'Aroclor 1221', 'Aroclor 1254' and 'Aroclor 1268', and one polychlorinated terphenyl, 'Aroclor 5460' (Monsanto) were used. The last two digits of the name give the percentage chlorination of each compound, but the exact composition is not available. All compounds inhibited ATPases in low concentrations (less than 1 p.p.m.) with the most pronounced inhibition on Mg^{2+} ATPase of muscle (Tables 1 and 2). The higher sensitivity of muscle Mg^{2+} ATPase to PCB compounds is consistent with the high sensitivity of this enzyme to DDT[13–15].

'Aroclor 1254' was the most effective inhibitor of ATPases, especially Mg^{2+} ATPase, from all tissues (Tables 1 and 2). 'Aroclor 1268', an effective inhibitor of muscle Mg^{2+} ATPase (Table 1), differed from the other PCB compounds in having minimal effects on Mg^{2+} ATPase activity from other tissues (Table 2). 'Aroclor 5460' seemed to be intermediate between 'Aroclor 1268' and the other 'Aroclor' compounds in its effect on Mg^{2+} ATPase activity (Table 2). The variation between tissues, most prominent with 'Aroclor 1268', indicates that the characteristics of Mg^{2+} ATPase differ depending on tissue source. A 30–40% inhibition of Na^+–K^+ ATPase measured only on brain and kidney preparations was consistently determined for all PCBs.

Table 2 Inhibition of Mg^{2+} ATPase and Na^+–K^+ ATPase from Tissues of Blue Gill Fish

Concentration (p.p.m.)	Percentage inhibition * 'Aroclor 1221'		'Aroclor 1254'		'Aroclor 1268'		'Aroclor 5460'	
	Mg^{2+}	Na^+–K^+	Mg^{2+}	Na^+–K^+	Mg^{2+}	Na^{2+}–K^+	Mg^{2+}	Na^+–K^+
(*A*) Brain								
0.03	0.3	4.6	4.2	16.9	0.4	29.4	2.3	26.9
0.33	8.4	10.5	0.7	19.1	+1.5†	27.6	2.9	26.3
3.33	7.2	20.4	27.7	41.5	+14.0	43.6	2.5	38.9
10.00	36.0	46.4	57.9	45.5	8.3	39.4	11.2	42.7
(*B*) Kidney								
0.33	7.4	15.1	1.3	35.2	0.0	25.4	0.0	22.3
3.33	5.0	24.3	33.4	45.9	+7.9	40.1	4.5	25.2
10.00	42.8	43.3	60.1	51.7	+4.8	48.0	+1.4	33.8
(*C*) Liver								
0.03	2.2		3.9		—		—	
0.33	2.6		19.4		+5.3		6.6	
3.33	2.9		24.3		+5.1		9.3	
10.00	35.8		53.0		+2.4		15.8	
16.70	45.9		74.1		6.2		—	

A, Specific activities for untreated brain, Mg^{2+} ATPase 25.2±1.0, Na^+–K^+ ATPase 35.8±2.1.

B, Specific activities for untreated kidney, Mg^{2+} ATPase 31.3±1.3; Na^+–K^+ ATPase 42.0±0.7.

C, Specific activities for untreated liver, Mg^{2+} APTase 28.5±3.0; Na^+–K^+ APTase 2.8±0.8; low specific activity of Na^+–K^+ ATPase in liver made accurate determinations of inhibition very difficult.

For reaction conditions see Table 1 except that temperature was 37° C.

* Each value represents the mean from three separate preparations.

† Plus values represent percentage activation of enzymatic activity.

In general, the PCB preparations do not reveal any obvious relationships between percentage chlorination and inhibitory effects. But the differences in action of 'Aroclor 1268' on Mg^{2+} ATPase from different tissues and the similarities in effects between PCBs and DDT require further study.

This research was supported by a grant from the US Department of Interior.

H. H. YAP
D. DESAIAH
L. K. CUTKOMP

Department of Entomology,
Fisheries and Wildlife,
University of Minnesota, St Paul

R. B. KOCH

Honeywell Research Center,
Hopkins, Minnesota 55343

Received January 11; revised May 24, 1971.

[1] Anderson, D. W., Hickey, J. J., Risebrough, R. W., Hughes, D. F., and Christensen, R. E., *Canad. Field Naturalist*, **83**, 89 (1969).

[2] Holmes, D. C., Simmons, J. H., and Tatton, J. O'G., *Nature*, **216**, 227 (1967).

[3] Koeman, J. H., Ten Noever De Brauw, M. C., and De Vos, R. H., *Nature*, **221**, 1126 (1969).

[4] Jensen, S., Johnels, A. G., Olsson, S., and Otterlind, G., *Nature*, **224**, 247 (1969).

[5] Risebrough, R. W., Reiche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N., *Nature*, **220**, 1098 (1968).

[6] Duke, T. W., Lowe, J. I., and Wilson, jun., A. J., *Bull. Environ. Contam. Toxicol.*, **5**, 171 (1970).

[7] Peakall, D. B., and Lincer, J. L., *Bioscience*, **20**, 958 (1970).

[8] Biros, F. J., Walker, A. C., and Medbery, A., *Bull. Environ. Contam. Toxicol.*, **5**, 317 (1970).

[9] Flick, D. F., O'Dell, R. G., and Childs, V. A., *Poultry Sci.*, **44**, 1460 (1965).

[10] Lincer, J. L., and Peakall, D. B., *Nature*, **228**, 738 (1970).

[11] Bitman, J., and Cecil, H. C., *J. Agric. Food Chem.*, **18**, 1108 (1970).

[12] Koch, R. B., *J. Neurochem.*, **14**, 269 (1969).

[13] Koch, R. B., *Chem.-Biol. Interact.*, **1**, 199 (1969/70).

[14] Koch, R. B., Cutkomp, L. K., and Do, F. M., *Life Sci.*, **8**, 289 (1969).

[15] Cutkomp, L. K., Yap, H. H., Cheng, E. Y., and Koch, R. B., *Chem.-Biol. Interactions* (1971).

[16] Matsumura, F., Bratkowski, T. A., and Patil, K. C., *Bull. Environ. Contam. Toxicol.*, **4**, 262 (1969).

[17] Matsumura, F., and Patil, K. C., *Science*, **166**, 121 (1969).

[18] Koch, R. B., *J. Neurochem.*, **16**, 145 (1969).

[19] Pullman, M. E., Penefsky, H. S., Datta, A., and Racker, E., *J. Biol. Chem.*, **235**, 3322 (1960).

[20] Fritz, P. J., and Hamrick, M. E., *Enzymologia Acta Biocatalytica*, **30**, 57 (1966).

[21] Yap, H. H., and Cutkomp, L. K., *Life Sci.*, **9**, 1419 (1970).

[22] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

Effect of Pregnancy on the Isoantibody Response in Rabbits

WHEN an Rh-negative woman becomes pregnant for the first time with an Rh-positive foetus, the immune response to Rh-positive foetal red cells entering the maternal circulation is usually manifested by the appearance of antibodies after delivery or by priming without detectable antibodies, which only appear during a subsequent Rh-positive pregnancy[1]. The appearance of antibodies during the first pregnancy, in the absence of any known prior stimulus with Rh-positive blood, is not as rare as was formerly thought. A minimum of 1% of such women can be found to have antibodies at the end of the first preganancy, and they appear during the later months of ABO-compatible pregnancies[2].

Although the passage of foetal cells into the maternal circulation is most frequent and intense as the result of labour, it also occurs during pregnancy particularly during the third trimester[2]. The relative infrequency of antibodies at the end of the first pregnancy and the frequent delay in their first appearance after delivery has led to the suggestion that women possess a diminished immune responsiveness to foetal red cell antigens during pregnancy[3,4], but the only direct experimental evidence is that of Merritt and Galton[5], who found that the antibody response of three different species of experimental animal to sheep red blood cells, bacterial endotoxin and bovine γ-globulin respectively, was the same in pregnant as in non-pregnant animals.

To obtain further information on this point, but using an iso-antigenic system, random-bred Californian virgin female Hg^{A}-negative rabbits were mated and injected intravenously with 2×10^{9} Hg^{AF} red cells at varying intervals after mating. One blood donor was used throughout the experiment. In an initial group of rabbits (group 1) the injections were given 14 days after mating, and in those that became pregnant the interval between the stimulus and delivery of the litters was found to be 17–20 days. A second group of rabbits (group 2) was injected at approximately 21 days after mating, the interval between stimulus and delivery being 9–13 days. Samples of venous blood were obtained from each rabbit at the beginning of the experiment and at 10, 20 and 30 days after the injection of red cells. The sera were tested for haemagglutinating anti-Hg^{A} antibodies using papainized Hg^{A}-positive red cells.

Table 1 Anti-Hg^{A} Response in Rabbits Injected 14 days after Mating

	Pregnant rabbits 20 days after stimulus	Pregnant rabbits 30 days after stimulus	Non-pregnant rabbits 20 days after stimulus	Non-pregnant rabbits 30 days after stimulus
Anti-Hg^{A} present	10 (55.6%)	10 (55.6%)	7 (41.2%)	9 (53.0%)
Anti-Hg^{A} absent	8	8	10	8
Totals	18	18	17	17

Tables 1 and 2 give the results of testing for antibodies in the two groups of rabbits, which are divided into those that became pregnant as a result of the mating and those that did not, the latter acting as controls. (The overall incidences of anti-Hg^{A} in the control rabbits was 51% which is very close to the 56% incidence found in a group of non-mated control animals in a previous study[6].) The incidence of positive tests for antibodies is given both for tests taken at the time when the pregnant rabbits reached the end of gestation and for tests taken 30 days after the stimulus. The latter are included because the pregnant state might have influenced not only the development of antibodies up to the point of delivery, but also their appearance in the post-gestational period. Previous experience[6] had shown that if antibodies were to develop they had done so by 30 days after the stimulus.

Table 2 Anti-Hg^{A} Response in Rabbits Injected 21 Days after Mating

	Pregnant rabbits 10 days after stimulus	Pregnant rabbits 30 days after stimulus	Non-pregnant rabbits 10 days after stimulus	Non-pregnant rabbits 30 days after stimulus
Anti-Hg^{A} present	7 (50%)	12 (85.7%)	11 (32.4%)	17 (50%)
Anti-Hg^{A} absent	7	2	23	17
Totals	14	14	34	34

In group 1, samples taken 20 days after the stimulus, that is, at a time when pregnant rabbits reached the end of gestation, showed that 41.2% of non-pregnant rabbits had antibodies, while these were detected in 55.6% of pregnant animals. The difference is not significant. During the subsequent 10 days, two further non-pregnant rabbits developed antibodies, the final incidences in the two groups becoming almost identical. In group 2, tests or samples taken 10 days after the stimulus, that is, at a time when the pregnant rabbits were at the end of their gestation period, showed that 32.4% of non-pregnant rabbits had antibodies, compared with 50% of pregnant animals. During the subsequent 20 days a further eleven developed antibodies, the final incidences being 50% for non-pregnant and 85.7% for pregnant animals.

There is thus no evidence that during pregnancy the rabbits showed any diminished unresponsiveness to Hg^{A}-positive red cells. The peak titres of antibody reached in responding rabbits varied from 1 : 1 to 1 : 256, and there was no difference in the distribution of titres between pregnant and non-pregnant rabbits. There is a suggestion of an increased responsiveness in group 2 pregnant rabbits, but the difference from controls is not significant. The litters included both Hg^A-positive and Hg^A-negative rabbits, and although it was not possible to blood group all the newborn animals, analysis of those that were grouped showed that whether or not the mother developed antibodies was unrelated to the blood groups of the litter. Spontaneous immunization of Hg^A-negative rabbits by their Hg^A-positive foetuses has not been demonstrated[7], and in the present experiment, animals carrying Hg^A-positive foetuses appeared no more tolerant of the injected Hg^A-positive red cells than did those carrying only Hg^A-negative foetuses.

Evidence for a depressed immune response during pregnancy comes largely from experimental allograft studies. It has been found that depression of allograft rejection during pregnancy may be (*a*) non-specific, that is, random grafts are better tolerated by pregnant than non-pregnant animals[8-10], or (*b*) specific in that allografts possessing paternal antigens are better tolerated than other allografts[9,10].

It has been suggested[10,11] that specific unresponsiveness may be related to the development in the mother of enhancing antibodies, and Currie was able to transfer such unresponsiveness to a paternal strain tumour with serum from interstrain pregnant mice. The ability to develop certain types of antibodies to paternal antigens may thus be an important event in pregnancy. Anti-leucocyte antibodies occur frequently in human pregnancy and are not uncommon even in a first pregnancy[12]. This study shows that iso-antigens on red cells of the rabbit are capable of producing a normal antibody response in pregnant females.

We thank Professor Carl Cohen for typing rabbits and supplying antisera.

J. C. Woodrow
C. J. Elson
W. T. A. Donohoe

Nuffield Unit of Medical Genetics,
Department of Medicine,
University of Liverpool

Received May 13; revised June 1, 1971.

[1] Woodrow, J. C., and Donohoe, W. T. A., *Brit. Med. J.*, **4**, 139 (1968).
[2] Woodrow, J. C., *Rh Immunization and its Prevention* (Munksgaard, Copenhagen, 1970).
[3] Gorman, J. G., Freda, V. J., Pollack, W., and Robertson, J. G., *Bull. NY Acad. Med.*, **42**, 458 (1966).
[4] Ascari, W. Q., Levine, P., and Pollack, W., *Brit. Med. J.*, **1**, 399 (1969).
[5] Merritt, Katherine, and Galton, M., *Transplantation*, **7**, 562 (1969).
[6] Elson, C. J., Woodrow, J. C., and Donohoe, W. T. A., *Vox Sang.* (in the press).
[7] Anderson, J. R., *Brit. J. Haematol.*, **2**, 44 (1956).
[8] Heslop, R. W., Krohn, P. L., and Sparrow, E. M., *J. Endocrinol.*, **10**, 325 (1954).
[9] Anderson, J. M., *Nature*, **206**, 786 (1965).
[10] Currie, G. A., in *Foetal Autonomy, Ciba Foundation Symposium* (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 32 (Churchill, London, 1969).
[11] Kaliss, N., and Dagg, M. K., *Transplantation*, **2**, 416 (1964).
[12] Overweg, J., and Engelfriet, C. P., *Vox Sang.*, **16**, 97 (1969).

Brain Catecholamines and Human Sleep

We have previously noted what seemed to be marked discrepancies between human and other mammalian species in the association of serotonin and sleep[1-3]. Furthermore, L-dopa, the immediate precursor of dopamine and noradrenaline, by producing a decrease in human rapid eye movement (REM) sleep[4] (an opposite effect from the predictions based on animal studies[5]), indicated that there might also be differences between human and other mammalian species in the adrenergic system. To further determine the role of the adrenergic nervous system in human sleep we studied the effect of depleting brain catecholamines by administration of alpha-methyl-*d* l-paratyrosine (AMPT) and alpha-methyl-*P*-phenylalanine (AMPHe), which are potent and specific inhibitors of catecholamine synthesis[6,7].

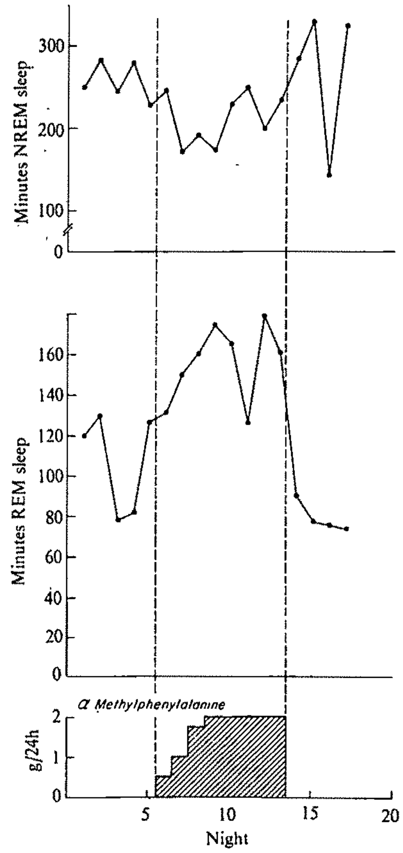


Fig. 1 Changes in rapid eye movement (REM) and non-rapid eye movement sleep (NREM) in patient (S. M., 42♀) taking alphamethylphenylalanine.

Five men and three women (two with essential hypertension, four with pheochromocytoma, one with Huntington's chorea and one with dystonia musculorum deformans) were studied by continuous all-night recording of electroencephalogram, electroculogram and electromyogram. Drugs were administered for therapeutic purposes. Each patient slept in his regular hospital room and, except for J. P. and B. F., who were allowed to sleep as late in the morning as they wished, was left undisturbed until awakened after 8 h of possible sleep. Previous medications were discontinued for at least 10 days before the beginning of the study. A constant number of identical capsules containing either placebo or active drug were administered four times each day with a maximum dose of 2–3 g per 24 h period. The protocol consisted of consecutive periods of pre-drug baseline placebo and active drug and post-drug placebo. Patients received no other drugs, and daytime naps were prohibited. Records were evaluated by standard criteria[8] by a scorer unaware of the patient's drug status. The first 2 nights were used to adapt the patients to the slightly unnatural sleeping conditions and were not included in the results. Sleep records during the first 5 nights after the daily drug dose exceeded 0.75 g were analysed. During pre-drug and post-drug placebo periods, data from 3–14 successive nights were examined.

In all eight patients, AMPT or AMPHe administration was associated with an elevation in total REM sleep (Table 1), ranging from 3–52%. This increase seemed to be related to an increase in the length of each REM period, because the total number of REM cycles was not affected. Three patients on AMPT had decreases in REM sleep after five nights, although the two other patients maintained the REM increase for the full period of drug administration (eight nights) as did the patients on AMPHe (Fig. 1). There was no change in non-rapid eye movement (NREM) sleep. When AMPT was discontinued there was a decrease in both types of sleep. The NREM decrease (20–80%) from pre-AMPT baseline was seen in three patients for only 1–2 nights and is not reflected in the post-AMPT NREM sleep mean (Table 1). There was a REM sleep post-AMPT decrease (3–77%) in the five patients which persisted for up to six nights. In one patient (Fig. 1), studied after discontinuation of AMPHe there was a 25% decrease in REM sleep accompanied by a 27% increase in NREM sleep. A Friedman's analysis of variance of the overall REM means for the three was significant ($P<0.05$). There were no significant changes for NREM sleep. All patients reported increased daytime sleepiness on AMPT but not on AMPHe.

Table 1 Effect of Alpha-Methylparatyrosine and Alpha-Methylphenylalanine on Duration of REM, NREM Sleep Time and Monoamines Metabolites

		Total min REM			Total min NREM							
	Patient	Pre-drug placebo	Drug treatment	Post-drug placebo	Pre-drug placebo	Drug treatment	Post-drug placebo	Drug dosage in g/24 h	Pre-drug HVA	Placebo 5HIAA	AMPT HVA	Treatment 5HIAA
AMPT	A. N.	65*	85	63	344	350	354	0.75–1.75	62	46	18	57
		(5)	(5)	(4)	(5)	(5)	(4)					
	B. R.	66	85	65	303	310	281	0.75–2.0	—	—	—	—
		(4)	(5)	(14)	(4)	(5)	(14)					
	R. L.	108	111	48	300	326	276	2.0–3.0	—	—	—	—
		(4)	(5)	(4)	(4)	(5)	(4)					
	J. P.	102	155	77	354	348	280	2.0	—	—	—	—
		(4)	(5)	(5)	(4)	(5)	(5)					
	H. D.	119	137	75	345	329	289	0.75–2.0	24	25	15	34
		(6)	(5)	(10)	(6)	(5)	(10)					
	B. S.	—	—	—	—	—	—	0.75–2.0	14	28	≤4	31
	L. D.	—	—	—	—	—	—	0.75–2.0	20	34	16	27
	M. R.	—	—	—	—	—	—	0.75–1.75	18	50	≤4	58
	Mean ±s.e.	92±11	$115\pm14^{+}$	$66\pm50^{\overset{+++}{++}}$	328±12	333±70	296±15		27±9	37±5	$11\pm3^{+}$	41±7
AMPHe	B. F.	106	142	—	348	400	—	2.0				
		(5)	(5)		(5)	(5)						
	S. M.	103	150	75	260	214	327	1.0–2.0				
		(5)	(5)	(4)	(8)	(5)	(4)					
	G. S.	91	100	—	285	246	—	2.0				
		(5)	(5)		(5)	(5)						

* Mean for number of nights given in parentheses. (+) $P<0.05$ for difference from pre-AMPT treatment concentrations. (++) $P<0.02$ for difference from AMPT treatment concentrations. (+++) $P<0.05$ for difference from pre-AMPT treatment concentrations.

As a test of the effect of AMPT on cerebral monoamine metabolism, cerebrospinal fluid concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were determined in two patients from whom sleep studies were available and in three further subjects who were receiving similar dosages of AMPT (Table 1). Lumbar punctures were performed in standard conditions[9] during the pre-drug period and at the time of maximum AMPT dose and assays were carried out by methods previously described[10,11]. The principal catabolite of dopamine (HVA), was reduced by 59%. The principal catabolite of serotonin 5-HIAA was increased in four of the five cases; however, these changes are not statistically significant. Because cerebrospinal fluid concentrations of monoamine catabolites seem to reflect central nervous system monoamine concentrations[12], these observations suggest that the AMPT in this study did produce a substantial reduction in brain catecholamines. The effect of AMPHe is presumably similar to that of AMPT, as it also is a potent inhibitor of tyrosine hydroxylase.

Our results indicate that central catecholamine-containing systems take part in the control of sleep in man. The finding of a substantial increase in REM sleep in the absence of a significant change in NREM suggests that these sleep states can be pharmacologically disassociated. These observations differ from the results observed in animal studies in which catecholamine synthesis was inhibited. There are no reports on the effects of AMPHe administration on animal sleep but AMPT had variable effects on REM sleep in rats[13,14] and cats[15,16], and decreased REM sleep in monkeys[17,18]. Conversely, administration of L-dopa to cats whose monoamine stores were previously depleted by reserpine, restored REM sleep[5]. In humans, L-dopa given with and without a peripheral decarboxylase inhibitor (MK 485) decreased REM sleep[4]:

in several patients, L-dopa at higher dosages also decreased NREM sleep.

In contrast, our previous studies with parachlorophenylalanine[1-3] and 5-hydroxytryptophan[19] indicate that in humans serotonin is intimately associated with the production of REM sleep, although a series of animal experiments suggest serotonin is associated with NREM[5,20-22].

We do not understand these apparent discrepancies between animal and human data, but they may be a result of differences in drug doses, route and period of administration or other parameters of experimental design[3]. It seems that in the human brain, however, the adrenergic and serotonergic brain systems act in opposite directions in the production of REM sleep, with the catecholamines decreasing, and serotonin augmenting, REM sleep.

Drugs were supplied by Dr Elmer Alpert, Merck Sharp and Dohme Co., Pennsylvania.

RICHARD J. WYATT
THOMAS N. CHASE
DAVID J. KUPFER
JIMMY SCOTT
FREDERICK SNYDER

National Institute of Mental Health,
9000 *Rockville Pike,*
Bethesda,
Maryland 20014

ALBERT SJOERDSMA
KARL ENGELMAN

National Heart Institute,
9000 *Rockville Pike,*
Bethesda,
Maryland 20014

Received August 11; revised December 17, 1970.

[1] Wyatt, R. J., Engelman, K., Kupfer, D. J., Scott, J., Sjoerdsma, A., and Snyder, F., *Electroenceph. Clin. Neurophysiol.*, **27**, 529 (1969).

[2] Wyatt, R. J., Chase, T. N., Engelman, K., Kupfer, D. J., Scott, J., Sjoerdsma, A., and Snyder, F., *Psychophysiology* (in the press).

[3] Lovenberg, W., Engelman, K., Carpenter, jun., W. T., Wyatt, R. J., and Gessa, G. L., *Ann. Internal. Med.*, 607 (1970).

[4] Wyatt, R. J., Chase, T. N., Engelman, K., Scott, J., and Snyder, F., *Nature* (in the press).

[5] Jouvet, M., *Science*, **162**, 32 (1969).

[6] Engelman, K., Horowitz, D., Jequier, E., Sjoerdsma, A., *J. Clin. Invest.*, **47**, 577 (1968).

[7] Torcliana, M. L., Porter, C. C., Stone, C. A., and Hanson, A. M., *Biochem. Pharmacol.* (in the press).

[8] *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects* (edit. by Rechtschaffen, A., and Kales, A.) (US Government Printing Office, Washington DC, 1968).

[9] Chase, T. N., Schnur, J., and Gordon, E. K., *Neuropharmacol.* (in the press).

[10] Cerbode, F., and Bowers, M. B., *J. Neurochem.*, **15**, 1053 (1968).

[11] Ashcroft, G. W., and Sharman, D. F., *Brit. J. Pharmacol.*, **19**, 153 (1962).

[12] Guldberg, H. C., and Yates, C. M., *Brit. J. Pharmacol.*, **36**, 535 (1969).

[13] Marantz, R., and Rechtschaffen, A., *Perception Motor Skills*, **25**, 805 (1967).

[14] Hartmann, E., Bridwell, I., and Shildkraut, J., *Psychophysiology* (in the press).

[15] King, C. K., and Jewett, R. E., *Psychophysiology*, **6**, 220 (1969).

[16] Iskander, T., and Kailbling, R., *Psychophysiology*, **6**, 219 (1970).

[17] Crowley, J. T., Pegram, G. V., Smith, E., and Lewis, D. F., *Primate Electrophysiology Particularly Related to Sleep* (*Rep. ARL-TR-69-5* (Holloman Air Force Base, New Mexico, 1969).

[18] Weitzman, E. D., McGregor, P., Moore, C., and Jacoby, J., *Life Sci.*, **8**, 751 (1969).

[19] Wyatt, R. J., Engelman, K., Sjoerdsma, A., and Snyder, F., *Psychophysiology* (in the press).

[20] Weitzman, E. D., Rapport, M. S., McGregor, P., and Jacoby, J., *Science*, **160**, 1361 (1968).

[21] Koella, W. P., Feldstein, A., and Czicman, J. S., *Electroenceph. Clin. Neurophysiol.*, **25**, 481 (1968).

[22] Torda, C., *Brain Res.*, **6**, 375 (1967).

Induction of Mesodermal and Endodermal Structures and Primordial Germ Cells in *Triturus* Ectoderm by a Vegetalizing Factor from Chick Embryos

A VEGETALIZING protein isolated from 9 day old chicken embryos[1-3] and implanted into the blastocoel of an early *Triturus* gastrula[4] induces the ectoderm of the host embryo to form mesodermal tissues (muscle, notochord, renal tubes). Larger quantities of this highly purified factor cause the invaginated endoderm to exovaginate and spread over the induced ectoderm at the end of gastrulation. This may result from a change of cell affinities in the induced ectoderm[5]. In the exovagination series voluminous mesodermal and endodermal structures have been shown to develop from gastrula ectoderm under the influence of the implanted substance (U. K.-B., unpublished).

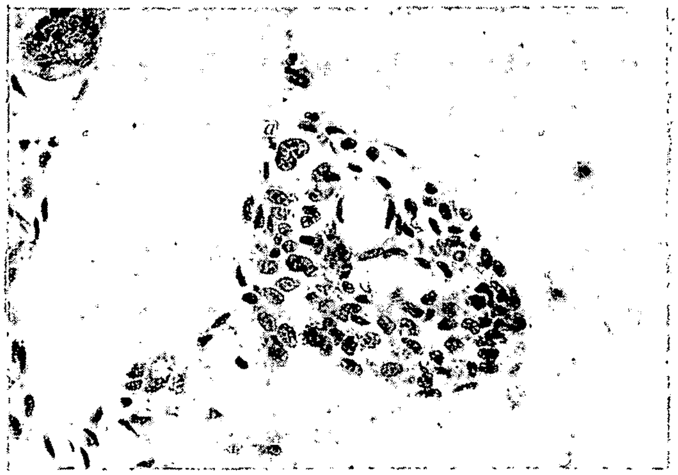


Fig. 1 Section through an induced gonad with an induced germ cell (*a*). Note the big cell with the typical potato-shaped nucleus. The explant was cultured for 41 days.

Effects of host embryos on the final outcome of the inductions are excluded if the inducer is tested on isolated gastrula ectoderm. To show the extent to which mesodermal and endodermal structures were induced by the factor, and to identify the induced endodermal organs, we used Holtfreter's sandwich technique[6]. Two pieces of gastrula ectoderm were sandwiched around a piece of the substance to be tested. Some explants were cultured at room temperature for 8–14 days, most of them for 3–7 weeks, to obtain good histological differentiation of endodermal organs. Most of the explants were spherical or cylindrical, compact and opaque and covered with endodermal epithelium.

As well as small unspecific neural structures, substantial but often poorly differentiated mesodermal and endodermal structures were induced in almost all cases. The induced mesodermal organs were notochord, muscle, renal tubes and sometimes mesenchym; the endodermal structures were mostly endodermal epithelium and intestine and, less frequently, liver, pancreas, stomach, oesophagus and pharynx. The relative frequency of all induced tissues varied between and within different experimental series, performed with various fractions of the vegetalizing factor. Though single explants consisted only of mesodermal tissues and others only of endodermal tissues and mesenteria, so far no fraction has induced mesodermal or endodermal organs exclusively. As well as these organotypic structures, in all series tissue complexes of large yolk-rich cells were found, which differed clearly from typical undifferentiated ectoderm. If the time of action of the inducing factor is shortened by removal of the implant after 1–24 h or if the factor is diluted, differentiation of the induced tissues improves.

Besides these organs and tissues, in eleven out of 125 explants primordial germ cells were observed for the first time as a result of induction. The primoridal germ cells were only found in explants which were cultured for at least 20 days. These especially large cells can be distinguished clearly from other cells by their histological appearance (Fig. 1). The explants in which primordial germ cells were found, contained also different mesodermal and endodermal tissues.

Both halves of the sandwich originate from gastrula ectoderm, so host germ cells, originating probably from lateral plate mesoderm[7,8], cannot have migrated into an induced germ ridge. The diagnosis of the primordial germ cells is based on their appearance and it has not been shown whether they are fertile. But the induction of these cells speaks strongly against the notion that the *keimbahn* is an absolute necessity for the development of germ cells in amphibians. Obviously germ cells can arise by regulatory mechanisms from somatic cells as do other cell types.

Probably only one factor induces the mesoderm–endoderm *anlage*, but the possibility that two factors are involved has not been excluded. All the observed differentiations including primordial germ cells certainly arise by secondary interactions.

URSULA KOCHER-BECKER

Heiligenberg-Institut,
Heiligenberg

HEINZ TIEDEMANN

Institut für Biochemie und Molekulare Biologie,
Freie Universität Berlin-Dahlem

Received March 22; revised June 14, 1971.

[1] Tiedemann, H., and Tiedemann, H., *Hoppe-Seylers Ztschr. Physiol. Chemie*, **306**, 7 (1956).

[2] Tiedemann, H., Born, J., Kocher-Becker, U., and Tiedemann, H., *Ztschr. Naturforsch.*, **20b**, 308 (1965).

[3] Geithe, H. P., Tiedemann, H., and Tiedemann, H., *Biochim. Biophys. Acta*, **208**, 157 (1970).

[4] Mangold, O., *Arch. Mikrosk. Anat.*, **100**, 198 (1923).

[5] Kocher-Becker, U., Tiedemann, H., and Tiedemann, H., *Science*, **147**, 167 (1965).

[6] Holtfreter, J., *Roux. Arch. Entw. Mech.*, **128**, 584 (1933).

[7] Nieuwkoop, P. D., *Arch. Anat. Microsc. Morphol. Exp.*, **39**, 257 (1950).

[8] Balinsky, B. I., *An Introduction to Embryology*, 45 (Saunders, London and Philadelphia, 1965).

Importance of Denitrification and Nitrate Reduction in Sediments to the Nitrogen Budgets of Lakes

DENITRIFICATION (biological conversion of nitrate and nitrite to nitrous oxide and molecular nitrogen) and nitrate reduction (assimilation of nitrate–nitrogen in microbial protoplasm) in lake sediments can play an important role in the nitrogen budgets of lakes. The hydrology of many lakes is such that ground water seepage through the floor constitutes a significant source of water income[1]. In such cases, nitrate in the incoming ground water must be considered when a nitrogen budget is calculated for the lake system. An excellent example is the estimate for Lake Mendota, Wisconsin[2], in which nitrate in seepage water constituted nearly 36% of the nitrogen added to the lake.

Lake sediments, being highly reduced and rich in organic matter, are ideal for denitrification. Fermentation gases emanating from lake sediments contain large volumes of molecular nitrogen, presumably biogenic in origin[3] and denitrifying microorganisms have been identified in marine and lake sediments (ref. 4 and unpublished results of R. L. Chen, D. R. K., J. G. Konrad, and A. J. Holding). Previous work in this laboratory[5] showed that the nitrate and nitrite contents of Lake Mendota sediment profiles increased with depth below the sediment surface, indicating that nitrate was moving through the profile and being reduced. But the only report attempting to quantitate the extent of denitrification and nitrate reduction in lake sediments is that of Goering and Dugdale[6] who in a laboratory incubation study with ^{15}N-labelled nitrate, found that nitrate added to mud–water samples from Smith Lake, Alaska, disappeared within 6 days and that reduction of nitrate to ammonia was insignificant compared with reduction to molecular nitrogen. Other work[7,8], has indicated, however, that significant nitrate reduction and denitrification will occur concurrently in anoxic lake waters.

We have evaluated the extent of denitrification and assimilatory nitrate reduction in the surface sediments of Lake Mendota. In June 1970, sediment samples were collected with an Eckman dredge and treated in the boat either with 5 ml./l. sediment of ^{15}N potassium nitrate solution (50.0 atom % ^{15}N) equivalent to 2.1 mg per l. nitrate–nitrogen on a volume basis, or with 5 ml. of distilled water (control samples). The treated sediment was added to 1 l. plastic bottles which were returned immediately to the lake bottom at the sampling site. The experimental sites were under 7 or 14 m of water and their temperatures were 18° C and 12° C; the dissolved oxygen contents of the water just above the sediment surface of the two sites were 9 and 7 mg/l. The bottles were left uncapped throughout the experiment. At 4, 8, 12 and 16 days after initiation of the experiment, two of the control bottles and two containing the treated sediment were carefully retrieved for analysis. Investigations conducted by lowering and immediately raising sterilized nitrate–nitrogen-treated sediment showed that loss of nitrogen by wash-out was negligible (average 99.8% recovery). Similar investigations showed that less than 1% per day of nitrate–nitrogen was lost by diffusion into the lake water.

Interstitial water ammonium, nitrate and nitrite and sediment exchangeable ammonium, as well as sediment organic nitrogen (the nitrogen in the sediment residue after exhaustive 2 M KCl extraction), were determined[5] and isotope ratioanalysis of interstitial and exchangeable ammonium-nitrogen and of sediment organic-nitrogen was conducted using a CEC 21-201 mass spectrometer by distillation and gas sample preparation methods[9,10]. Physical and chemical properties of Lake Mendota sediments have been described[5,11].

We found that nitrate disappeared rapidly from the sediment samples; only trace levels of nitrate existed 4 days after initiation of the experiment and nitrite did not accumulate. Interstitial and exchangeable ammonium-nitrogen levels in the 14 m sediment samples (13 and 184 p.p.m. respectively) were about four times higher than those in the 7 m sediment and remained fairly constant throughout the experiment at both sites. Also, these values did not differ significantly between the control and the treated sediment at each site.

The ^{15}N distribution data (Table 1) show that significant assimilatory nitrate reduction as well as denitrification was occurring in these sediments and that nitrogen turnover was quite rapid. Considerable incorporation of nitrate–nitrogen into microbial protoplasm occurred immediately on addition of nitrate, but in general the results reflect a microbial turnover of nitrogen coupled with sediment–water interchange. At both sites microbial immobilization was maximum at 4 days but was accompanied by considerable mineralization of protoplasm nitrogen. About 37% of the ^{15}N was recovered at 4 days, declining to about 20% at 16 days. The increase at 16 days was probably due to re-incorporation of soluble organic nitrogen compounds into microbial protoplasm, as soluble forms of nitrogen were not measured in our experiment. But this increase might be due to fixation of molecular ^{15}N produced during denitrification[12,13].

Table 1 Distribution of ^{15}N in Lake Mendota Sediment Samples at Various Times after Treatment with ^{15}N-labelled Potassium Nitrate

Time after initiation of experiment (days)	Recovery (%) of added ^{15}N in			
	7 m samples		14 m samples	
	Organic-N	Ammonium-N	Organic-N	Ammonium-N
0.4	8.1	0.3	14.3	1.5
4	30.8	6.9	29.1	7.6
8	9.3	8.0	13.5	6.9
12	5.2	6.1	9.2	3.8
16	14.5	4.7	14.4	5.4

These data point out the fact that denitrification and assimilatory nitrate reduction occur concurrently in sediments and must be considered when evaluating the nitrogen budget of lakes receiving nitrate from ground water. Lee[2] has estimated that Lake Mendota receives about 36% of its nitrogen from ground water seepage. This amounts to about 77,000 kg nitrogen per annum for the lake. It is clear that if this figure were reduced by using the 4 day results (37% of the nitrate–nitrogen immobilized and 63% denitrified), the ground water portion of the Lake Mendota nitrogen budget could be revised accordingly. Such a revision shows that the nitrogen contribution would decrease by 49,000 kg due solely to consideration of sediment denitrification. This illustrates the point that the fate of ground water nitrate in lake sediments must be considered when nitrogen budgets of seepage lakes are calculated.

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, and by a grant from the Federal Water Quality Administration. We thank Professor R. H. Burris for use of the mass spectrometer and Mrs K. Chen for assistance in the analyses.

D. R. KEENEY
R. L. CHEN
D. A. GRAETZ

Department of Soil Science,
University of Wisconsin,
Madison, Wisconsin 53706

Received May 6; revised June 4, 1971.

[1] Hutchinson, G. E., *A Treatise on Limnology*, 231 (Wiley, New York, 1957).
[2] Lee, G. F., *Eutrophication*, 17 (Univ. of Wisconsin Water Resources Center, Madison, 1970).
[3] Allgeier, R. J., Peterson, J., Juday, W. H., and Birge, E. A., *Intern. Review Hydrobiol.*, **26**, 444 (1932).
[4] Chamroux, M. S., *Ann. L'Inst. Pasteur*, **109**, 424 (1965).
[5] Konrad, J. G., Keeney, D. R., Chesters, G., and Chen, K., *J. Water Poll. Contr. Fed.*, **42**, 2094 (1970).
[6] Goering, J. J., and Dugdale, V. A., *Limnol. Oceanog.*, **11**, 113 (1966).
[7] Brezonik, P. L., and Lee, G. F., *Environ. Sci. Technol.*, **2**, 120 (1968).
[8] Goering, J. J., *Deep Sea Res.*, **15**, 157 (1968).
[9] Bremner, J. M., and Edwards, A. P., *Soil Sci. Soc. Amer. Proc.*, **29**, 504 (1965).
[10] Bremner, J. M., in *Methods of Soil Analysis* (edit. by Black, C. A.), 1256 (American Society of Agronomy, Madison, Wisconsin, 1965).
[11] Keeney, D. R., Konrad, J. G., and Chesters, G., *J. Water Poll. Contr. Fed.*, **42**, 411 (1970).
[12] Howard, D. L., Frea, J. L., Beeister, R. M., and Dugan, R. P., *Science*, **169**, 61 (1970).
[13] Brooks, R. H., Brezonik, P. L., Putman, H. D., and Keirn, M. A., *Limnol. Oceanog.* (in the press).

GENERAL

Uniform Distribution of Objects in a Homogeneous Field: Cities on a Plain

THE uniform distribution of objects in a homogenous field is familiar in all kinds of circumstances—atoms in liquids[1-3], fibrils in cells[4], hairs on insects[5,6] and mammals[7], stomata on leaves[8], trees in forests[9-10] and cities on plains[11,12]. In many of these circumstances the relative positions of the objects may be described by a radial distribution function. In what follows we shall show that such a function may be used to study the distribution of cities on a plain.

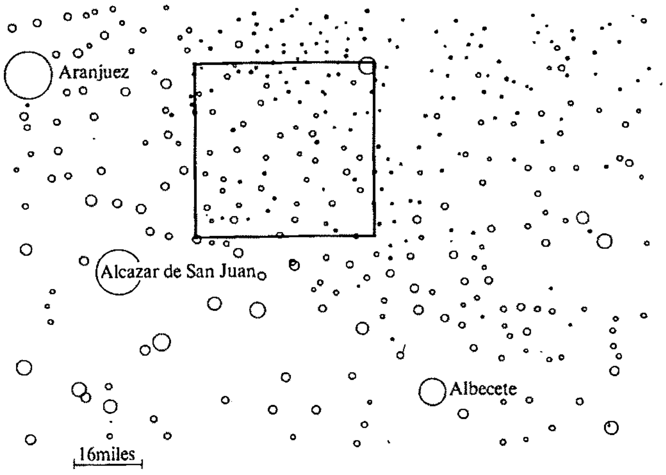


Fig. 1 A map of part of the Spanish Plateau lying south-east of Madrid, bounded by latitudes 0° 53′ W, 3° 41′ W and longitudes 38° 48′ N, 40° 16′ N. The radial distribution function of the cities lying in the square has been computed as described in the text. The locations of the towns were taken from the US Army Map Service topographic sheets of the Iberian Peninsula, 1 : 250,000, dated 1944.

Fig. 1 is a map of part of the Spanish Plateau lying south-east of Madrid and bounded by latitudes 0° 53′ W, 3° 41′ W and longitudes 38° 48′ N, 40° 16′ N. This area is homogeneous in climate, physiography, transportation, population density and economy. The towns are represented by circles whose radius is an approximate measure of physical extent[13]. We have chosen for analysis the 40 mile square centred at latitude 2° 30′ W and longitude 39° 47′ N which is especially homogeneous in town size and density.

The radial distribution function $g(R)$ is defined as the probability that a structure will be in a unit area a distance R away from an arbitrary structure, divided by the number probability density. It may be computed for the town lying in the square in Fig. 1 in the following way. Place each town in turn at the origin and surround it by a series of concentric circles spaced at a distance of $\Delta R = 1$ mile. Determine the area, $K_i(R)$ (lying wholly within the square in Fig. 1) of the annulus whose boundaries are at distances of $(R - \Delta R/2)$ and $(R + \Delta R/2)$ from town i. Let the number of towns lying within this annulus be N_i (R). If N is the total number of towns in the square and K is the area of the square, then

$$g(R) = \frac{N(R)}{\rho K(R)}$$

where

$$N(R) = \sum_{i=1}^{N} N_i(R)$$

$$K(R) = \sum_{i=1}^{N} K_i(R)$$

$$\rho = \frac{N}{K}$$

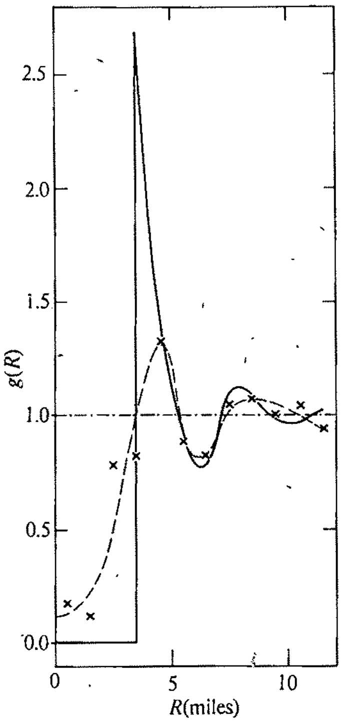


Fig. 2 The radial distribution function computed for the towns in the square in Fig. 1 (- - -), for a totally random distribution (—.—.—), and for a random distribution of hard-core disks with $\bar{\rho}=0.5$ and $d=3.46$ miles (——).

In Fig. 2 the computed distribution function is indicated by the dashed curve drawn through the crosses which represent the points at which the determination was made; the radial distribution function for a random distribution is depicted by the dashed-dot line in Fig. 2.

The diminished density near the origin and the subsequent damped oscillations in density are characteristic of distributions in which objects are fundamentally randomly dispersed, but are subjected to repulsive interactions which tend to diminish the probability of finding two objects close to each other. If the distribution is formed by a dynamic process which guarantees that the distribution is maximally random subject to the constraints of the interparticle interactions, then the theoretical concepts developed in the study of the statistical mechanics of equilibrium liquids may be applied.

An example is useful. Assume that the towns are randomly distributed subject only to the constraint that no two town centres may be closer than a distance, d. Similar models have been proposed as prototypes for spatial interaction and pattern formation in biological systems[6,7,9]. Because the resulting distribution also arises in the hard-core model of classical liquids at equilibrium[2,3], the radial distribution functions for such distributions have been intensively studied. The results are tabulated[3] for different relative packing densities, $\bar{\rho}$, where $\bar{\rho}$ is the number density divided by the maximum close packed hard-core density, and is given by

$$\bar{\rho} = \frac{\sqrt{3}}{2}\,\rho\, d^2$$

The best agreement is given when $\bar{\rho}=0.5$, which corresponds to a hard-core town diameter of $d=3.46$ miles (solid line in Fig. 2). Although there is qualitative agreement, the hard-core interaction is too stringent to give detailed quantitative agreement with the experimental results. A more realistic interaction between towns would be graded, in which the probability of finding two towns close together would be low, but would continuously increase as the distance between the towns increased. This is what would be expected from the geographic theory of central places[11,12]. Lawrence, in a study of hair placement in *Oncopeltus*, has hypothesized the existence of a very similar repulsive interaction which leads to the uniform distribution of hairs[6].

No matter what system is considered, this hypothesized repulsive field plays much the same role as the intermolecular potential in the theory of liquids. If the distribution of structures is maximally random subject to the constraints imposed by this repulsive field, then the equations developed in statistical mechanics may be applied. It is possible to compute theoretical radial distribution curves for a given hypothesized field (ref. 1, chapter 5) and also to determine, approximately, the interaction between structures if the radial distribution function is known (ref. 1, page 80). Future work may characterize the hypothesized repulsive fields and explore their physical, biological and social origins in those diverse disciplines where uniform distributions are observed.

L. G. thanks Professor H. C. Longuet-Higgins for his help and the US Public Health Service, National Institute of Health, for financial support. The data were assembled by G. Aspbury during tenure of a National Science Foundation grant.

LEON GLASS

Department of Theoretical Biology,
939 *E 57th Street,*
University of Chicago,
Chicago, Illinois 60637

WALDO R. TOBLER

Department of Geography,
University of Michigan,
Ann Arbor, Michigan 48104

Received March 29, 1971.

[1] Egelstaff, P. A., *An Introduction to the Liquid State* (Academic Press, London, 1967).
[2] Alder, B. J., Frankel, S. P., and Lewinson, V. A., *J. Chem. Phys.*, **23**, 417 (1955).
[3] Chae, D. G., Ree, F. H., and Ree, T., *J. Chem. Phys.*, **50**, 1581 (1969).
[4] Farrell, R. A., and Hart, R. W., *Bull. Math. Biophys.*, **31**, 727 (1969).
[5] Wigglesworth, V. B., *J. Exp. Biol.*, **17**, 180 (1940).
[6] Lawrence, P. A., *Dev. Biol.*, **19**, 12 (1969).
[7] Claxton, J. H., *J. Theoret. Biol.*, **7**, 302 (1964).
[8] Ursprung, H., in *Major Problems in Developmental Biology* (edit. by Locke, M.) (Academic Press, New York, 1966).
[9] Pielou, E. C., *J. Ecol.*, **48**, 575 (1960).
[10] Pielou, E. C., *An Introduction to Mathematical Ecology*, ch. 10 (Wiley Interscience, New York, 1969).
[11] Christaller, W., *Central Places in Southern Germany* (Prentice Hall, New Jersey, 1966).
[12] Berry, B., and Horton, F., *Geographic Perspectives on Urban Systems* (Prentice Hall, New Jersey, 1970).
[13] Tobler, W. R., *Area*, **1**, 30 (1969).

BOOK REVIEWS

Oppenheimer in Dock

The Oppenheimer Case, Security on Trial. By Philip M. Stern. Pp. xiv+591. (Rupert Hart-Davis: London, May 1971.) £3.50.

"THIS began as a book about a security case," writes Mr Stern in his preface. "It ended as a book about the security system. The case is now history. The system is still with us." A baleful opening for anybody old enough to remember the witch-hunting McCarthy period in the United States and the accusing of J. Robert Oppenheimer, theoretical physicist, so-called father of the atomic bomb, of being a Soviet agent. And not encouraging for anybody young who gives thought to the secret dossier-collecting activities of security organizations today—an estimated 14 million dossiers totted up by the US Department of Defense, 42 million by an American private investigation agency. . . . Mr Stern describes them: "Permanent collections of secret information, many of which may contain misinformation that the 'subject' has no way of rebutting or correcting—because he is not allowed access to it."

In the case of Robert Oppenheimer there were 3,000 pages of secret material, from which large black notebooks were made and circulated in advance of the hearings not just to the counsel who was going to lead the prosecution, but also to the judges—a tribunal called the Gray Board, set up by the US Atomic Energy Commission. So, by a reversal of the American "blank pad" (for the judges) rule in trials at law, Oppenheimer had laid on him from the start the burden of proving his own innocence.

Oppenheimer and his counsel were not allowed to see what was in the notebooks; and "for reasons of security" his counsel was sent out of the court while certain extracts were dealt with—not that this stopped the subsequent reading out in open court by the prosecution counsel of some of those which Oppenheimer's counsel had been denied. So Oppenheimer had to prove his own innocence in the face of accusatory material known to the judges that was never revealed in full to his side. Furthermore, several of the principal charges against him, including a crucial security charge, had been taken into account when he was given his Q-clearance seven years earlier; so he was in effect being "tried" twice over for the same thing. Furthermore, some of his "offences" were, at the time he was supposed to have committed them, not unlawful. . . . Security on trial indeed! If there was one thing security was *not*, it was on trial. Safe behind closed doors, it totally evaded any critical, let alone judicial, investigation of its own processes and nature.

The history of the case is hair-raising. With an unforced combination of the historian's organization of fact and the novelist's interest in people, Mr Stern tells it brilliantly: each of the 500 pages simply has to be read. In the last fifty pages, when he specifically turns his attention to "security on trial", it loses a little of its coherence and grip, but that is a minor defect. He begins his story in 1936, when the young Oppenheimer, rich, cultivated, solitary-natured bachelor son of a Jewish East Coast family joined the Faculty of the University of California at Berkeley; and began in the "liberal" fashion of the times to associate with local Left wing organizations, near-Communists, Communists. With a sharp pertinence, Mr Stern remarks that when Oppenheimer was "tried" in 1954 these associations were set by his accusers and judges entirely against the current cold war situation of the early 1950s, and never judged against the historical background in which they occurred. To be fair to his accusers and judges, when reading about them now, one has to avoid the comparable trap of judging their behaviour entirely against the *détente* ambience of today, forgetting the paranoia and hysteria which was gripping the United States (not to mention the Soviet Union) then.

Throughout his career Oppenheimer showed an intellectual brilliance which permanently captivated his friends and his followers—and roused permanent hatred and alarm in the men he made his enemies. In his theoretical physics, though, he never made any fundamental discoveries: he was the quintessential solver of other people's problems rather than creator of his own. At first sight it seems odd that a scientist of this kind should succeed so astonishingly as the director of the scientists at the Los Alamos weapons laboratory who actually were making discoveries. Even odder that a man of his physical and temperamental type—essentially ectomorphic, fine-drawn, a creature of quick, nervous apprehensions—should be so masterful in the hurly-burly of power. And should continue to be so, later on, when from the eminence of the Princeton Institute for Advanced Study he engaged in controversy over whether the hydrogen bomb should be developed, and then whether it should be made the centrepiece of US defence—controversy in which he made an enemy of Edward Teller, father of the hydrogen bomb, and, more fatally, aroused the hatred of the US Strategic Air Command.

In fact the Gray Board's final judgment was that Oppenheimer "seemed to have had a high degree of discretion, reflecting an unusual ability to keep to himself vital secrets." Did most of them, and successive ratifiers of the verdict, ending with the Atomic Energy Commissioners and then the Government, bring him to his knees by revoking his security clearance really out of fear that at some future date he would give away secrets to the Russians? Or, Mr Stern asks: "Would Oppenheimer have been charged as a security risk if he had not held controversial opinions; if he had not advocated them with extraordinary persuasiveness, if he had not aroused (and on occasion sorely offended) persons in high places?"

A very good question. Given the answer is "No", it is disappointing not to find oneself whole-hearted about Oppenheimer as a man. It seems that in the last resort he lacked some kind of ballast—moral ballast, psychological ballast, possibly even intellectual ballast. Throughout the book examples abound to such an extent that it cannot be ignored. There was something very lightweight about the way he got himself mixed up with Communists before the Second World War. When he saw the first atomic device exploded he murmured a poetic quotation from the *Bhagavad-Gita.* And after having been so appalled by the destructive possibilities of the hydrogen bomb that he wanted America to forswear working on it and to get the mutual consent of Russia to do the same (what a hope!)—after all that he said, when Teller triumphantly showed it could be made, that it was "so technically sweet that you could not argue about it". So technically sweet. . . There is a point at which lightness of weight, absence of ballast, can become faintly sickening. Yet it must be stressed that men who knew him, men such as I. I. Rabi and Hans Bethe, possessors of great ballast themselves, could reiterate years later their admiration and respect for him as a whole man. Mr Stern's attitude towards him, I may say, is impeccably detached and fair.

However, nobody's reading of Oppenheimer's character solves the enigma which overhangs the crucial security charge against him. Sometime in January or February 1943, Oppenheimer and his friend Haakon Chevalier were making martinis in Oppenheimer's kitchen. "There is no dispute over the fact," Mr Stern records, "that Chevalier reported that Eltenton said he had means of getting technical information to the Russians. It is also agreed that Oppenheimer made it immediately clear that he would have no part in such an affair." The astonishing, inexplicable thing is that Oppenheimer later gave different accounts of the incident; and that in particular he gave an egregiously untruthful account to a security agent called Boris T. Pash, in which he refused to disclose Chevalier's name while saying that several other men were involved—a cock-and-bull story that was much more injurious to himself and Chevalier than the truth, and which at his "trial" he allowed to be called "a tissue of lies". It was an incredible thing to do; and when asked why he did it, he replied: "Because I was an idiot." It did him critical harm.

What could be the explanation? Nobody among his friends or his enemies has found a credible answer by looking at the character of Oppenheimer. Is it to be found, I propose, by looking instead at the character of the incident? Is that kind of interrogation liable to induce that kind of aberration? Oppenheimer's egregious performance *vis-à-vis* Colonel Pash was recorded through a hidden microphone and it makes disturbing reading—because of the peculiar intimacy existing between interrogator and interrogated. A fulsome, ingratiating duplicity on the interrogator's side; a fulsome, yielding over-accord on the interrogated's—"God bless you," he says at the end of it. One wants to turn one's head away . . . But that would mean turning one's head away from a very serious matter, where security really on trial could well be required to prove its case. The matter at issue is not the unnaturalness of the intimacy: it is the trustworthiness of the situation—its trustworthiness not to throw a man off balance, not to produce, while he is in it, almost a personality change. Is the solution to the Oppenheimer enigma not that he was an idiot, but that such interrogations are liable to bring idiocies to a man's lips? Really on trial, security is not invulnerable.

WILLIAM COOPER

Early Trains

Civil Engineering: Railways. By Bryan Morgan. Pp. xvi+176+45 Photographs. (Longman: London, April 1971.) £2.96.

THE admirable series of studies of industrial archaeology edited by Mr. L. T. C. Rolt has now reached railways. Wisely, he has decided that the subject is too big to be comprehended in a single volume, such as the one he wrote himself on *Navigable Waterways*, and that it must be treated in two. This book is therefore complemented by another on mechanical engineering, written by Mr J. H. Snell.

Mr Morgan's study is comprehensive, clearly arranged, and most pleasantly written. Within the modest limits enforced on him he packs a great deal of information and lively comment. He rightly stresses the importance of the early years of railway building, before the locomotive appeared and during its primitive, fitful development. He pays proper attention to the track, commenting with a severity that is well justified on the treatment usually accorded to "these unglamorous lengths of scrap iron" in museums, and taking trouble to explain the reasons that lay behind the adoption of different forms of rail, sleeper, and ballast. His illustrations and diagrams are a well integrated adjunct to his text.

The book has some faults. Its proportions may be criticized here and there. Scotland receives on the whole scanty attention. Of more than a hundred structures mentioned in the gazetteer at the end of the book, only four lie north of the border. Perhaps more serious, more than four-fifths of the text is devoted to the period before 1865. That was indeed the heroic age of railway building in this country, but notable work has been performed since, which is passed over here: for example, the extraordinary series of bridges, in Staffordshire blue brick, erected over the Midland Railway's line south of Kettering in the 1880s and 1890s, where the engineers were treating the bricks in an almost plastic way that seems to anticipate the handling of reinforced concrete in the twentieth century. And by way of a final criticism it must be said that there are rather more small mistakes here than there ought to be: obvious misprints uncorrected, names mis-spelt and places wrongly cited—Ashburton for Ashton, for example. It was not the North Midland Company that tried to reach Manchester (page 117); nor was Tite the architect of the station at Shrewsbury (page ix).

These small blemishes can be rectified when the book goes into a second edition. It deserves to be kept in print for a long time as an excellent introduction to its subject, which will not lose its value. Mr Morgan is generous in searching out distinction among his engineers and architects, whether in pioneering forms and techniques or in aesthetic sensibility, and he communicates his enthusiasm so attractively that he will inspire his readers to follow him in the direction of inquiry he has laid down. His book achieves its purpose very well indeed.

JACK SIMMONS

Röntgen Revealed

The Life of Wilhelm-Conrad Röntgen. By W. R. Niske. Pp. xi+355. (University of Arizona: Tucson and Arizona April 19, 1971.) $8.50.

WHEN in 1895 Röntgen made his great discovery he was already fifty years old, and the rest of his life was in a sense an anticlimax; it seems that he genuinely hated fame and publicity, and regretted his move from Würzburg, where he had been happy, to Munich, where he was not. After his three papers describing the phenomena of the X-rays, he played no important part in the practical development of the apparatus or in the explanation of the phenomena; in this he reminds one of Volta, who made his discovery at much the same age. It is clear from this biography that Röntgen's discovery, while unexpected, was in no sense an accident. He had been carefully trained under A. E. E. Kundt to make very accurate determinations of physical quantities; first investigating the specific heats of gases, and then studying electromagnetism, and the physical properties of crystals. His academic position—he had held a chair at Giessen before being called to Würzburg to follow Kohlrausch—and his reputation for sober accuracy ensured that his preliminary paper on X-rays was taken seriously.

The study of cathode rays was an obvious field for investigation; mercury pumps were available to give very low pressures, and it seems that others had noticed fluorescence near cathode ray tubes and fogging of photographic plates, but had not seriously investigated the matter. In November and December 1895, Röntgen investigated the X-rays in an extremely capable manner, proving that the effects could not be due to cathode rays, and taking a number of X-ray photographs. He submitted his preliminary paper on December 28, 1895; it was published with a rapidity that moderns might envy, for the offprints were ready to be sent out on new year's day 1896. Röntgen was transformed from a man with a high reputation within his field into a celebrity; and was in 1901 awarded the first Nobel Prize for Physics.

Reading this sympathetic biography, one feels that Röntgen had somehow got out of his depth; like Faraday, he had the "ability to improvise laboratory equipment, which enabled him to make observations that less skilled men were unable to do", but we find little evidence that like Faraday he was a solitary voyager in strange seas of thought. His orderly life, with the spring and summer vacations spent in Italy and Switzerland, does not have that appearance of complete devotion to the struggle with the mysteries of nature which characterizes those few scientists of the first rank. Röntgen's example can encourage us all, for he seems to have been a rather ordinary scientist who made an extraordinary discovery; and this biography is valuable in bringing before us such a man and casting light on his times. In spite of a tendency to degenerate into travelogue, the book is readable and interesting; we encounter both the crusty and forbidding professor and the shy man at ease holidaying with his friends. We even find him in 1888 complaining of the declining number of students because for scientists "the outlook is gloomy".

D. M. KNIGHT

Illuminating Letters

The Born Einstein Letters. By M. Born, Translated by I. Born. Pp. xi+240+4 plates. (Macmillan: London and Basingstoke, May 1971.) £3.85.

THIS volume consists of 120 letters between Einstein and Max and Hedwig Born, the first written by Einstein in 1916 as the General Theory was breaking on the world, the last by Born in January 1955, as both men became involved in the events leading up to the Russell–Einstein Manifesto. Commentaries written by Born in 1965, when he was already in his eighties, fill in details, add later judgments, and provide a background to the men, and the occasional woman, who lived through the dramatic era of physics between the two world wars.

The principal scientific interest in the correspondence lies of course in the long debate between the two men, and most, although not quite all, of their long discussions can be followed here. Their first meeting was at Salzburg in 1909 when Einstein, justy thirty, giving his first big invited paper, declared "a profound change in our views of the nature and constitution of light" to be indispensable. Born himself finds it hard to believe that he did not correspond with Einstein when working on the latter's theory of specific heats three years later.

The letters touch on the anti-Einstein movement of the early 1920s in Germany and on the confrontation between Einstein and Lenard at Bad Nauheim in 1920, in some ways comparable with the Wilberforce–Huxley dispute at Oxford in 1860. (Interestingly enough, no full verbatim account of either seems to have survived.) They add to the material on the birth of quantum mechanics already available in the Einstein–Sommerfeld letters and in the *Letters on Wave Mechanics* by Einstein, Schroedinger, Planck and Lorentz published four years ago. And they carry the debate on quantum mechanics into the 1950s.

Yet their interest does not lie solely in the contemporary comments of two masters, made as the new physics evolved. There is also their parallel reaction to events in Germany, to the birth of nuclear weapons and to the increased responsibility of scientists for the ways in which their work is utilized. While Einstein signed the momentous letter to Roosevelt which helped launch the American atomic effort, Born found the idea of nuclear weapons so distasteful that he avoided personal involvement and tried—unsuccessfully—to dissuade one of his workers in Edinburgh from joining the British effort: Klaus Fuchs. And when Born returned from Britain to Germany after the Second World War, Einstein noted that he was going back "to the land of the mass-murderers of our kinsmen".

There is a third interest in the correspondence, in some ways almost as important as the light cast on the argument between the standard-bearers for two opposing scientific views. Both Max Born and Albert Einstein reveal in these letters not only great intellects, but a warmth and humanity sometimes considered—rightly or wrongly—to be rare among scientists.

R. W. CLARK

Physics Performed

Physics: Demonstration Experiments. Edited by Harry F. Meiners. Vol. 1: Mechanics and Wave Motion. Pp. x+1–654. Vol. 2: Heat, Electricity and Magnetism, Optics, Atomic and Nuclear Physics. Pp. iv+655–1395+36. (Ronald: New York, 1970.) $30 per set.

ONE of the opening chapters of the two volumes entitled *Physics, Demonstration Experiments* was written by the late Sir Lawrence Bragg. His association alone makes these two books not just another pair of books on physics demonstrations but an inspiration—an inspiration to all those who wish to make a physics course in school or at university really alive.

The two volumes are divided into a subject classification of, volume I: *Mechanics and Wave Motion*, and volume II: *Heat, Electricity and Magnetism, Optics, Atomic and Nuclear Physics.* A great bonus in each book is an additional chapter: in volume I the whole philosophy and art of the use of demonstrations to teach physics is discussed, while in volume II the additional chapter discusses the use of closed circuit television, overhead projectors, stroboscopic effects and other modern teaching devices which are now becoming commonplace in the lecture room.

Not since *Demonstration Experiments in Physics* by R. M. Sutton has there appeared a work which deals with the subject so well. Professor Harry F. Meiners, the editor, has gathered the cream of international physics demonstration techniques and methods into these two volumes. Contributions from the United States, Britain, Israel and Russia are all included, together with many others.

From this mine of information for teachers and lecturers, it would be impossible to summarize, or even give a list of the demonstrations in these volumes, which together add up to 1,400 pages. It is easier to say that from a simple demonstration of shadow projection to solid state physics or the application of a laser, all are included.

On reading these books the complexity of some of the apparatus is a little worrying until it is realized that apart from the description of the demonstration in the text, full constructional details and drawings, almost down to the last nut and bolt, are given in an appendix at the end of each volume. If commercial apparatus is used a list of suppliers or manufacturers is given. This publication was sponsored by the American Association of Physics Teachers, and being American, most of the suppliers are American. This need not deter the English reader as equivalent equipment is obtainable in this country.

These books, which have some 2,000 photographs as well as line drawings, would be excellent additions to any Science Library.

To conclude, Sir Lawrence Bragg's advice to a lecturer was to not just talk about science, but, if possible, to demonstrate it. These books show the way.

W. A. COATES

Anaesthetic Collection

International Encyclopedia of Pharmacology and Therapeutics. Section 8, Vol. 1: Local Anesthetics. Section edited by P. Lechat. Pp. ix+377. (Pergamon: Oxford and New York, April 1971.) £6; $16.

THIS volume of the *International Encyclopedia of Pharmacology and Therapeutics* comprises ten chapters written by internationally known authors, two of whom are now unfortunately deceased. It amply justifies the title encyclopaedia, and covers in exceptional detail the various aspects of local anaesthetics, which range from historical development to current clinical application, and deals with structure–activity relations, mode of action, pharmacological properties and their modification, methods for comparison of activity and finally the toxicity and side effects of these substances.

Each chapter is very fully referenced and the individual authors are to be complimented on the fullness of their text which is amply illustrated with photographs, diagrams and tables, although the first chapter on the historical development of local anaesthesia tends toward over illustration. A strong emphasis is placed throughout the book on historical aspects and early work, and in several chapters careful reading is required, as the general theme passes through a maze of previous ideas, experimentation and thought, before more recent concepts are expounded and further compared. However, even though the book is often an exposition of previous and current work, an attempt is made when appropriate to provide critical assessment.

The part local anaesthetics play in the temporary alleviation of pain during surgery is well known, and the continuing search for newer and better agents serves to illustrate the importance of these drugs and the fact that even those of more recent development suffer from certain clinical deficiencies, a point stressed in the final chapter. Much knowledge has, however, still to be gleaned and this textbook with its well documented account of past development, the deficiencies and properties of current drugs, and the experimental techniques used for the assessment of new drugs, should do much to stimulate further interest in the subject and provide an excellent comprehensive reference and reading text, not only for pharmacologists, both graduate and undergraduate, but anyone with an interest in local anaesthetics.

A. J. DUXBURY

Pesticides Around Us

Pesticides in the Environment. By Robert White-Stevens. Pp. xv+270. (Marcel Dekker: New York, June 1971.) $23.50.

THE current widespread interest in all forms of environmental pollution by chemicals ensures an extensive potential audience for any work with the title *Pesticides in our Environment.* This is the first of five books to be published as parts of two volumes under this title. This first part of the full volume, and the part yet to be published, together deal with the more theoretical aspects of pesticidal chemicals, whereas the second volume will deal with the more practical problems of handling pesticides in the environment. The editor of the series, Dr R. H. White-Stevens, now heads the Bureau of Conservation and Environmental Science in New Jersey, and has had considerable experience of research in the agricultural chemical field.

The professed aim of the series, as stated by the editor, is to present in authentic objective terms, the properties, functions, utility and contributions of pesticidal chemicals to human welfare, and to provide data that will enable the optimum combination of efficacy, safety, reliability, economy and environmental improvement to be attained. Unfortunately, it is impossible to judge accurately from this first book in the series whether these ambitious aims will be achieved when both volumes have been published.

This book comprises three sections all written by well qualified authors. They discuss the chemistry and biology of pesticides (R. L. Metcalf), the metabolism of insecticides and fungicides (T. R. Fukuto and J. J. Sims) and the metabolism of herbicides (J. E. Loeffler and J. van Overbeek). The chapters are well written and serve as an excellent, chemically oriented, introductory text and reference work on pesticides. However, they are written so as to appeal more to practising scientists than to the layman, at whom they also profess to be aimed. It is a great pity that the book lacks an index, because this greatly lessens its value as a rapid reference text. One other shortcoming is that, if the discussion is to be really pertinent to the title of the series, the relative persistence of pesticides should be discussed, but it is not; perhaps this omission will be rectified in the second volume of this series. However, these criticisms are minor and do not detract from any appreciation of the efficient and workman-like way in which the sections are written.

The standard of production is good and the layout pleasing and suitable for the subject matter, but the price will probably prove a considerable obstacle to many potential readers. There is no doubt that this book is a useful new addition to the literature on pesticides, providing information that may not be readily available elsewhere.

CLIVE A. EDWARDS

Feathered Friends

Man and Birds. By R. K. Murton. (The New Naturalist.) Pp. xx+363 +32 plates. (Collins: London, May 1971.) £2.50.

CONFUSINGLY, an earlier volume in this New Naturalist series, by E. M. Nicholson, appeared under the title of *Birds and Men.* However, while Nicholson in 1951 could point out how little was known about the precise relationships of birds and men, Murton can show how much has been discovered. It is salutary that less than one-sixth of the references cited here were available to Nicholson when he wrote his book twenty years ago, and many of these are merely old, unquantitative records showing that a particular problem is not new. To quote but one example, Nicholson does not mention the oyster-catcher, which is thought by some to have a serious effect on the cockle industry; twenty years later, after the Ministry of Agriculture, Fisheries and Food has spent perhaps £100,000 investigating the matter, Murton can devote thirteen pages to the problem based on many published works, mostly dating from about 1957.

Man and Birds contains an amount of theoretical population biology which the amateur may find somewhat heavy going, particularly the second and third chapters (on ecological considerations and predators and prey). Notwithstanding this, there are few places where such matters have been so forcefully put. The fundamental concept of population ecology is probably that of density dependent mortality; at certain seasons of the year, animals, as a result of reproduction, are usually commoner than some resources that they need. A number (usually relatively large) of them must die as a result of competition for the resource. If man kills a proportion of the animals it does not follow that breeding numbers will be reduced; usually they are not because individuals which would otherwise have died in competition with their fellows are now able to survive in place of them. This point, together with the rapid rate of increase (or recovery), when birds are scarcer than usual, is still often overlooked by people who seek to control or to protect birds.

Some 180 pages of the book cover, by species, the birds which impinge on man's activities; the section is of great value, for the author clearly knows his literature. Two final chapters cover birds and industrial man (including fouling of buildings and bird strikes and aircraft), and wildlife management (future methods of control, such as tape recorded alarm calls and distasteful compounds).

This is a very valuable review which will be widely read. However, since it was written for a market which is, to some extent, "popular" it could have been more simply written, and fewer digressions into methodology (and subjects such as photoperiodic physiology) would have made it more concise. So vast is the scope that no two people would agree on what should be included, but I would have included more about man's affect on the birds. Even the most ardent bird-hater must appreciate that massive mortality of birds may give him an early warning of something that he is doing which may ultimately back-fire on himself; large losses of sea birds, particularly the auks, are potentially important from this aspect. Also there is relatively little about bird problems outside Europe. The small finch *Quelea* in Africa does perhaps many times more damage than any birds in Europe. Problems in North and South America and elsewhere on rice-eating birds have been studied in some detail as have those of birds in vineyards, but these are barely mentioned.

CHRISTOPHER PERRINS

CORRESPONDENCE

Freedom of Scepticism

SIR,—In his letter (*Nature*, **232**, 355; 1971) Dr Higginson raises a number of points that are perhaps representative of a certain kind of contemporary view and which I shall endeavour to answer.

All of us are influenced by a respect for the authority of properly qualified scientists and by the beliefs of our colleagues. But it is a voluntary alignment and every man must have the right to re-examine sceptically the verities of conventional wisdom.

There are also many well-qualified scientists who set constraints on these freedoms and react unfavourably when the expression of scepticism generates heat rather than light. But public disclosures are the primary means by which we mutually regulate our affairs! Friction may be generated by this exchange. But I believe this comes about when the sceptic is not afforded the same courtesies as is the true believer, and Dr Higginson's letter is a case in point.

For instance, there exist well-documented critiques of most of the published evidence concerning smoking and lung cancer and other health matters—critiques, moreover, that appeared in respected and rigorously reviewed journals. In the past few months articles have appeared by Dr Yerushalmy, evaluating the data on the effect of smoking on the weight and growth of infants born to mothers who smoke (*Amer. J. Epidemiol.*, **93**, 443; 1971), and carefully re-examining and rejecting the claim advanced by a number of prestigious (and unreviewed) US Government reports that smokers suffer from a larger incidence of morbidity than do nonsmokers (*J. Amer. Stat. Assoc.*, **66**, 251; 1971). The trouble, however, is that Dr Higginson does not care to acquaint himself with the literature of dissent or even acknowledge its existence (although demanding that we document our objections—*again?*).

Or Dr Higginson speaks of the "widely explored area of cigarettes and lung cancer"; but is this impression of such a thorough exploration not created in part, too, because Dr Hammond and others have published over and over again their findings based on a single study? Dr Hammond has produced over a dozen published papers, besides innumerable talks and testimonials, all based on his confidential 1960 population survey. (His procedures had been felt to be inadequate by a number of statisticians who, incidentally, have published their criticisms all along.)

On the other hand, how often can one publish a critical review?

Again, Dr Higginson betrays his extreme displeasure by equating a courteous request by fellow investigators to meet and discuss the possibility of a public review of data, including assurances of all possible safeguards, with a "smear" and hints that the perpetrator of this dark deed somehow is motivated by "the grant reported to have been made recently to his university by certain industrial interests". (I am sure that if Dr Higginson will address a proper request to the administration of Washington University and specify which industrial interest he seeks to expose, this information will be made available to him.)

I understand that centuries ago a number of members of the faculty of Oxford University protested the establishment of laboratories for students. Being men of the highest repute and integrity, they saw no need for students to verify their claims about what was observed in their laboratories at the cost of great bother to them and needless expense to the university. But this took place many years ago; and even at Oxford the faculty has proved to be wrong occasionally.

Yours faithfully,

T. D. H. STERLING

Department of Applied
Mathematics and Computer
Science,
Washington University,
St Louis, Missouri

Weaned from Confusion

SIR,—"The cow suckles the calf" and "the calf sucks the cow" are descriptions of the one phenomenon, so clear that there should be no difficulty; but there is. During recent fairly extensive reading about this and related topics, I was sometimes so confused by the haphazard use of the two verbs that I was at a loss to know who had been doing what to whom.

Among this reading was a letter[1] written by Cowie and his colleagues and published by you in 1951, which must evidently be blamed for some of the confusion, since some authors have quoted it as their authority for writing "suckle" when they have meant "suck". Others have muddled the words quite indiscriminately and still others have adopted standard usage. Is it too late to agree that the language of the Psalms, St Matthew, Shakespeare and Lamb is good enough and that we should all use it to record our observations and express our thoughts as clearly as we can?

Yours faithfully,

F. R. SPRATLING

School of Veterinary Medicine,
Cambridge

---

[1] Cowie, A. T., Folley, S. J., Cross, B. A., Harris, G. W., Jacobsohn, Dora, and Richardson, K. C., *Nature*, **168**. 421 (1951).

Alluvial Gold

SIR,—In the issue of *Nature* for July 23 (**232**, 214; 1971) there is a brief item on the proposed exploration for gold and copper by Rio Tinto Zinc in the Mawddach estuary and Snowdonia. I was particularly interested in the statement that RTZ plans to sift or dredge the sediment of the estuary for alluvial gold.

During my doctoral studies from 1961 to 1964, I had occasion to study in detail the sediment from the estuary proper, from the freshwater rivers running into it, and from the offshore area in the immediate vicinity. In particular, I examined the texture and the accessory minerals of the sediments. The results of this examination showed significant similarities in the sediments from the estuary and the offshore environments and significant differences in the sediments from these two areas and that from the freshwater rivers (Mawddach and Wnion). This led me to the conclusion that ". . . it is very probable that the offshore areas are the major source for the sediment in the estuary, at least as far up as Penmaenpool . . ."[1]. Put more simply, this means that most of the sand in the Mawddach estuary is brought in from the sea and is *not* derived through the erosion of the gold-bearing rocks near Bontddu.

Also, as part of the overall study, I examined the records of the Barmouth Harbour Trust in the National Library of Wales, Aberystwyth. These records showed that a European firm had been granted a concession to dredge for gold in the Mawddach estuary just prior to World War II, but that the firm had abandoned the project as uneconomic.

The success of any dredging projects for alluvial gold in the Mawddach estuary, or even the need for preliminary exploration, are therefore questionable.

Yours faithfully,

R. M. McMULLEN

951 *Pinewood Crescent,*
Ottawa, Ontario K2B 5Y3

---

[1] McMullen, R. M., thesis, Univ. Reading (1964).

Obituary

Sir Lawrence Bragg

Fig. 1 William Lawrence Bragg as a young graduate at Cambridge. (By courtesy of the Nobel Foundation.)

SIR LAWRENCE BRAGG, who died on July 1 aged 81, had the unique distinction of having himself created the science to which he devoted his life's work, and lived long enough to experience its revolutionary impact, first on inorganic chemistry and mineralogy, then on metallurgy, and finally on organic chemistry and biochemistry. At the same time X-ray analysis itself advanced from the ingenious puzzle-solving of the early pioneers to the almost blindfold automation of the present day.

Röntgen discovered X-rays when Bragg was five years old, and von Laue, Friedrich and Knipping demonstrated X-ray diffraction by crystals in the spring of 1912, when Bragg was taking his physics degree at Cambridge (Fig. 1). Von Laue, who had predicted the effect, also succeeded in developing a theory of scattering from a three dimensional lattice, but he assumed that the crystal of zincblende used for diffraction had a primitive cubic lattice, and therefore could not explain why spots corresponding to certain indices were present while others with closely related indices were absent. He speculated whether the diffraction effects had arisen from fluorescence excited in the atoms of the crystal by the "aperiodic pulses" of the primary radiation or whether five prominent wavelengths pre-existing in the primary radiation had been separated out to produce the different diffraction spots.

Bragg's father, then Professor of Physics at Leeds, heard of the German work in the summer of 1912 and discussed it with his son, who set about re-interpreting the X-ray pictures of zincblende published by the Munich group. Within a few weeks he presented the correct answer to a meeting of the Cambridge Philosophical Society. The German group had noticed that spots which were round when their photographic plate was close to the crystal became elliptical as the plate was moved further away. By a remarkable feat of imaginative insight Bragg realized that such a focusing effect would arise if the X-rays were reflected by successive atomic planes (Fig. 2), and he reformulated von Laue's conditions for diffraction into what became known as Bragg's Law, which gives a direct relationship between the crystal structure and its diffraction pattern. Bragg then recognized that sets of parallel lattice planes would select from a continuous spectrum (or pulse, as he called it) those wavelengths which corresponded to integral multiples of the path difference between reflexions from successive atomic planes, so that each Laue spot would be made up of several harmonics of some selected wavelength. Finally, he demonstrated that the presence of spots with certain combinations of indices, and the absence of others, could be accounted for by assuming a face-centred rather than a primitive cubic lattice.

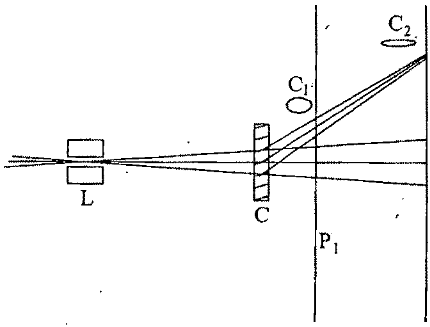


Fig. 2 Bragg's interpretation of the focusing of the diffracted spots observed by von Laue, Friedrich and Knipping which led to the formulation of Bragg's law: $n\lambda = 2d\sin\theta$. L, Lead screen; C, crystal; P_1P_2, positions of photographic plate; C_1C_2, cross sections of pencil of rays at P_1P_2. (From *Proc. Camb. Phil. Soc.*, **17**, No. 1, 43; 1913.)

Why did this twenty-two year old student succeed in correctly interpreting the diffraction pattern predicted and discovered by an accomplished theoretician eleven years his senior? Bragg himself modestly attributes it to a "concatenation of fortunate circumstances" but his paper soon convinces you that its success owed more to Bragg's astute powers of penetrating through the apparent complexities of physical mechanisms to their underlying simplicity.

This first paper was quickly followed by another, written in collaboration with his father, on their newly developed X-ray spectrometer, and a third, written by himself alone, solving the structure of common salt and showing how the Laue pictures of several other simple minerals could be indexed. There follows the structure of diamond, solved, as he relates, largely by his father, and the structures of fluorspar, zincblende, iron pyrites, calcite and dolomite solved by himself alone. Finally, on July 16, 1914, he communicated a paper on the structure of metallic copper. In view of this published record and the fact that for most of the relevant period the father was at Leeds and the son at Cambridge, it seems hardly believable that the scientific public tended to attribute most of the credit for these discoveries to his father, sometimes with the undertone that the son had cashed in on the father's success. The son must have suffered a great deal from these thoughtless judgments.

The outbreak of the First World War put an end to further research. Bragg joined the cavalry and was soon detailed to help in the development of sound ranging for the location of enemy batteries. At first he could not separate the report of the gun from the noise of the projectile travelling at supersonic speed. Then he suddenly spotted that the firing of the gun gave rise to a pressure wave, a "rush of air" as he called it, which he was able to detect by its momentary cooling of a hot wire. This simple device made an important contribution to victory.

He resumed his research when, aged twenty-nine, he was appointed Rutherford's successor as professor of physics at Manchester. His early research had replaced the concept of the inorganic molecule by the new one of a three dimensional array of close-packed and electrically compensating ions. The next step was a reformulation, on a structural basis, of the chemistry of the silicates. For example, diopside had been assigned the formula $CaMg(SiO_3)_2$, but B. E. Warren and Bragg discovered that there was no such thing as an SiO_3^{2-} ion. The structural units consisted of SiO_4 tetrahedra as in quartz; these shared corners so that they formed infinite chains bound to their neighbours on all sides by Ca and Mg ions. The varying properties of the many different silicates were found to be a consequence of the ways in which SiO_4 tetrahedra can be linked, sharing corners, edges or faces, and forming long chains as in asbestos, sheets as in mica, or three dimensional networks as in the feldspars, so brilliantly solved by W. H. Taylor. Silicates make up the bulk of the Earth's crust, and Bragg was proud that he and his associates had explained their properties on the basis of their atomic structure. The results of the Manchester school also led Pauling to formulate general rules for the structures of inorganic crystals: atomic arrangements are governed by the ratios of the ionic radii and are stable only if all charges are locally compensated.

When the main structural principles of the silicates had been discovered, Bragg's interest turned to metals. He did not actually solve any metal structure himself, but became absorbed in the X-ray analysis of alloys by A. J. Bradley and others in the Manchester laboratory. His greatest papers of that period are those with E. J. Williams on order–disorder phenomena. For example, in brass at room temperature, copper occupies the corners and zinc the centres of a cubic lattice, but when heated they become more randomly distributed over the two sites until at a critical temperature all long range order ceases abruptly. Why does the approach to disorder suddenly become catastrophic? Bragg and Williams argued that "the decrease in order lessens the forces which tend to keep an atom in its 'right' place, since when disorder is nearly complete the criterion between 'right' and 'wrong' tends to vanish. The effects of increasing thermal agitation and lessening distinction between right and wrong so interact that on approaching a critical temperature the order drops rapidly to zero". Their physical explanation of the catastrophic approach to disorder in alloys later formed the basis for the interpretation of other cooperative phenomena in solids.

I first met Bragg in 1939 at the height of his metallic period, and he put me to work on the X-ray microscope, a neat optical device which he had just invented for the summation of two dimensional Fourier series. He was also keenly interested in my X-ray pictures of haemoglobin, but he did not actually work on the problem until the early fifties when he deduced the correct shape of the haemoglobin molecule from my data. Shortly afterwards he introduced the principle of minimum wavelength which limits the combination of signs in the Fourier transform of a molecule of given size. In the event, the limitations proved insufficient to solve the phase problem in haemoglobin, but the principle later helped D. L. D. Caspar and Rosalind Franklin to solve it in a radial projection of tobacco mosaic virus.

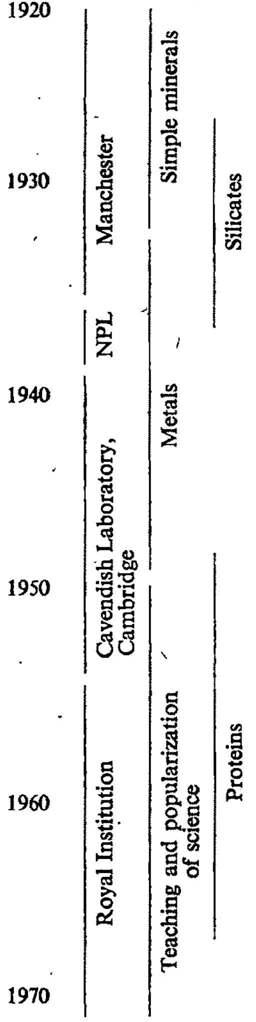
Bragg's last original paper concerned the determination of phase angles by the method of multiple isomorphous replacement with heavy atoms. In monoclinic crystals such as myoglobin or haemoglobin the location of the heavy atoms in the centrosymmetric projection was straightforward, but the determination of their third coordinate, which is not related to any symmetry element in the crystal, posed a difficult problem. Bragg devised an elegant method, based on a principle of physical optics, of deducing these coordinates from the changes in intensity produced by different isomorphous replacements in the same reflexions. As the correct values of the phase angles of several myoglobin reflexions emerged from his calculations he realized that the problem of protein structure, that seemingly hopeless venture which he had backed against all odds for the past eighteen years, could now be solved, and tears of emotion streamed down his face. Bragg's coordinates served as an independent check for those which Kendrew and his collaborators deduced from Patterson syntheses. K. C. Holmes recently used his method to find the heavy atom coordinates in tobacco mosaic virus.

I have tried to summarize Bragg's many other contributions to science in a table which also shows the periods of his different activities in various places (Table 1). The sciences have developed so much faster than the arts. If we think of Bragg as an artist and compare him to, say, Giotto, it is as though he had himself invented three dimensional representation, and then lived through all the styles of European painting from the Renaissance to the present day, to be finally confronted by computer art.

So often men of genius were hellish to live with, but Bragg's creativity was sustained by a happy home life; typically one would find him tending his garden, with Lady Bragg, children and grandchildren somewhere in the background, and before getting down to business he would proudly demonstrate

Table 1 A Chronology of Bragg's Life and Progress

Places and periods of activity



	Important works not mentioned in the text, and books
1921	Intensity of reflexion of X-rays by rock-salt (with R. W. James and C. H. Bosanquet)
1922	Absolute intensities and the determination of structures with several independent parameters (with J. West)
1924	Theory of double defraction in calcite
1924	*X-rays and Crystal Structure* (with W. H. Bragg)
1927	Isomorphous replacement (J. M. Cork)
1929	Fourier projections
1933	*The Crystalline State, A General Survey*
1934	Order–disorder in metals (with E. J. Williams)
1936	Structure factor graphs (with H. Lipson)
1937	*Atomic Structure of Minerals*
1939	X-ray microscope
1940	Structure of cold-worked metal
1942	Bubble model of plastic flow
1945	Fly's eye
1947	Dynamic model of a crystal structure (with J. F. Nye)
1957	Determination of heavy atom parameters in proteins
1965	*Crystal Structure of Minerals* (with G. F. Claringbull)
1971	*The Development of X-ray Analysis*

sity Library SCIENCE COLLEGE. 92 Upper Circular Road CALCUTTA

his latest roses. To the present young generation Bragg was an avuncular figure who showed them that science can be fun. At the Royal Institution he instituted a series of physics lectures for sixth formers, making use of the institution's excellent experimental facilities to bring home the laws of optics, electricity and magnetism. These lectures were attended by 22,000 London school children each year. He also gave a remarkable series of television broadcasts on the properties of matter which brought him popular fame. He was intrigued when the greengrocer woman in Soho told him that he was "the spitting image of a man she saw on the telly last night" and modestly signed the bill for her to keep as a souvenir. Bragg's superb powers of combining simplicity with rigour, his enthusiasm, liveliness and charm of manner, and his beautiful demonstrations all conspired to make him one of the best lecturers on science that ever lived. Yet few realized the formidable intellect that lay behind his genial façade or the decisive and revolutionary contributions to several fields of science for which he had been directly or indirectly responsible. His scientific papers still stand out as models of lucidity and conciseness. They generally contain little mathematics but are based on elegant physical and geometric arguments. His book on *The Crystalline State* once opened a new world to me and remains one of the best elementary introductions to X-ray crystallography.

Most X-ray crystallographers all over the world are "descended" from his or his father's pupils. He regarded them as one family and took the first steps in founding the International Union of Crystallography soon after the end of the Second World War. J. C. Kendrew's and my own early attempts at protein crystallography could not have been sustained throughout the many lean years without his support, and our small group at the Cavendish Laboratory in Cambridge became the nucleus from which the present MRC Laboratory of Molecular Biology later developed. Several other schools, including H. Lipson's at Manchester and W. Cochran's at Edinburgh, are offshoots of his group at the Cavendish. D. C. Phillips's flourishing Molecular Biophysics Laboratory at Oxford stems from the small protein crystallography group which Bragg brought together at the Royal Institution in the 1950s. We all owe him a great debt.

M. F. PERUTZ

British Diary

Tuesday, September 7

Computers for Analysis and Control in Medical and Biological Research (three-day conference) Institution of Electrical Engineers, in association with the Institute of Electrical and Electronics Engineers, the Institute of Mathematics and its Applications, the Institution of Electronic and Radio Engineers, the Biological Engineering Society and the British Computer Society, at the University of Sheffield.

Displays (four-day conference) Institution of Electrical Engineers; and the Institution of Electronic and Radio Engineers, at the University of Technology, Loughborough.

Human Locomotor Engineering (four-day conference) Institution of Mechanical Engineers, at the University of Sussex, Falmer, Brighton, Sussex.

Thursday, September 9

Electrical Plasmas as Free-Atom Generators, and Reservoirs for Analytical Atomic-Absorption, Emission and Fluorescent Spectroscopy (2.30 p.m.) Professor V. A. Fassel, Society for Analytical Chemistry, Midlands Region, at Henry Wiggin and Co., Ltd, Holmer Road, Hereford.

Sunday, September 12

Cell Nucleus (Third Harden Conference, six days) Biochemical Society in collaboration with the Biological Council, at Wye College, Ashford, Kent.

Monday, September 13

Fundamentals of Software for Computer Engineers (vacation school, thirteen days) Institution of Electrical Engineers, in association with the Institution of Electronic and Radio Engineers, at the University of Manchester.

Surface Chemistry of Oxides (three-day discussion) Faraday Society, in the Chemistry Department, Brunel University, Uxbridge, Middlesex.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

West of Scotland Agricultural College, Grassland Husbandry Department. Experimental Records. No. 22: Comparison of Productivity of Eight Varieties of Cocksfoot. By I. V. Hunt, J. Frame and R. D. Harkess. Pp. 16. No. 23: Varietal Productivity. Meadow Fescue, Series II. By I. V. Hunt, J. Frame and R. D. Harkess. Pp. 15. No. 24: Comparison of Productivity of Varieties of Tall Fescue. By J. Frame, R. D. Harkess and I. B. Hunt. Pp. 45. No. 25: Comparison of Productivity of Varieties of Timothy. By I. V. Hunt, J. Frame and R. D. Harkess. Pp. 38. (Auchincruive, Ayr: West of Scotland Agricultural College, 1971.) [26

The Prospects for a European Security Conference. By Michael Palmer. (European Series, No. 18.). Pp. 107. (London: Chatham House and PEP, 1971.) £1. [36

The Birds of Exmoor. By N. V. Allen. Pp. 644+14 plates. (Dulverton, Somerset: The Exmoor Press. 1971.) 50p. [36

Department of Health and Social Security. Buildings for Mentally Handicapped People. Pp. 99. (London: HMSO, 1971.) £2. [36

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:
12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature + Nature Physical Science	**£24**	**$83**	**$90**
Nature + Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office
Copyright © Macmillan Journals Limited, September 3 1971

NATURE Volume 233
SEPTEMBER 10 1971

Prosperity and Science as Chicken and Egg

SIR ALEC CAIRNCROSS, President of the British Association at its annual meeting last week, has probably lost a good many friends among professional scientists with his stern declaration that postwar British governments may have been misguided in supposing that investment in scientific research would ensure prosperity and competitiveness with advanced economies such as that of the United States. After all, a great many people have invested their reputations in various versions of this belief, and nobody likes to be told that he has backed the wrong horse, especially when so much time has gone by that he personally may be unable to back another. It is also unfortunately true that much of what has been written in the past, and many government policies on research and development, have been based on the assumption that it might be possible to win extremely quick economic returns from investments in research and development, and to that extent Sir Alec's declaration, reactionary though it may have seemed to some, is entirely well grounded. The expectation that investment in research and development on nuclear power stations in the late fifties would create an internationally dominant nuclear power industry was one of the more conspicuous miscalculations. But it would be a great misfortunate for everybody if Sir Alec Cairncross's complaints that people have expected too much too quickly of scientific research and development should confuse the issue of what connexion there may be in the long run between research and development on the one hand and prosperity and amenity on the other. In particular, it is to be hoped that Lord Rothschild and his helpers, now by all accounts engaged on a comprehensive review of the British government's involvement in research and development, will not use the spirit of Sir Alec's declaration as an excuse for parsimony.

Much of the growth of scientific research in Britain in the last few decades stems from the way in which, in the late forties, a succession of public inquiries drew attention to the great scarcity of skilled manpower in large parts of British industry. But this was also a period in which governments and others hoped that it might be possible in the postwar world to stimulate deliberately the kinds of developments which had given Britain a great reputation for scientific innovation during the Second World War—radar, jet engines and such things as penicillin. Sir Alec Cairncross pointed out last week that Whittle, the man who made the first jet engine, was not a scientist in the ordinary sense but, rather, a man in the more straightforward tradition of the classical inventors. Moreover, the argument goes, jet engines saw the light of day not merely because Whittle was an enthusiast but also because there was an urgent military need of such a development in technology. The implication, of course, is that consumer demand will somehow evoke appropriate technological developments from the community of inventively inclined people, and there is of course some truth in this description of the incentives for invention. How else is it possible to explain the way in which many new developments must be reinvented on half a dozen occasions before they find an economic climate in which full-scale exploitation becomes profitable? From this point of view, the British government's investment in research and development in nuclear power was frustrated not so much by technical difficulties as by the lack of a market. Yet none of this implies that the recognition of the need for some technical development will instantly insure that the necessary inventions are whistled up from thin air, as it were. On the contrary, there is a host of circumstances in which an apparent need for a new technical development has been satisfied not by the local invention of a suitable device in Britain but, rather, by its invention elsewhere. Questions about the relationship between investment in research and development and industrial innovation must be answered by a definition of the economic and social circumstances in which need will stimulate the appropriate developments. The question has been too much ignored.

The most obvious danger, and the most common fault, is to make too sharp the connexion between scientific research and the social or economic need that it must satisfy. This was the British government's fault with nuclear power. Now that advanced communities acknowledge that motor cars can clog up the streets of cities, there is a temptation to spend money on the development of hardware for other kinds of transport such as railways or travelling pavements. Similarly, because exhaust fumes are a nuisance, there is a temptation to spend money on steam engines and electric storage batteries. Yet it may well be that in the long run cities will benefit more from building techniques that make possible and cheap a more complete separation of people and transport, or even from systems analysis that makes transport in the sense of the physical movement of people from one place to another less necessary than at present. It is even possible that the best cities will in future owe less to research on transport as such than to the embodiment in working systems of some of the techniques of electronic communication which have already done much to replace messenger boys by switchboard girls and which still have a long way to go. This is eminently a field in which an attempt to meet a proper and clamant demand for cities that are better organized may encourage inappropriate innovations. Everything depends on whether the vision that inspires the research is of the right breadth, neither too pragmatic nor too abstract.

Strictly industrial developments, electronic computers for example, provoke or should provoke similar considerations. One of the minor scandals of the 1960s is that computer manufacturers made and sold all kinds of equipment with great promises of immediate economic benefit but without means of making sure that the customers would reap the benefits they had been promised. The trouble, in retrospect, is not simply excessive enthusiasm among salesmen but also a failure by computer designers to appreciate the complexity of many of the tasks for which they had commended their machinery. On the face of things, of course, it is paradoxical that there should be few complaints about the way in which computing machinery functions in complicated military innovations such as anti-ballistic missile systems, and endless complaints at the way in which computers function when dealing with practical commercial problems such as the calculation of weekly payrolls, but this should be no surprise; the second situation differs from the first in that it requires computing machinery to be made to play a part in a framework previously designed entirely for people, and to do this for comparable cost. But the persistent failure of computer manufacturers to live up to their promises in the past few years is of course also a definition of a technological need, and there is no doubt of handsome profits for whichever manufacturer of computers finds himself able to make machinery that functions as it is said in the brochure to function.

In countries such as Britain, there are particular economic and social needs which cry out to be satisfied. What is to be done about shipbuilding? Does Britain, either separately or as a part of the European Community, have a future as a manufacturer of aircraft or of the electronic systems with which modern aircraft are filled? Is there some way of making the automobile industry competitive in circumstances in which it seems all too probable that manufacturers on the mainland have already won the race, both in technical quality and in the cost of production? And if sober consideration does suggest that traditional industries will have very little to contribute to British success in the European economic competition for the few decades during which the European Community will be a club of economic competitors, not a federation of like-minded states, what new industries will give the British economy grist for its mill in the years ahead? It is impossible to see how any of these questions could be answered without an intellectually strong scientific community in Britain able to provide the men and women capable of solving not merely the problems which exist but those which have not yet emerged.

Quite apart from over-optimism about the probable connexion between research and prosperity, the past few decades have seen in Britain the reflexion in science policy of some of the problems which have confused international relations as a whole—the tendency to believe that even small countries such as Britain could remain creative and successful in all possible industrial fields, the tendency to suppose that native wit and intelligence would be a match for the systematic deployment of imagination and skill, backed by money, in countries such as the United States, and the suspicion that the inherent virtues of British manufactures would somehow make unnecessary attention to such skills as marketing, still regarded in Britain as techniques appropriate to second-rate commodities. In the past few years, as luck will have it, British society seems to have acknowledged that these principles are now untenable. One of the dangers of too literal a reading of what Sir Alec Cairncross had to say last week is that the tendency towards braggadocio in British science policy, which he has mistaken for a more pedestrian if less reprehensible wish to turn research into gold, may now be replaced by the equivalent in research policy of what the Little Englanders would do in politics. In short, there is a danger, which the present government so far has given no sign of acknowledging, that public policies on the scale and balance of research and development will in future be guided by the most pragmatic and expedient calculations.

This is why the British government, and Sir Alec Cairncross as well, should read carefully the speech on Monday this week in which Sir Brian Flowers, chairman of the Science Research Council, made the first official attempt so far to anticipate the effects on British policy for research and development of entry into the European Community. Over the years, British governments have taken an entirely misguided view of what the European relationship entails. A decade ago, when the first attempt was being made to win entry into the Common Market, people such as the then Minister of Aviation, Mr Peter (now Lord) Thorneycroft, hoped that it might be possible to make the process easier by offering the rocket Blue Streak as a basis for the development of the European Launcher Development Organization. By now, there is plenty of evidence that Mr Thorneycroft's calculation was mistaken. In Mr Harold Wilson's time, the British government offered an even more ambitious version of the same prospectus, proclaiming the advantages of what it called a technological community in Europe, but what was intended was simply a more grandiose system in which European countries would collaborate with each other on a multitude of projects, no doubt hoping against hope somehow to resolve the perennial problems of how best to share out contracts and kudos fairly among participants and non-participants.

The merit of what Sir Brian Flowers has to say is that it recognizes the importance (see *Nature*, **230**, 198; and **231**, 3; 1971) that European collaboration should allow participants to concentrate on the things that they do best without fearing that competitors will in the process steal a march on them. Although most discussions of British membership of the European Community have been based on the assumption that the six present members, and the ten that there will be if the new applicants eventually join, are a group of nations bound together by artificial rules in such a way as to make economic competition more wounding than it would otherwise be, the truth is that the European pattern is one that may enormously simplify the European economy. The fact that tariff barriers disappear implies that once chauvinism has been eroded by the passage of time, customers will buy the cheapest products without concern for where they come from, and industries will therefore find themselves concentrating on a few manufacturing plants, not a random scattering throughout Europe. But in this state of affairs, it will be important that there should be opportunities for skilled technical people to move freely to where their ingenuity can best be employed (which is also one of the conditions of the Treaty of Rome) by which time it will be apparent that if British industry is unable to use the skills of its graduates in science and technology, other countries will be eager to do so. But from this it follows, as night from day, that attempts will have to be made to coordinate the policies on research and development which the separate governments pursue. In the long run, the result is bound to be an integration of the university systems as well as of policies for research and development. The British government is lucky that Sir Brian Flowers has now pointed out the need for moving in this direction. It is unfortunate that Sir Alec Cairncross had nothing to say on the subject last week.

Where Real Trouble Lies

In the past several months, all kinds of fears have been expressed about the damage that might be done by pollution of various kinds to species of animals which are either of economic importance or of some other value to society at large. By now, for example, it is known that DDT and other organochlorine pesticides can damage the reproductive capacity of birds, particularly falcons and eagles. Similarly, there have been fears that pesticides and other chemicals in the sea may cause irreparable damage to breeding stocks of fish or other sea animals. It is therefore ironical but important that signs are now rapidly accumulating to show that the most serious and rapidly growing threat to the survival of these species lies not in any form of pollution but in the activities, sometimes illegal, of people armed with nothing more unfamiliar than guns and trawling nets.

The case of the American eagle is already something of a scandal. Over the years, the populations of the bald eagle and the golden eagle have steadily declined to 2,000 and 8,000 respectively and, because both birds are protected by federal legislation, it has been assumed that the decline in their populations is to be attributed to the hostile influences of the contemporary environment, DDT and all. But now it turns out that farmers in states such as Wyoming, anxious to protect flocks of sheep and other livestock against predators, have been hiring helicopters so as to destroy eagles in flight. One estimate is that close on a thousand eagles have been destroyed by these techniques in Wyoming in the past year, and it is clear that others have been killed by poisoned bait of various kinds. On the face of things, at least, it looks very much as if the illegal operations of the farmers are in themselves sufficient to account for the decline in the populations of the eagles, and that they are sufficient to jeopardize the future of the two species. Because there are bound to be serious limits to the efficiency with which the law can be enforced, is there not a strong case for asking that some of the ecologists who worry about the effects of pesticides on the breeding stocks of eagles in North America should turn their attention to such a close understanding of the habits of these splendid creatures that means might be evolved for keeping them out of reach of their most serious enemies, the farmers of the north-west?

The case of vertebrates in the North Atlantic is potentially much more serious. It is already well established that at least two species of whale have been exterminated as a result of hunting in Antarctic waters, and others are in danger (see *Nature*, **232**, 80; 1971). There is now a serious danger that species of commercial fish may be exposed to insupportable pressure from fishing vessels. It is already well documented that the catch of herring in the North Sea and around the British Isles has declined steadily since the Second World War, and this is one of the reasons why the British government is sensitive about the limits preserved for national fishing in coastal waters, due to be negotiated in Brussels this week with the members of the European Community. But it is also becoming harder for fishing vessels to keep up their productivity in catches of cod and haddock, two of the principal catches in the North Atlantic. Figures recently made available by the Fisheries Research Board of Canada (ref. Technical Report 260) illustrate how sharp has been the decline of the population on what is called the Scotia Bank, the shallow region of the continental shelf east and south of Nova Scotia. By means of comprehensive samples in the region, it has been possible to show that for both species, the present rates of exploitation are comparable with or even exceed the maximum sustainable yield of the two species. But there is at present no limit to the intensity of fishing in regions such as this; instead, there is a constant incentive to travel further afield in search of fish. Although not nearly enough is known of the population dynamics of cod and haddock, there is at least a strong danger that over-fishing could bring about a marked reduction of the stocks of fish available within a decade.

One obvious moral is that some attempt should be made to set up international controls of the intensity of fishing in the North Atlantic. If the snail's pace at which similar attempts to restrict the sovereignty of nations and the independence of fishermen is any guide, a decade leaves hardly any time for manoeuvre. The second important lesson is that those who fear the way in which pollution of various kinds may damage marine life should acknowledge that whatever the rightness of their case, there are still more urgent problems to be tackled.

---

100 Years Ago

The Aurora

I HAVE just read Mr. Wilson's interesting paper entitled "Some Speculations on the Auroras," published in your periodical for September 7. In the *Philosophical Magazine* for July 1870 I made a suggestion as to the origin of auroras similar to that just published by Mr. Wilson.

The periodicity in auroral displays noticed by Mr. Wilson had not attracted my attention. It would doubtless, if it were well established, be confirmatory of the views independently put forward by Mr. Wilson and myself. A. S. DAVIS

Meteor

ON Saturday, September 2, at 8.14 or 8.15 P.M., I saw a fine meteor under very favourable circumstances. I was standing with several friends at the door of Mr. W. F. Moore's house at Croakbourne, in the Isle of Man, and we were looking up at the western sky at the moment when the meteor came. It started between, I think, γ and π Herculis (it was too cloudy to see those stars), descended nearly vertically, passing through Corona Borealis, and vanished a little below ζ Bootis, at about 15° above the horizon. It moved slowly but continuously, taking from two to three seconds in travelling over 45°. It broke into three, which followed one another, connected and followed by a luminous train which was visible for about one second. The first part of the three was brilliant white, and was estimated by Mr. A. W. Moore and myself independently as equal in size to $\frac{1}{8}$th of the moon's surface. It was very brilliant, being mistaken by the Rev. John Howard, who was looking in another direction, for a flash of lightning. The two latter globes were blue.

Rugby, September 6 J. M. WILSON

From Nature, 4, 385, *September 14, 1871*

OLD WORLD

BRITISH ASSOCIATION

Cross Roads Again

from our Special Correspondent

Swansea, September 8

THE 135th annual meeting of the British Association, now finished, has been more than usually a demonstration of how much the association has to offer and a source of anxiety that it might not be able to continue. The scientific programme has been imaginative if unspectacular. The association has also gone a long way to meet the criticism that it has done too little in the past to provide a forum for the discussion of important public issues in the management and exploitation of science. But a cloud, now very much bigger than a man's hand, continually hangs over the association's future. It remains to be seen whether the decisions taken this week will provide an administrative framework in which the association will be able to survive.

The mood of the annual meeting, a cautious blend of enthusiasm and scepticism, was in part determined by the opening address of the president, Sir Alec Cairncross, who argued that economic history contains very little evidence to suggest that "the process of innovation owed much directly, even if it owed a great deal indirectly, to advances in scientific knowledge". The result, he said, is that those "who put their faith in science as a means of our economic salvation exaggerate the part played by the body of formulated knowledge in the generation of technological change". He went on to say that it is consumer demand—the interests of governments as well as of individuals—and not advances in scientific knowledge that "gives innovation its motive-force", and that in the moulding of innovation by consumer demand, what matters most is the way in which industrial management exploits the potentiality of techniques.

The moral which Sir Alec Cairncross drew from this analysis is that there is no correlation between expenditure on research and development and the rate of economic growth. The suspicion that there must be has, in Britain, moulded government policy towards innovation in such a way that "the problem was misconceived, the strategy mistaken, the tactics unsatisfactory . . .". For the British and for other governments, the social climate is also an important determinant of the pace of innovation. Britain has been unlucky in this respect. But for all governments, according to Sir Alec, innovation "comes far more from below than above". Governments may be able to cajole but they cannot direct.

Sir Alec's declaration has drawn a number of opponents in the past week. At a public discussion on Monday evening, Sir Brian Flowers, the chairman of the Science Research Council, said that he could have written a similar address to the Royal Institute of Economics, replacing the word "science" by "economics". Sir Alec himself is anxious to insist on the distinction between science and technology, so that in that sense his argument is not relevant to all the things that British governments have done in the past few years to foster innovation in industry.

One of the ingredients missing from Sir Alec's address was a sense of what might happen or should happen in the planning of government policy. From a quite different point of view Sir Brian Flowers spelled out on Monday some of the steps which in his view must be taken to foster the development of science within the European Community, with luck enlarged by the accession of Britain and the other applicants. (The full text of this address will appear in *Nature* in the near future.) On the creation of common institutions he asked that innovators should insist "the science must be good science" and the purpose of the institution must be clear. But the initiative for creating a new organization must come from scientists and there must be good reasons for thinking that international collaboration is essential. With these conditions satisfied, Sir Brian considers that there are great benefits to be won in the development of the European economy, especially if ways can be found of encouraging the nations of Europe to "give up the idea of alternative and purely national ventures".

Elsewhere, various sections of the association have sought to lay down the law on what should be done to brighten or to make more liberal the education of science graduates at British universities—these days, it seems, the advocates of liberal courses are the only ones who dare to speak in public. Several of the meetings in the past week have been designed to draw attention to social problems inherent in scientific developments of all kinds, and there has been a particularly successful discussion, led by Professor R. R. Porter, of the social problems occasioned by medical research. The same question was argued over at a discussion to which members of the general public were invited—an innovation for the British Association.

Controversy about the place of science in the modern world has sprung up at several sessions, sometimes by accident and sometimes by design. Nobody is in a position to count heads and thus identify the winner. On balance, however, this year's meeting has been distinguished by much more open discussion of the social antecedents of science, which is why the statement issued by the British Society for Social Responsibility in Science that "a few tame symposia on environmental things, a few public lectures on the ethical problem of this or that are not a real response to this crisis" has fallen a little flat.

This "crisis" is whether the British Association will somehow manage to survive. The association seems to have gained very little from the year long study by the Committee of Review (see *Nature*, **231**, 412; 1971). The financial position is now even more desperate.

Letter from Rothschild

SIR,—In your leading article on August 20 you referred to "inexcusable delays" on the part of the Government in reaching important decisions. You cited the study of certain aspects of Government research and development.

The Central Policy Review Staff was asked by the Government at the end of March to report on this subject by October. Given the complexity of the issues involved, to which your article testifies, six months is surely not too long for a study of the organization and management required for the annual expenditure of £650 million of public funds. This study has obviously entailed "the ample discussion with interested parties" which you imply the Government has been neglecting.

You also argue that in the management of the Government's policy on research and development, "the machinery that exists is a hindrance, not a help". It is precisely this question of the machinery which the CPRS has been studying.

Yours faithfully,
LORD ROTHSCHILD

11 Herschel Road,
Cambridge

It is good to know that the Rothschild inquiry is well on with its work, but must everything wait until its report has been digested? And will the Dainton report on the Research Councils be published?

EDITOR.

RADIO TELESCOPES

Jodrell Bank in Wales

by our Astronomy Correspondent

THE Science Research Council has earmarked £250,000 for a detailed design study of the telescope which the Nuffield Radio Astronomy Laboratory at Jodrell Bank, and in particular the laboratory's director, Sir Bernard Lovell, have looked forward to building for years. But it is clear that Sir Bernard's original aspirations have had what—instead of the 400-foot dish the Jodrell Bank group had hoped to build, the design study is for a telescope with an aperture of 375 feet. Steel prices have also made it economic to replace much of the steel in the original design by concrete.

Nevertheless, the Mark VA, as it will be called to distinguish it from the original design, will be the largest fully steerable radio telescope. Its diameter will be 47 feet greater than the current holder of the record, the telescope that is at present being brought into operation at Effelsberg, West Germany, by the University of Bonn.

But the design philosophy of the new Jodrell Bank telescope will be radically different from that of the Bonn telescope. The Bonn telescope has a light structure which is designed to distort under the forces of gravity and wind loading in such a way that the surface always remains a paraboloid to a sufficient accuracy for observations down to wavelengths of a few centimetres. In the design for the Mark VA the effects of wind are to be reduced by using a heavier structure, and the position of the reflecting membrane will be adjusted during operation by means of screw jacks attached to the backing structure to allow for gravity distortions at different elevations.

The steel backing structure which will carry the membrane of the Mark VA will pivot in elevation on bearings supported by a beam which in turn will be supported by a concrete turntable. It is hoped that the telescope will be operable down to three centimetres.

Last week's announcement by the Science Research Council should put European radio astronomers at a further advantage compared with radio astronomers in the United States, where the lack of new radio telescope projects is widely lamented. In Europe, on the other hand, significant new telescopes have recently begun operation at Westerbork in Holland and at Effelsberg, and a three-mile interferometer is under construction for Sir Martin Ryle's group at Cambridge. But it will clearly be past the middle of the decade before the Mark VA could be completed—it will be a year before the design study will have progressed far enough for tenders to be invited, and it is expected that the telescope will take three or four years to build, at a cost that is likely to be more than £4 million. Consulting engineers for the Mark VA will once again be Husband and Company, who were responsible for the 250-foot telescope at Jodrell Bank.

Although most radio astronomers will undoubtably welcome last week's news, some astronomers have argued that the wealth of instruments becoming available to radio astronomers in Europe makes the construction of a radio telescope able to operate down to millimetre wavelengths more urgent than the Mark VA (see *Nature*, **227**, 107; 1970). Such a telescope would primarily be valuable for work on interstellar molecules which have characteristic lines at centimetre and millimetre wavelengths, and would allow European astronomers to participate in a field which up to now has been dominated by the Americans. It is understood, however, that such a telescope may be under consideration by the Science Research Council, although for reasons of atmospheric transparency it could not be situated in Britain.

The site chosen for the Mark VA is at the village of Meifod in Montgomeryshire, fifty miles from Jodrell Bank, and to a considerable extent the telescope will be controlled automatically from Jodrell Bank. Land was bought at Meifod three years ago for the laboratory, with a view to Meifod being the site of the Mark V telescope, although planning permission for the dish has not yet been applied for. Although the people of Meifod are reported as being in favour of the plan, Meifod also happens to be the headquarters of the Council for the Protection of Rural Wales, which has made available the following statement: "The telescope will be completely out of scale with the Meifod valley, a countryside of small and intimate detail and exceptional beauty. The nearest hill to the site rises little more than the diameter of the dish.

"Sir Bernard has said recently that the whole of Britain was searched for a suitable site. We understand that only scientific criteria were taken into consideration in these searches and the appearance of the landscape was ignored. No consultations were held with the Countryside Commission. Local opinion is in favour of the telescope but it is now realized that our countryside is a matter of concern for everyone in Britain. Local opinion believes that Meifod will be 'put on the map'. However, the benefits may be illusory since employment will be minimal once the site is built. Sir Bernard has told us that tourism will not be encouraged because of electrical interference and tourist facilities have already been provided at Jodrell Bank. The danger of electrical interference will also inhibit the natural development of the area within range of the site."

POLLUTION

Canberra Conference

from a Correspondent

Some of the guidelines for the long awaited United Nations Conference on the Human Environment to be held in June 1972 in Stockholm became clearer during a thirteen nation meeting of the UNESCO advisory committee held in Canberra on August 23–27. The recommendations of the committee on Natural Resources were couched in general terms but they give some idea of the directions which UNESCO would like the Stockholm conference to take.

The committee proposed that interdisciplinary projects should be emphasized including both social and natural sciences and that priority should also be given to continuing and expanding those projects begun under the aegis of the International Biological Programme which have proved to be successful. The committee also favoured an early start to some projects which are likely to be productive in order to get the forthcoming "Man and the Biosphere" programme, that is being sponsored by UNESCO, firmly established. Also it recommended that projects should "concentrate on the study of dynamic rather than static phenomena".

The committee further recommended that a set of ecological guidelines for development projects should be prepared to encourage planners of major works, such as large dams, to include ecological considerations in their planning. The director of UNESCO's Natural Resources Division, Dr Michel Batisse, said that he hoped that UNESCO would publish some definitive guidelines after the Stockholm conference. The administration of Man and the Biosphere was criticized by the advisory committee, as was the administration of the International Geological Correlation Programme (IGCP). The committee recommended that the IGCP administration should come under a single coordinating body whose members would be appointed jointly by the Director-General of UNESCO and the President of the International Union of Geological Sciences (IUGS). The IGCP is a joint project of UNESCO and IUGS that is planned to begin in 1973. Its aims are to correlate geological knowledge of different areas—partly in the hope of encouraging new mineral finds. These recommendations will be put to an inter-governmental conference to continue preparations for the IGCP which will be held in Paris on October 19–29.

Dr Michel Batisse said that Man and the Biosphere was receiving strong support from developing countries, communist countries and most western nations but that the United States appeared only moderately enthusiastic with Britain also treating the programme very coolly. Professor J. N. Black of the Forestry and Natural Resources Department, Edinburgh University, was elected chairman of the advisory committee, which possibly signifies an increase in Britain's support.

SRC Members

THE Secretary of State for Education and Science, Mrs Margaret Thatcher, has this week appointed two new members to the Science Research Council to replace Professor Sir Ronald Nyholm and Professor P. A. Sheppard, who have retired from the council. They are Professor H. Elliot of Imperial College, London University, and Professor R. Mason of the University of Sussex. The composition of the council from October 1 will then be: Professor Sir Brian Flowers (chairman), Dr A. H. Chilver, Dr D. S. Davies, Dr E. Eastwood, Professor H. Elliot, Professor H. Ford, Professor J. C. Gunn, Professor F. Hoyle, Professor H. L. Kronberg, Professor R. Mason, Professor P. T. Matthews, Dr J. W. Menter, Professor E. W. J. Mitchell, Mr D. L. Nicolson, Dr E. J. Richards and Professor M. M. Swann.

NUCLEAR REACTORS

All Set at Dounreay

ALTHOUGH the construction of the British prototype fast breeder reactor is running about eighteen months behind schedule, it is still ahead of similar projects in other countries. The prototype is being built by the Nuclear Power Group at the United Kingdom Atomic Energy Authority Experimental Reactor Establishment at Dounreay in the North of Scotland, and should be completed at the end of this year.

The original plan was that the reactor system should deliver power to the North of Scotland Hydroelectric Board during the first half of this year, but because of trouble with the construction of the roof of the reactor vessel, commissioning is now expected to take place early in 1972 with the first output of elecricity at the end of the year. By contrast with the smaller Dounreay fast reactor which first went critical twelve years ago and has been producing about 14 megawatts (MW) of electricity as well as providing a valuable irradiation facility, the prototype fast reactor (which has cost about £38 million) will generate at least 250 MW and earn about £4 million a year from electricity sales. A further £2 million a year will probably accrue from rental of irradiation space in the core.

The Dounreay prototype fast reactor represents an important link between the small experimental fast breeder and the reactors needed for a new generation of nuclear power stations. The Central Electricity Generating Board is seriously considering the feasibility of fast breeder reactor stations and proved commercial systems could also be sold abroad provided that the designs are not so geared to the requirements of the Central Electricity Generating Board that they are unattractive to other potential customers (a situation that may account in part for the poor response to the British advanced gas-cooled reactors). Discussions between the Atomic Energy Authority and the Central Electricity Generating Board have led to a proposal that a 1,300 MW fast reactor power station should be started in 1974. This would cost about £130 million, but any decision to go ahead with such a project cannot be taken until the satisfactory operation of the Dounreay prototype fast reactor is assured and the thorny problem of siting fast reactors near centres of population is at least partially resolved. The Central Electricity Generating Board would certainly not be happy with a remote station because of the high costs of electricity transmission. A definite choice of site for the first commercial fast reactor will, however, have to be made during 1972 if the rather ambitious 1974 deadline is to be met.

In spite of the interruptions in the Dounreay prototype reactor programme, only the Russian 350 MW prototype reactor at Shevchenko is being built on a comparable timescale; a full-size Russian system is, however, unlikely to be a serious commercial competitor in the West. A French 250 MW prototype reactor project is under way at Marcoule, but the core is not expected to go critical before 1973. In the United States General Electric and Westinghouse have designed a "demonstration" fast breeder reactor but construction has not yet started.

Britain is also involved in two collaborative fast reactor ventures in Europe. In 1969, the Nuclear Power Group joined representatives of eight European countries in a project to investigate gas cooled fast reactors (as opposed to the sodium cooled fast reactors such as the Dounreay prototype); the group is also involved with Kraftwerk Union (through its associated company Interatom), Belgonucleaire and Neratoom in an agreement to market fast reactors eventually on a worldwide basis. Interatom, Belgonucleaire and Neratoom are at present working on the design of a 300 MW fast reactor to be built in West Germany.

The Dounreay Experimental Reactor Establishment. The prototype fast reactor is in the right background, and the older Dounreay fast reactor is in the left foreground.

SPACE SCIENCE

Probing Mars

from our Soviet Correspondent

THE Soviet Mars-2 and Mars-3 probes, launched on May 19 and 28, 1971, and now half-way through their six-month voyage to their target, not only carry experiments for the study of Mars and circum-Martian space, they also have on board a number of experiments intended to operate during the voyage itself. These are intended to measure magnetic field strength, the flux of charged particles and study the solar wind.

The solar wind experiment, which appears to be the most important to the Soviet space programme at this stage, is designed to determine the velocity, temperature and composition of the principal components of the solar wind and the variation of these parameters with time and distance. It includes an ion-electron spectrometer unit, capable of measuring the spectral distribution of the particles from 30 eV to 10 keV. This unit consists of eight individual spectrometers, each consisting of an electrostatic analyser, a high-gain electron channel multiplier (the first to be produced in the Soviet Union), sampling schemes and a signal recording device.

The first results from the solar wind experiment are now being analysed, and are providing good evidence of the satisfactory design of the unit. Some typical data so far obtained, for the first 10 days of the flight of Mars-3, are that velocity of the solar wind fluctuated between 300 and 600 km s^{-1}, relative content of alpha-particles varied from 5 to 10 per cent. The temperature of the solar wind particles and the considerable velocity gradients recorded are the subject of further and more detailed study.

The time-gap between the launching of the two probes and the availability of readings from other Soviet projects makes it possible to estimate which fluctuations are due to variation along the trajectory and which are due to variation in time. The solar wind data obtained from Lunokhod-1 and from the Earth-orbiting Kosmos satellites are providing reference data against which the Mars-2 and 3 data may be more clearly evaluated.

ENVIRONMENT

A-level Development

THE third attempt in recent years to develop an A-level syllabus in environmental studies was launched last week by a party of school teachers from Hertfordshire (*Environmental Studies—The Construction of an A-Level Syllabus*; National Foundation for Educational Research in England and Wales). The other two attempts, in 1968–69 and 1970, were unsuccessful but the present proposal has been constructed in greater depth. The Hertfordshire County Adviser for Environmental Studies, Mr S. McB. Carson, said this week that he was optimistic that the present submission would be accepted by the examination boards and the Schools Council.

There are twenty-two schools and two colleges in Hertfordshire that are planning to start teaching the new syllabus in September 1972 if approval is granted by then, and there are four examination boards that have already been approached with a view to instituting the syllabus. These are the University Entrance and Schools Examination Council of London University, the Associated Examining Board, the Cambridge University Local Examination Syndicate and the Oxford Delegacy of Local Examinations. With the exception of the Associated Board which is not sympathetic to the new syllabus, the other boards are considering the proposal. Before the syllabus can be instituted, however, the boards must submit it to the Schools Council for approval. If approval is granted it is unlikely that more than one examination board will eventually offer the examination and arrangements will probably be made, as is currently done in other subjects for that one board to offer the examination to the schools attached to the seven other boards.

The development of an A-level course in environmental studies is a natural consequence of the O-level and CSE examinations in similar subjects that are already recognized by several examination boards. A problem in instituting the syllabus at A-level will be to find qualified teachers. Mr Carson said this week that the plan in Hertfordshire is to have the teaching done by biologists and geographers, or by specialist environment teachers—either those who are now involved in teaching the O-level subjects or by graduates of one of the several universities that now offer courses in environmental studies. The overlap of the new syllabus with other topics already being taught in schools is not a problem, according to Mr Carson, and of the fifteen university faculties that have in principle approved of the subject as an entry requirement, only one expressed reservations about a candidate offering both environmental studies and biology.

One of the arguments in favour of the new syllabus, says Mr Carson, is the increasing awareness of today's sixth-formers about ecological problems. Unlike most A-level syllabuses, A-level environmental studies will be as relevant as anybody could wish. Another potential advantage of the syllabus is that it offers career opportunities at non-degree level in teacher training and in Town and Country Planning.

BIOMEDICAL RESEARCH

Science Looks Forward ?

by our Special Correspondent

"Too plainly we are—in common with the whole of humanity—confronted with the question of where (the development of biology and medicine) leads." This was the reasoning which led Hoffman-La-Roche to celebrate their 75th anniversary by holding a symposium, "The Challenge of Life", in Basle last week. The aim of the symposium was outlined by Dr Alfred Pletscher (Hoffman-La-Roche) as "to define more clearly the problems created by biomedical progress and to find, if possible, essential facts or clues for their solution". Although the illustrious assembly of speakers, which included not only biologists but also sociologists, philosophers and even theologians, was, of course, unable to provide definitive answers to this ambitious question, this proved to be one of the very few conferences where a consensus of opinion emerged to pinpoint with some clarity the essential problems which need to be resolved.

Some fascinating clashes of temperament dominated the symposium, in particular the conflict between the idealism of Dr Philip Handler (President, US National Academy of Sciences) and the pragmatism of Solly Lord Zuckerman (former Chief Scientific Advisor to the British Government). Whilst Professor Joshua Lederberg (Stanford University) and Professor Arnold Burgeon (Director, National Institute for Medical Research, Mill Hill) strongly urged that it is essential for scientists to communicate with the public and to reverse the alienation which many laymen now feel from science, Professor Niels Jerne (Director, Basle Institute for Immunology) maintained that it is impossible and, indeed, perhaps undesirable, for scientists to popularize their work.

Any curtailment of basic research would be tantamount to the book burning of the Nazis, said Dr Handler and Professor René Konig (University of Cologne), whilst others, in particular Professor Bernard Barber (Columbia University), were adamant that, to some extent at least, the sociologist, as a representative of public opinion, should be allowed to sit in judgment on the scientist. And Professor Margaret Mead (American Museum of Natural History) wished that the scientist would listen to the warnings that the social sciences can provide of reactions such as the backlash to science.

The consensus of opinion was with Professor Salvador Luria (Massachusetts Institute of Technology) that the critical point for control is not the direction of basic research but its technological development. And as Lord Todd (University of Cambridge) said, the problem of overpopulation loomed large over the entire proceedings. The right of the individual to expect medical science to maintain him in good health was defended by doctors, including Professor Burgeon and Max Lord Rosenheim (President, Royal College of Physicians of London), but the meaning of this was challenged by Professor Jeanne Hersch (University of Geneva). One of the main talking points was Lord Zuckerman's insistence that governments simply lack the resources to keep up with the demands for expensive treatments, and that medical science is aggravating this problem by devising more of these treatments.

A more detailed account of these discussions will appear in *Nature* next week.

GRADUATE EMPLOYMENT

A Question of Degree

CONCERN over graduate employment prospects and over the efficiency and effectiveness of the service that appointments boards provide marked the meeting of the Standing Conference of University Appointments Services (SCUAS) held in Cambridge last week. The conference, which meets every two years, was attended by 150 appointments officers and 70 guests, and took as its theme "Appointments Services in a Changing World".

Professor R. V. Jones, Professor of Natural Philosophy at the University of Aberdeen, outlined some of these changes in his opening address to the conference. After tracing the history of university education, Professor Jones suggested that the increase in the number of graduates had not been matched by an increase in quality. He went on to say that this factor combined with the present overproduction of specialist scientists will result in increasing numbers of science graduates following non-specialist employment.

To adapt to this situation science courses may have to be altered to produce more generally employable graduates. Professor Jones argued for a common course in the early stages of university education to prepare three types of graduates—those who would go on to be professional scientists, including teachers, those who would be professionally employed but not in the fields of research or design, and those who would almost immediately move outside science. He also suggested that demand would again equate to supply in the mid-1980s.

But, for the present, opinions varied as to the seriousness of the situation, Mr H. B. Putt, the retiring chairman of SCUAS, thought it possible that by the end of the year the situation "will be less disturbing than we had at one time feared". Mr Bernard Holloway, of the Manchester University Board, SCUAS's very own Job's comforter, felt otherwise. He predicted that by the end of the year the situation would be "worse than our worst fears", that 1972 would be at least as bad, and that 1971 was "the first of the seven lean years".

The duty of appointments officers, he said, was to be pessimistic, so that everyone became aware of the problems facing graduates. Mr Tom Snow, of the Oxford Board, while admitting that there are more graduates than traditional graduate jobs, denied that the situation was doomladen, and argued that "the function of education is not to fill gaps in the economic system". The impression must not be produced that higher education is a waste of money. Graduates, he felt, were still a very saleable product. He pointed out that by 1981, 30 per cent of men and 25 per cent of all eighteen-year-olds will be receiving higher education, and that the appointments boards should draw attention to the need they are, and will be, filling.

But it became clear from the conference that, saleable or not, to accommodate the growth in graduate numbers in the next ten years there will have to be a number of basic changes in attitude by students, schools, employers, unions and academics. All will have to become more flexible. University education will have, in the words of Mr Neil Scott of Nottingham University Board, to become an "education for citizenship", and much less a passport for a job. Graduates, particularly those who are not first-class material in either research or industrial terms, will have to be prepared to start at the bottom of their chosen ladder, and spend some years training. Students will have to realize this before they go to university, or, as Mr Holloway pointed out, graduates with unfulfilled expectations may produce a lot of resentment in society.

The expectation of what university has to offer will have to change at school level. This means alerting headmasters, of whom there were only two at the conference, as well as career teachers to the new realities. Academics too may have to be more flexible and provide broader based courses, and the conference will suggest to appropriate bodies that when new courses are planned appointments boards might have something to say. Employers will also have to accept that many jobs now filled by A-level entrants will in future be filled by graduates. Some employers are well aware of this—the GPO's traditional A-level white collar entry is now two-thirds filled by graduates.

Mr W. P. Kirkman, President of SCUAS, and Secretary of Cambridge University Appointments Board. "SCUAS", he says, "must be very well aware of changes, and move to meet them, but beware of rushing up any side track that comes along".

In fields where graduates have rarely if ever penetrated before there will have to be radical changes. Pay and promotion structures will have to be altered to allow graduate entry—at present their degree can put graduates automatically on to too high a pay and promotion scale for the employer to be able to afford to train them. Potential problems with unions who will object to the intrusion of graduates in their ranks will have to be ironed out.

But if the conference was aware of the limitations of other organizations, it was also aware of its own. The need for a central service unit which would provide badly needed centralized organization and prevent pointless duplication of work was reiterated, and it is likely that some such organization will soon be established. The American placement offices have had a central organization for the past fifteen years.

The use of a computer matching service, to cut out much of the routine paperwork, is being considered. This would allow the student to find early in the year what jobs are available in the fields in which he is interested. The system requires comparatively little personal information, and the National Union of Students, subject to certain safeguards, is cautiously enthusiastic.

A computer would allow more time for actual counselling, to help find graduates the kind of job suited to their talents, and, as Miss Audrey Newsome of Keele University pointed out, it is just that that the appointments boards must provide if they are not to become irrelevant to student needs. Miss Newsome also pointed out that appointments officers, although they are often experienced, are essentially an amateur body, and she called for training courses in counselling. Students, she said, must be met and helped before they come to feel that appointments boards are irrelevant.

Overall the conference was active without being pushed into panic by one particularly bad year, and they established a number of long-term studies to continue to examine their role. Their main problem is that while they have a close and constructive relationship with employers, particularly through the 300 members of the Standing Conference of Employers of Graduates, and, on the whole a good relationship with students, the appointments boards relationship with the universities, although improving is by no means uniformly good. They feel that now universities might listen more to what they have to say, and possibly spend more on them. The country spends about £80 getting a student to university, £3,500 educating him, and about £22 finding him a job. That balance is going to have to change if appointments boards are to provide the graduate with the service he needs.

NEW WORLD

AEC Causes Anger and Delight

by our Washington Correspondent

ACCUSED by the Court of Appeals of foot-dragging in its implementation of the National Environmental Policy Act, and of making a mockery of the act's intentions, the Atomic Energy Commission has reacted with a swiftness and boldness that suggest that the court hit a particularly sensitive spot. Little more than a month after being told by the court to take another look at its licensing policies, the AEC has come up with a new set of regulations which include every environmental safeguard that the court demanded, and which threaten to shut down five plants that are already operating and to halt construction on forty-six others while their environmental effects are reviewed. The new regulations also threaten to land the AEC in a flood of court actions brought by angry electricity companies who will have to foot the bill for costly delays and who face the prospect of adding expensive modifications to their plants to reduce environmental damage. When James R. Schlesinger, who took over as chairman of the Atomic Energy Commission on August 16, promised to be sympathetic to the demands of environmentalists, few expected him to demonstrate his sympathies quite so quickly or so dramatically.

The new regulations were made in response to a ruling of the US Court of Appeals which, on July 23, took the AEC to task for not considering environmental issues as a matter of course when an application to construct or operate a nuclear plant is given a public hearing; for excluding environmental issues from being considered in any public hearing announced before March 4, 1971 (fourteen months after the National Environmental Policy Act came into force); for failing to evaluate and balance certain environmental factors by abdicating its responsibilities to other agencies, and for allowing electricity companies to carry on construction of plants which have not been reviewed under the procedure laid down by the National Environmental Policy Act (NEPA).

It took the AEC more than eleven months after NEPA came into force to draw up regulations to comply with the act's intentions, but only one month to design new regulations to comply with the court's ruling. Some indication of the haste with which the AEC moved to meet the objections of its environmentalist critics can be gauged by the fact that when the new regulations were announced last week, the commission was unable to say how many plants at present being constructed, or which have recently been given operating licences, would be affected by each section of the new regulations. Hurried discussions at the AEC's headquarters, however, produced a list some seven hours after the regulations were made public.

AEC's "Mockery"

THE AEC's new environmental regulations sprung from a decision of the US appeals court on July 23, 1971, in which a group of environmentalist organizations accused the AEC of paying insufficient attention to the National Environmental Policy Act, particularly in regard to construction of a nuclear power plant on the Chesapeake Bay in Maryland. The following extracts are taken from the opinions of the appeals court:

The environmentalists charged the AEC with not submitting its detailed environmental impact statement to a review process unless it was specifically required to do so by public challenge. The court decided:

> We believe that the Commission's crabbed interpretation of NEPA makes a mockery of the Act. What possible purpose could there be in the Section 102(2) (C) requirement (that the "detailed statement" accompany proposals through agency review processes) if "accompany" means no more than physical proximity—mandating no more than the physical act of passing certain folders and papers, unopened, to reviewing officials along with other folders and papers? What possible purpose could there be in requiring the "detailed statement" to be before hearing boards, if the boards are free to ignore entirely the contents of the statement? NEPA was meant to do more than regulate the flow of papers in the federal bureaucracy. The word "accompany" in Section 102(2) (C) must not be read so narrowly as to make the Act ludicrous. It must, rather, be read to indicate a congressional intent that environmental factors, as compiled in the "detailed statement", be *considered* through agency review processes.

On the AEC's decision not to consider environmental issues for permits issued before March 14, 1971, the court said:

> Strangely, the Commission has principally relied on more pragmatic arguments. It seems an unfortunate affliction of large organizations to resist new procedures and to envision massive roadblocks to their adoption. Hence the Commission's talk of the need for an "orderly transition" to the NEPA procedures. It is difficult to credit the Commission's argument that several months were needed to work the consideration of environmental values into its review process. Before the enactment of NEPA, the Commission already had regulations requiring that hearings include health, safety and radiological matters. The introduction of environmental matters cannot have presented a radically unsettling problem. And, in any event, the obvious sense of urgency on the part of Congress should make clear that a transition, however "orderly", must proceed at a pace faster than a funeral procession.

Finally, the court instructed the commission to consider calling a halt to construction of some plants:

> . . . In order that the pre-operating licence review be as effective as possible, the Commission should consider very seriously the requirement of a temporary halt in construction pending its review and the "backfitting" of technological innovations. For no action which might minimize environmental damage may be dismissed out of hand. Of course, final operation of the facility may be delayed thereby. But some delay is inherent whenever the NEPA consideration is conducted —whether before or at the licence proceedings. It is far more consistent with the purposes of the Act to delay operation at a stage where real environmental protection may come about than at a stage where corrective action may be so costly as to be impossible.

The new regulations place firmly in the hands of the AEC the responsibility for evaluating likely environmental effects of a nuclear power plant, including the so-called thermal pollution of cooling waters, and the commission is also charged with the task of balancing these likely effects with the growing need for electric power and with assessing alternative approaches. Environmentalists have in the past accused the AEC of being unresponsive to environmental questions because it has been in no position to evaluate alternatives; and therefore any attempt at a cost benefit approach to the licensing of a new power plant has been chiefly left to the electricity companies which cannot be expected to take a wholly unbiased attitude.

Environmental effects of proposed nuclear power plants will now be considered as a matter of course at two different stages of a licence procedure —when a company applies for a licence to construct a plant, and again when it applies for a licence to operate it. At the construction stage, the applicant must submit to the AEC a report spelling out in detail the environmental effects of the proposed plant, any adverse environmental effects that cannot be avoided should the proposal be implemented, alternative approaches to the proposed construction, "the relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity", and any irreversible and irretrievable commitment of resources which would be involved in the proposed action should it be implemented. The last two obscurely worded requirements are designed to help the AEC to conduct its own cost benefit analysis of the project, in terms of alternative methods for reducing environmental damage. An applicant for a licence to operate a nuclear plant must also submit to the AEC a report detailing the environmental effects of the plant, but only where they differ from the effects spelt out in the application for a licence to construct the plant.

The next stage in the process requires the AEC's Director of Regulations to draw up his own draft statement on the likely environmental effects of the plant, open the statement up for public comment, in the light of which he will draw up a final detailed statement and give his opinion on whether or not the permit should be issued. The detailed statement will include a cost benefit analysis balancing the environmental effects of the facility with its economic and technical effects.

The court of appeals chided the AEC for simply turning over the applicant's environmental report to other federal and state agencies, such as the agencies approved to regulate water quality standards under the Federal Water Pollution Control Act, for assurance that the proposed plant will meet their standards. "The upshot is," the court pointed out, "that the NEPA procedures, viewed by the Commission as superfluous, will wither away in disuse, applied only to those environmental issues wholly unregulated by other federal, state, or regional bodies." This abdication of responsibility also prevented the AEC from considering the environmental effects of the proposed plant in the light of alternative actions. But the new regulations specifically require that the AEC's detailed statement includes a discussion of the effects "irrespective of whether a certification from the appropriate authority has been obtained".

The detailed environmental statement will then be offered in evidence if a public hearing is held, and the Atomic Safety and Licensing Board has been charged with the task of determining whether the requirements of the National Environmental Policy Act have been carried out in the proceedings. The licensing board will then have the honour of determining whether the licence should be granted, denied or modified to protect the environment. That decision would, of course, be open to public appeal, and all construction permits and operating licences would be issued on the condition that the licensee observes environmental conditions laid down by the federal government, the state in which it is to be built or operated and the Atomic Energy Commission.

The upshot of these new regulations is that the Atomic Energy Commission itself will be directly responsible for ensuring that the environment will not be abused by the construction or operation of a new plant, that environmental considerations are given prominence at every stage of the licence application, and that there is ample time for the public to offer a comment or a challenge on every report and decision made during the process. Another effect is that the regulations will add a further bottleneck to the processing of applications and place an extra burden on the commission's staff. Asked last week whether the regulations will slow up the granting of a licence which will permit operation or construction, Harold L. Price, the AEC's Director of Regulations, said that he hopes the present delay of eighteen months between application and granting of a construction licence will not be extended. Others, particularly the electricity producers, were not so optimistic, however, and the spokesman for one company said last week that licensing delays are already placing an intolerable burden on the utilities in trying to meet the consumers' power needs. (The Federal Power Commission states, however, that licensing procedures caused delays to only eight out of 114 major power plants that started producing electricity between 1966 and 1970.)

Of even greater concern to the electricity producers is the provision in the new regulations for submitting to a detailed environmental review all the plants at present under construction or which have received operating licences since January 1, 1970, the date on which the National Environmental Policy Act came into force. Not only will these plants be reviewed, but the AEC is also asking that construction or operation be stopped until the review is completed. The electricity companies have been given thirty days to show why construction or operation should not be stopped before the AEC throws its powerful spanner in the works, and their only hope is to be able to show that continued construction or operation will not damage the environment significantly, that it will not preclude the adoption of environmental safeguards should they be necessary, and that delay would damage the public interest by failure to meet power demands or by causing undue costs to the licensee or the consumer. Five nuclear power stations that are already producing electricity and forty-six others that are under construction are threatened by this provision. Environmental reports must be submitted to the AEC detailing the environmental impact of the plant, and the Director of Regulations of the AEC must then go through the complete procedure of issuing a draft and later a final detailed environmental report on the plant. It is then up to the Director of Regulations to decide whether the licence or permit should be continued, modified to protect the environment, or be revoked, and his decision will be open to challenge in a public hearing.

In backtracking so vigorously on its past regulatory policies, the AEC has opened itself wide to court action from disgruntled electricity producers, and the sharpening of knives could clearly be heard in the industry last week. A spokesman for the Consolidated Edison Company, although reserving his comments until the company has had a chance to study the regulations in greater depth, accused the AEC of plunging the electricity industry into a crisis, and he pointed out that several electricity companies are intending to appeal against the appeals court decision which precipitated the AEC's new environmentally conscious image. Even if that appeal is successful, however, it is unlikely to affect the new regulations, although it would place the AEC in the embarrassing position of enforcing regulations that would no longer be upheld by a court decision, and this would serve to strengthen the hand of the electricity companies in any future court skirmish with the commission

Of more immediate concern to the AEC, however, is the possibility that electricity companies will file a suit for losses caused by construction or licensing delays, or for the installation of expensive equipment such as cooling towers. In that case, the AEC would undoubtedly be on very sticky legal ground, for the companies have all proceeded under licences or permits issued legally by the AEC under its old regulations, but they now face the possibility of having those licences modified or revoked because the commission now regards them as inadequate for the protection of the environment. The AEC's policy switch has therefore put both the commission itself, and the industries it regulates, in an extraordinary position, but while the courts are being kept busy, environmentalists can take heart that the new chairman of the commission is not deaf to their pleas.

EMPLOYMENT

Unemployed PhDs

by our Washington Correspondent

MORE evidence about the tight job market for those holding a PhD in science has been published this week by the National Research Council. A survey, conducted between December 1970 and March 1971, of scientists who received their PhD degree in 1970, showed that 1.6 per cent were out of work, and that a further 1.2 per cent held jobs for which their academic training was unnecessary. Moreover, there are signs that many more PhDs are looking towards postdoctoral training as an escape from the realities of the job market, but the indications are that they will fare no better when they finally leave the groves of academe, for the National Research Council has discovered that of the PhDs that had been engaged in postdoctoral training in 1970, 2.2 per cent were unemployed in the following winter and 0.9 per cent were employed in an "inappropriate position".

The results of the survey, which in many respects bear out the findings of an employment analysis conducted by the National Science Foundation (*Nature*, **232**, 150; 1971), are a clear indication that the prospect of a dire shortage of PhDs that was predicted for the 1970s has not materialized, and that the universities are already turning out more PhDs than the market can absorb. The Office of Education, for example, predicted in 1964 that there would be a desperate shortage of PhDs by 1974, and a great drive was undertaken in the universities to make up the predicted shortfall. Last year, for example, 29,436 scientists received PhDs—an increase of 14 per cent compared with the previous year. Although, compared with the national unemployment rate, which has been running at about 6 per cent for the first half of 1971, the unemployment rate for PhDs is small, prospects for the rest of the decade look grim. The 1970 figure, for example, showed that the number of scientist PhDs out of work is increasing at an alarming rate (the 1970 figure was double the 1969 total), and the NSF has recently predicted that by 1980, at present rates, there will be about 45,000 more PhDs in America than there are suitable jobs.

The NRC survey has also brought out the fact that physicists are the worst hit single group of scientists, with 2.9 per cent of the PhDs and 3.3 per cent of the postdoctorals out of a job. Mathematicians, geoscientists and social scientists, on the other hand, reported lower than average unemployment rates, with 1.2, 0.7 and 0.5 per cent of their PhDs out of work in 1970. Even these subjects, however, reported greater unemployment rates for 1971 than for the previous year. There was also a sharp increase in the number of foreign-born scientists unemployed in the United States—the figure rose from 0.9 per cent in 1969 (the same as for US citizens) to 2.3 per cent in 1971 (compared with 1.6 per cent for US nationals)—a figure that can only be partly accounted for by the fact that fewer foreign-born holders of the PhD left after completing their academic training in 1971 than in 1969.

One of the most significant findings in the survey is that the number of new recipients of the PhD who went on to postdoctoral work in the universities in 1970 increased by 4.5 per cent over the 1969 figure. This fact undoubtedly hides much of the unemployment, and disillusionment among PhDs fresh from the university production line, but with few prospects of appropriate employment outside the universities. Moreover, the situation will probably be made considerably worse by the fact that predictions made two years ago by the Office of Education, that graduate enrolment would have risen by 99 per cent by 1980, now seem to be vast overestimates—the NSF, for example, now predicts that the expansion may be nearer 45 per cent—thereby reducing drastically the predicted number of jobs for PhDs in the universities themselves.

The National Research Council itself concludes from the survey that "various market accommodations are undoubtedly responsible for preventing what would otherwise be a gross oversupply". Nevertheless, with an eye clearly on the mandarins in the Office of Education, the NRC says "any deficit of jobs must . . . be a matter of national concern, and reliance solely on market adjustments to an oversupply would be both callous and unwise. The large investment in graduate education and the valuable human resources at stake demand that any underemployment or unemployment of doctorate recipients be corrected".

Percentage Distribution, 1971 Activity of 1969 and 1970 Graduates, by Citizenship, by Gross Field Categories

1971 activity	Citizenship	1969 graduates				1970 graduates			
		EMP*	Bio.	Soc. Sci.	Total	EMP	Bio.	Soc. Sci.	Total
Postdoctoral training	US	8.8	20.8	1.8	9.9	12.4	30.9	3.0	14.4
	Foreign	8.0	15.3	0.4	8.7	13.6	22.1	1.6	13.5
Appropriate jobs in USA	US	82.8	69.8	89.0	81.4	78.4	58.7	88.5	76.5
	Foreign	51.8	31.0	36.7	44.6	47.6	23.7	42.5	41.6
Employed in inappropri- work	US	0.6	1.0	0.4	0.6	1.3	1.2	0.8	1.2
	Foreign	1.3	0.4	0.0	0.9	1.3	2.6	0.8	1.5
Unemployed, total	US	0.7	1.1	1.0	0.9	1.4	1.6	1.2	1.4
	Foreign	0.8	1.3	0.4	0.9	2.7	2.0	0.8	2.3
Left US	US	1.6	2.5	2.4	2.0	2.0	2.7	2.3	2.2
	Foreign	28.8	48.8	54.9	37.4	30.5	44.2	48.5	36.4
All other	US	0.7	0.8	0.3	0.5	1.0	1.1	0.6	0.9
	Foreign	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0
Unknown	US	4.8	4.0	5.1	4.7	3.5	3.8	3.6	3.6
	Foreign	9.2	3.2	7.6	7.5	4.2	5.3	5.8	4.7
Total No. in sample	US	5,262	2,208	2,296	9,766	5,740	2,429	2,477	10,646
	Foreign	1,190	471	275	1,936	1,420	493	365	2,278

*EMP, Engineers, mathematicians and physicists.

NEWS AND VIEWS

Of Mice and Men

As the number of research workers setting their sights on the discovery of human tumour viruses increases—as it seems certain to do, especially in the United States where money for cancer research is becoming so easy to come by and money for anything else commensurately difficult to find—it can confidently be anticipated that there will be numerous repetitions of the sequel of events which has followed the report by Priori, Dmochowski and their colleagues (*Nature New Biology*, **232**, 61 ; 1971) that a cell line from a child with Burkitt's lymphoma produces a C-type RNA virus and which has culminated in the report by Gilden, Parks, Huebner and Todaro (see page 102 of this issue of *Nature*) that this is probably a mouse virus, presumably a contaminant.

Dividing cells, whether in an animal or maintained *in vitro* in cultures, provide an ideal environment for the replication of all sorts of viruses which have nothing whatsoever to do with malignancy. Any virus which by chance reaches a population of such cells is likely to become permanently established in it. And those biologists who are now turning to tumour virology, if only to keep themselves in a job, would do well to remember that whenever they find a virus in a fresh biopsy of tissue from a tumour or in a population of tumour cells in culture, the first priority is not to call a press conference but to recognize that the virus is probably a contaminant. As recent events seem to have shown, even such old hands in the game as Priori and Dmochowski can be tripped over by contamination, and their story is salutary.

Priori *et al.* established a monolayer cell culture from the pleural effusion of an American child with Burkitt's lymphoma. During the tenth passage of these cells they were found to bud typical C-type particles with all the morphological characteristics of the avian and animal RNA tumour viruses. Well aware of the problem of contamination, they apparently sent samples of their virus to Dr Old's group at the Sloan–Kettering Institute to have them classified by serological tests. The crucial question was simply does this virus have the group-specific antigen, which indicates species specificity, of any of the known animal sarcoma and leukaemia viruses or does it have a unique group-specific antigen which would strongly suggest that it is indeed a human C-type virus? Although Priori *et al.* gave few details of the tests performed in Old's laboratory they reported that their virus seemed to have a group-specific antigen different from any of the antigens of the known animal leukaemia viruses. Not surprisingly this result encouraged them to believe that they might well have isolated a human C-type virus, but both this result and its interpretation have now been seriously challenged by Gilden *et al.*

Gilden *et al.* obtained from Priori and Dmochowski some of the virus producing cells at their forty-first passage and taking every precaution to prevent any contamination of the cells in their two laboratories, they performed three sorts of immunochemical tests to see whether the virus was related to any of the known animal C-type viruses. The results of both complement fixation and radioimmunoprecipitin inhibition tests strongly suggested that something in the human, virus-producing cells carried the group-specific antigens of the mouse leukaemia viruses. Pursuing this lead, they then performed immunodiffusion tests with sera specific for the mouse virus group-specific antigen and sure enough found a precipitin line of identity between highly purified murine group-specific antigen and concentrates containing the virus liberated by the human cells. In short, these results must mean either that the virus being produced by Priori and Dmochowski's cells is murine leukaemia virus, which presumably somehow contaminated the cultures, or mouse and human C-type viruses have group-specific antigens which cannot be differentiated by these tests. This second alternative seems to be most unlikely and, if the results of Gilden *et al.* can be independently confirmed, it will be hard to avoid the conclusion that the virus Priori *et al.* have isolated is anything other than a contaminant and a red herring.

But as one putative human cancer virus is eliminated, others are turned up. The two virus-like particles from cultures of cells from patients with Hodgkin's disease, which Kingsley Sanders's group describe on page 104 of this issue of *Nature*, for example, must be suspect until they have been further characterized. Perhaps stimulated by the fascinating epidemiological study of a rash of Hodgkin's disease cases among the 1954 graduating class of a New York State high school, which lead Vianna, Greenwald and Davie (*Lancet*, i, 1209 ; 1971) to conclude that Hodgkin's may well be an infective disease with a long incubation period and a carrier state, Kingsley Sanders and his colleagues established a number of long-term cell cultures from lymph node material of Hodgkin's patients and searched for virus particles. The cells in the cultures underwent some bizarre changes including a "blastoid transformation" during which "practically every reticular cell could be seen to have one or more round cells within it", but they also began producing two sorts of virus-like particles. One of these is apparently a DNA containing particle which under the electron microscope resembles a herpes virus and there is evidence that the cells after the blastoid transformation have herpes virus antigens. The second particle which has so far defied all but the most preliminary characterization apparently contains RNA but under the electron microscope nothing with the characteristic morphology of a virus has yet been seen.

Obviously these observations are extremely preliminary; whether or not these cells are producing an RNA virus remains to be seen and the significance of the herpes virus is at present anybody's guess. Nevertheless, the findings of Vianna *et al.* strongly suggest that a virus which plays a part in the aetiology of Hodgkin's disease awaits discovery and it can be certain that Kingsley Sanders and his colleagues will soon find themselves in a crowded field, for if so many workers are set on searching for human cancer viruses they may as well concentrate on one of the extremely few forms of human cancer for which there is at least some epidemiological evidence to suggest the existence of a virus. And it scarcely needs adding that a more extended investigation of the epidemiology of Hodgkin's disease would not come amiss.

CANCERS

Dangers of Affluence

from our Cell Biology Correspondent

THE fig and prune producers of the world might do worse than start an advertising campaign with the slogan "a bowl a day keeps bowel cancer away" to judge from what Burkitt has to say in the current issue of *Cancer* (**28,** 3; 1971). Burkitt has, of course, spent many a long year trying to pin down environmental factors responsible for particular cancers, always arguing that the incidence of the disease can be significantly reduced more easily by eliminating or evading the causative agents than by trying to fathom out their mode of action and devise a cure. And his latest foray, this time into the epidemiology of cancer of the colon and rectum, leads him to the conclusion that the refined diets of the world's developed nations, in particular their lack of indigestible fibre, may, together with particular gut floras, be responsible for the comparatively high incidence of bowel cancers and other non-malignant and non-infectious diseases of the bowel in affluent societies.

The incidence of bowel cancer, which in most western countries is second only to the incidence of lung cancer, is correlated with the stage of economic development, and Burkitt claims that the age adjusted incidence of this disease is ten-fold greater in Western Europe and North America than in most African countries. Epidemiologists when faced with such findings frequently resort to the incantation "dietary differences" and leave it at that with the real problem, the identification of the crucial dietary components, unsolved. Burkitt to his credit has given hostages to fortune by suggesting what the crucial factors may be, namely the transit time of the stools through the bowel and the nature of the bowel flora.

By recruiting the help of African and English boarding school boys, who were provided with radio-opaque plastic pellets as a condiment for their school meals, he and his collaborators have shown that there is a clear inverse relationship between the transit time and bulk of the stools and the fibre content of the food eaten—the more fibre the shorter the transit time. African boys passed 80 per cent of the plastic pellets in 45 h whereas their English counterparts took 89 h. Burkitt suggests therefore that carcinogens produced by the bacterial flora in the bowels of westerners, whose foetid stools contrast markedly with the odourless stools of Africans, are held at high concentrations for prolonged periods in contact with the bowel mucosa with the net result of an increased incidence of bowel cancer in western populations. Like a prophet in the wilderness Burkitt writes: "In view of the evidence, it seems justifiable to issue a warning against the removal of so much of the unadsorbable fibre from our food, and the associated over ingestion of refined carbohydrate".

Another and related warning was published recently by Hill, Goddard and Williams (*Lancet*, i, 472; 1971) who suggest that differences in fat consumption together with differences in gut flora may account for the geographical variations in the incidence of breast cancer. It is well known that mammary carcinoma is far more prevalent among North American and North West European women than among African, Asian and South American women, and surveys of the Japanese populations in Japan, Hawaii and the West Coast of the United States show that differences in the incidence of breast cancer depend on geographic rather than genetic factors. Dietary differences have been discussed, and Wynder, for example, has postulated that the consumption of large amounts of fat may affect hormone production and/or storage in adipose tissue and thus be responsible for an increased incidence of breast cancer.

Hill and his colleagues now suggest an alternative mechanism. They have shown that people eating high fat diets have in their guts more active and strictly anaerobic bacteria than people eating low fat diets. Moreover these bacteria are able to produce oestradiol, oestrone and several other steroid sex hormones. If one or more of these steroids is carcinogenic or acts synergistically and stimulates the growth of tumour cells, anaerobic gut bacteria may be the link between high fat diets and a high incidence of breast cancer, and for that matter colonic cancer. If the much discussed expansion of cancer research materializes, the distributors of the dollars might do worse than promote further investigations of the metabolic activities of gut bacteria.

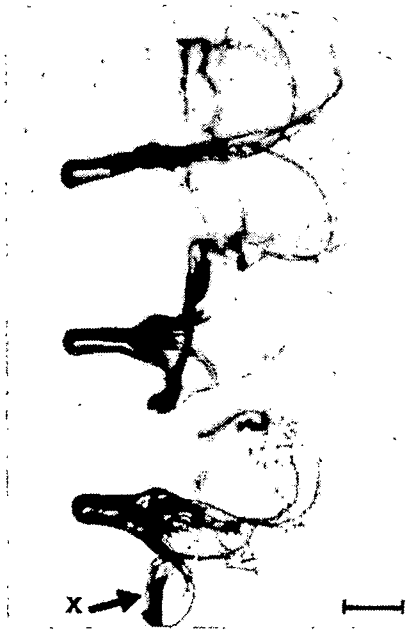
Liquid Drops in Shock Tubes

THE photograph below shows some of the bizarre shapes that drops of water can take up in a shock tube. The drops are falling through a flow that is from left to right, and the aerodynamic and surface tension forces involved have given the drops an umbrella-shaped canopy. The photograph is one of several in next Monday's *Nature Physical Science* that have been obtained by P. G. Simpkins of Bell Telephone Laboratories, Whippany, New Jersey, illustrating the successive deformations that drops undergo when exposed to a sudden increase in the external flow.

The crucial parameter in work of this nature is the ratio of the force due to aerodynamic pressure to the force due to surface tension—the Weber number—and the photograph was taken at a Weber number of 45. But the region in which Simpkins is interested has a lower limit at a Weber number of about 7.3—above this critical value the non-uniform distribution of surface pressure exerted on the drops causes deformations which eventually lead to the bursting of the drops.

The steps in this process, as charted by Simpkins, are as follows. At a Weber number of 19 drops that initially had a diameter of 1 mm are flattened by the flow into thin disks. The centres of the disks then blow out in the direction of the external flow, so that the drop takes on the appearance of a bag supported by a liquid annulus. Simpkins presents graphs showing how the distortion proceeds, and discusses the deviations in behaviour from those predicted by simple theory.

The deviations from expected behaviour become more severe at higher Weber numbers. Umbrella-shaped distortion of the drops like that in the photograph occurs at Weber numbers above twenty. The mechanism by which the drops form a narrow stem and subsequently a canopy is, however, unknown.



Drops in a shock tube at a Weber number of 45, 1,660 μs after the start of the flow. The flow is from left to right and the bar represents 1 mm. The arrow points to a satellite drop which is showing the bag response.

PROTEINS

The Mighty Photon

from our Molecular Biology Correspondent

THE use of the photon as a reagent, or as a trigger that will set off other reagents at a chosen point in space or time, is an approach that will undoubtedly find increasingly wide application in molecular biology. One—albeit qualified—advantage is that short-lived photochemically generated species are inclined to have very high reactivity, and when unleashed on an enzyme, for example, are apt to react even with aliphatic side chains. Too indiscriminate a reactivity of course restricts the value of a reagent, and Sperling and Elad (*J. Amer. Chem. Soc.*, **93**, 3839; 1971) have addressed themselves to ways of controlling the specificity of photochemical alkylation. They have found that on irradiation of a system containing a peptide, an unsaturated species, such as butene or toluene, and an initiator, substitution occurs at the α-carbon of glycine residues, butene giving rise to norleucine, toluene to phenylalanine. When proteins are allowed to react under these conditions, however, histidine, cystine, methionine, tyrosine and tryptophan residues also react. This is a consequence of the formation of various free radicals, and if radical scavengers are added to the reaction mixture the majority of these side reactions are suppressed. It proved impossible, however, to prevent instant death to the tryptophans. In ribonuclease, which has no tryptophan, glycine reacted and, though a certain amount of other damage occurred, 20 per cent of enzymatic activity was retained.

Of more immediate biological moment is the design of photochemically triggered affinity labels, which hold out the advantage that they will not react fruitlessly on their way to the specific binding site. Brunswick and Cooperman (*Proc. US Nat. Acad. Sci.*, **68**, 1801; 1971) have prepared analogues of the biochemical elixir, cyclic AMP, labelled with tritium and bearing diazomalonyl groups in the purine and in the sugar. On irradiation a ferociously reactive carbene is generated, which is expected to react with almost any group within reach. The analogues will bind to the enzyme phosphofructokinase, for which cyclic AMP is the natural activator. They both activate and compete with cyclic AMP, and after irradiation are covalently integrated into the enzyme.

This work now opens the way to the isolation of a labelled peptide and the characterization of the activator binding site. A more exciting prospect for the biochemist, however, is the use of the same reagents to label cyclic AMP receptors in cells, and indeed Brunswick and Cooperman have already succeeded in introducing a label into a protein component of rat testis. The reagent is thus permitted to traverse the membrane and diffuse through the cell to its target protein before it is irradiated and caused to react. The advantages of this strategy in labelling components in intact tissue need no labouring.

Erlanger and his colleagues have meanwhile continued their attempts to simulate the photochemical systems of nature in the laboratory. By the use of acetylcholine analogues capable of light-induced *cis-trans* isomerization they achieved partial control over the activity of an excitable tissue preparation, because the isomers will not in general be expected to possess precisely the same binding characteristics. Bartels, Wassermann and Erlanger (*ibid.*, 1820) have now brought this essay in biochemical engineering to a considerable state of refinement. They have, apparently by a process of serendipity, arrived at a species—an azobenzene derivative—of which the *trans* isomer is one of the most powerful known activators, and the *cis* isomer is largely, perhaps completely, inactive. They have moreover built into such a molecule the means for attaching it covalently to its receptor. This is based on the earlier observation by Karlin that affinity labelling of the acetylcholine receptor of the electroplax membrane could be achieved with a thiol-reactive function, if the tissue was first treated with a reducing agent to break a disulphide bond, evidently located hard against the binding site.

Substantial *cis-trans* interconversion of the azobenzene derivative can be achieved by ultraviolet irradiation, and is reversed by white light. Thus when the system containing electroplax tissue, stimulated by the *trans* isomer, is exposed to the near-ultraviolet light, precipitous depolarization ensues. Competition with carbamylcholine and curare occurs as expected. When the thiolreactive version of the analogue is covalently attached to the reduced membrane, the competitive effect of carbamylcholine is lost. This then is a completely self-sufficient light-regulated system. The reactive *trans* isomer is reported to inhibit acetylcholinesterase, but so equally does the *cis* form. The inferred message is that the acetylcholine receptor and acetylcholinesterase are distinct and unrelated forms.

Plasma Current Amplifier

IN next Monday's *Nature Physical Science*, P. C. Stangeby and J. E. Allen describe a new device which contains a narrow metal-walled electric discharge vessel and which is capable of high current amplification. The metal walls behave as a cathode and electrons are extracted from the metal by surface recombination with ions that arrive from the plasma.

According to the theoretical description of the device, the ion current travelling to the walls of the conducting cylinder is related to the discharge arc current in an expression involving the radius of the cylinder, the electron and ion masses and other constants which are of the order of unity. The cylinder is biased in such a way that the electrons produced by ionization of the neutral particles which carry them away from the wall are retained in the arc discharge; the ions can then return to the cylinder to pick up other electrons. The plasma current thus increases exponentially with distance down the cylinder and, for small cylinder radii and small ion masses, the multiplication factor is very large (for H_2 in a 5 mm diameter cylinder, for example, a current amplification of about 10^7 can be obtained).

The prototype apparatus used by Stangeby and Allen comprised a metal tube 6 mm in diameter and an anode which could be moved inside the tube. There were two alternative cathodes—a mercury pool type and a thermionic type. A 2 A current from the mercury pool cathode was made to flow across the mouth of the metal discharge tube and was collected by an auxiliary anode; the required current input to the metal tube itself was then tapped off.

The thermionic cathode gave similar experimental results, but one of its advantages was that the tube current could be measured directly with an ammeter. There was also a facility for introducing a quantity of argon gas to examine the effect of reducing the ion mass (which should increase the amplification). Measurements with the apparatus have shown that the current amplification at a mercury pressure of 1 mm Hg and an argon pressure a few hundred times this value is as expected theoretically. Although they did not measure the argon flow rate accurately, Stangeby and Allen found that an increased rate was associated with a distinct increase in the amplification.

There are probably several uses for current amplifying devices of this type, including applications to high power lasers; they may also be useful as high current controls.

PERMO-TRIASSIC

Problems left Unsolved

from a Correspondent

AN international conference, with problems of the Permian–Triassic boundary as its principal theme, was held from August 23–26 at the University of Calgary, Alberta, Canada. The keynote of the conference was set by Dr A. McGugan, speaking on behalf of Dr N. D. Newell (American Museum of Natural History, New York), who outlined some of the major problems of the late Permian and early Triassic. These included the almost universal occurrence of discontinuities or paraconformities at or near the system boundary, the general poverty of the contemporary fauna and the wide extent of essentially barren continental red beds.

The concept of a universal hiatus in marine strata at or near the system boundary was emphatically supported by Dr E. T. Tozer (Geological Survey of Canada, Ottawa), and, although there was some disagreement on exact placing, it was generally conceded that a break is present in each of the classic mid-Tethyan sequences of Armenia, Kashmir and the Salt Range of West Pakistan. The duration of the hiatus was thought on some palaeontological evidence to be of the order of one to two stages, but Dr W. G. Sweet (Ohio State University) considered that the break could not be recognized in conodont assemblages. In an unscheduled and characteristically forthright paper, Dr R. Assereto (University of Milan) asserted that the hiatus might have been shorter in some eastern parts of the southern Alps than in the classic sections farther east, and Dr H. Taraz (Geological Survey of Iran), whose view was challenged in discussion, suggested that no breaks were present in a thick late Permian and early Triassic marine sequence in central Iran. He proposed a loosely defined Abadeh stage for the time represented by post Djulfian pre-Triassic strata.

The staggered but progressive decline in late Permian floras and faunas was documented by several speakers, including Drs R. Toryama (Kyushu University) and G. L. Wilde (Humble Oil and Refining Company, Houston) who described the final stages in the evolution of the fusulinids, and Dr G. L. Batten (American Museum of Natural History, New York) who showed how a dramatic decrease in marine gastropod genera took place in the late Permian and was not fully recouped until the Ladinian. Among plants, Dr J. M. Schopf (US Geological Survey, Columbus) demonstrated a sharp unexplained change from the dominant *Glossopteris* flora of the Permian to a Triassic flora dominated by *Dicrodium*.

Several speakers speculated on the cause of the floral and faunal decline in the late Permian, without reaching agreement. Drs J. Pattison, D. B. Smith and G. Warrington (Institute of Geological Sciences, Leeds) suggested that a major cause might be the virtual elimination of shelf seas, during a prolonged phase of tectonic stability and peneplanation following rapid build-up of evaporites and progradation of coastal plain sediments. This view was supported by Dr B. Waugh (University of Hull) and, with variations, by several other speakers. There were tentative suggestions that the changes in the contemporary biota might have been related to the beginning of the break-up of Laurasia, and Dr G. F. Stehli (Case Western Reserve University) showed that seafloor spreading could be reconciled with the distribution of known Permian and Triassic biotas as well as that of evaporites and red beds.

The effects of late Permian and early Triassic plate movements along the Pacific margin of the United States were graphically interpreted by Dr N. J. Silberling (Stanford University), but Dr A. A. Meyerhoff (Tulsa), opposing the concept of continental movements, claimed that the biotic crisis could have been caused by cosmic ray and temperature maxima associated with rare galactic events of a type calculated to have prevailed near the end of the Permian. Finally, the view that late Permian floras and faunas might have declined following nutrient depletion and a progressive failure of lower members of the food chain was persuasively argued by Drs Helen Tappan and A. R. Loeblich (University of California).

In the closing sentence of his introduction, Dr N. D. Newell had expressed the wish that the conference would leave unsolved at least some of the Permian and Triassic problems: his wishes, perhaps unwittingly, were duly gratified.

How SV40 DNA Replicates

THE mechanism of replication of such covalently closed circular DNA molecules as the chromosomes of *Escherichia coli* and the coliphages lambda and ϕX 174 has long been a source of fascination to molecular biologists. And there continue to be arguments for and against the Cairns's model of replication, which predicts that none of the DNA strands in the replicating structure is longer than a parental strand and the two progeny strands are of equal length, and the rolling circle model, which predicts the occurrence of some DNA longer than parental strands and progeny strands of unequal length. The replication of the genomes of the DNA tumour viruses SV40 and polyoma virus, which are not only closed, circular, double stranded DNAs but are also supercoiled, presents, of course, the same topological problems as the replication of circular phage and bacterial DNAs. And the experiments published in *Nature New Biology* next week by Jaenisch, Mayer and Levine clearly indicate that it is by the Cairns's mechanism that these molecules duplicate.

Jaenisch and his colleagues used the Hirt procedure to extract preferentially the SV40 DNA from infected African green monkey kidney cells, which 40 h after infection had been exposed to ^{3}H-thymidine for periods ranging from 3.5 min to 120 min. They then analysed the viral DNA on sucrose, ethidium bromide–caesium chloride and alkaline gradients and examined it by electron microscopy. In summary they find that replicating SV40 molecules contain parental closed circular DNA strands as well as linear fragments of newly made DNA. Moreover, the DNA labelled during the shorter pulses is shorter than one complete SV40 strand and is not linked to parental strands; the rolling circle model, of course, predicts the opposite of these findings.

Molecules which are partially (10–70 per cent) replicated sediment faster than anticipated, but under the electron microscope the reason for this becomes obvious; these molecules have two untwisted branches of equal length attached to a third twisted or supercoiled branch and, as expected, the length of one untwisted strand plus the twisted strand is about the same as the length of a single relaxed SV40 genome. The electron microscope also reveals single stranded discontinuities at the forks and some 34 per cent of the molecules examined have these regions at both forks. If a single stranded region at a fork indicates a site of replication then at least some SV40 DNA molecules must be replicated in two directions from an origin, although this cannot be obligatory, for 41 per cent of molecules had only one fork with single stranded DNA.

By showing in reconstruction experiments that relaxed SV40 DNA molecules are not converted into covalently closed, double stranded circles during the experimental procedure, Jaenisch *et al.* have disarmed critics who might have suggested that *in vivo* the template for replication is a nicked DNA strand, which, during the experiment, is closed into a ring. And so it is hard to escape the conclusion that at least some SV40 DNA molecules replicate by the Cairns's mechanism.

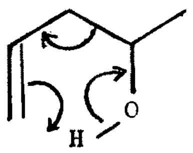
REACTION MECHANISMS

Where is the Proton?

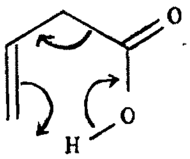
from our Biological Chemistry Correspondent

Kinetic isotopic effects provide a specialized tool for investigating transition states of reactions which involve slow proton transfers. At the qualitative level, the technique of exchanging hydrogen for deuterium has many successes to its credit, not least in the field of enzymatic mechanisms. The problems emerge when attention is directed to the significance, if any, of the quantitative aspects of primary isotopic effects.

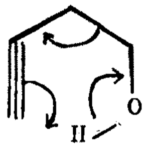
The early theoretical appraisals, notably due to Westheimer and to Biegeleisen, developed the concept that the magnitude of the kinetic change observed on isotopic substitution should reflect the extent of hydrogen transfer in the transition state: "maximum" effects correspond to those reactions in which the hydrogen is symmetrically located between the donor and acceptor groups in the transition state. Although this idea has been widely applied, direct supporting data have been sparse and the concept has been challenged often, most recently by F. G. Bordwell and W. J. Boyle (*J. Amer. Chem. Soc.*, **93**, 512; 1971) who searched in vain for a simple correlation between isotopic effect and extent of proton transfer.



(1)



(2)



(3)

H. C. Kwart and M. C. Latimore (*ibid.*, **93**, 3770; 1971) have now provided strong validation of Westheimer's concept by investigating three gas-phase reactions. The thermolytic fragmentation of 4-penten-1-ol (1), 3-butenoic acid (2), and 3-butyn-1-ol (3) all proceed through concerted bond-reorganization processes which unquestionably involve transfer of hydrogen from oxygen to carbon—formally indicated by the "curly" arrows, but probably sigmatropic processes. In spite of this similarity, the three cases provide a significant variation both of the acidity of the hydrogen being transferred and in the electronic structure around the acceptor-carbon. If therefore these factors influence the isotopic effect, differences should become apparent.

The rates of all three reactions, measured over a range of temperatures, show a 2-fold to 2.5-fold rate retardation on replacement of hydrogen by deuterium. "Maximum" values for the isotopic effects were calculated on the assumption that the zero-point energy difference, O-H *vs* O-D, alone determines the kinetic isotope effect. In each case, observation and calculation agree within the limits of experimental error.

These results thus provide one of the strongest supports for the continued use of kinetic deuterium isotopic effects as a criterion for concertedness in hydrogen transfer reactions.

HEPATITIS

Active Immunization

from our Medical Virology Correspondent

It has been repeatedly demonstrated in many parts of the world that the injection of normal human immunoglobulin (gamma-globulin) during the incubation period of infectious (epidemic) hepatitis can prevent jaundice among contacts. Although the incidence of the icteric form of hepatitis was thus reduced, infection was not prevented—it was attenuated to a variety of hepatitis without jaundice or to the subclinical form of the disease and faecal excretion of the virus still occurred.

The precise mode of action of immunoglobulin is not completely known but it is presumed that partial passive, and therefore short term, immunity against infectious hepatitis is obtained by the antibodies present in the globulin prepared from large pools of plasma. Active immunity may, of course, be acquired through subclinical infection, so-called passive–active immunity.

The problem with serum hepatitis, on the other hand, seems to be entirely different, and immunoglobulin has not reduced significantly the incidence of post-transfusion hepatitis. The discovery of the association between Australia antigen and serum hepatitis has provided the crucial key for studies of this form of hepatitis. S. Krugman and his associates (*J. Amer. Med. Ass.*, **217**, 41; 1971) now report the results of limited studies in volunteer children on active immunization against serum hepatitis caused by the MS-2 strain. Thirty-nine children were divided into two groups: one group of twenty-five susceptible children were injected with MS-2 serum; the second group included fourteen susceptible children who received either one or two inoculations of heat inactivated preparation of a 1 in 10 dilution of MS-2 serum followed by an inoculation of untreated infective serum 4 to 8 months later. It was found that hepatitis induced by MS-2 serum was prevented by active immunization and that two spaced inoculations were more effective than one. Nevertheless, one inoculation gave enough protection to prevent some cases of serum hepatitis and to modify others. Krugman and his colleagues emphasize that these obser-

---

"Unblocking" Sera in Anti-tumour Immunity

During the past few years evidence has accumulated that animals and human patients with progressively growing tumours often have immune cells that are capable of reacting against their own tumour cells, as shown by cytotoxicity tests *in vitro*, but they also have in their sera factors that can specifically block the anti-tumour reactions. To increase anti-tumour immunity it would be useful if ways could be found for overcoming such blocking effects. The possibility that another group of serum factors can "unblock" immune reactions against tumours arises from the work of the Hellströms and their associates on sera from mice with regressing Moloney sarcomas, and of Bansal and Sjögren on polyoma virus-induced tumours in inbred rats. The latest report from these last authors will appear in next Wednesday's *Nature New Biology*.

Bansal and Sjögren have found that when sera from rats immune to polyoma tumours are mixed with homologous blocking serum, they counteract the inhibition produced by the blocking serum on lymphocyte-mediated cytotoxicity against polyoma tumour cells *in vitro*. Still more remarkable, such sera inoculated into five rats bearing transplanted polyoma tumours brought about regression of the tumours in four of the animals. These effects are not caused by anti-viral antibody, and it seems unlikely that they are caused by serum-mediated autotoxicity because the transplanted cells grew into palpable tumours before regressing—such regression is never seen in untreated rats. Further work is required to establish how common unblocking activity in serum is, whether it is caused by a particular kind of antibody, and how it unblocks. One interesting possibility is that blocking is caused by antigen–antibody complexes, with which unblocking could react. Whatever the mechanism, there is no doubt that unblocking is of general interest as well as potential therapeutic significance.

vations are based on relatively small numbers.

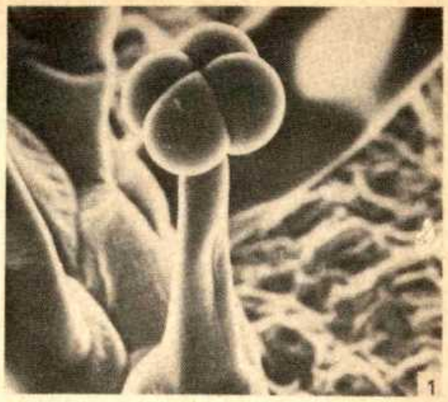
These studies represent the culmination of many years of research using human volunteers. The use of mentally handicapped children and prisoners in such studies has been criticized on many occasions and the background and justification for such studies were recently summarized by S. Krugman and J. P. Giles (*J. Amer. Med. Ass.*, **212**, 1019; 1970). Apart from the ethical issues involved, however, several questions are inevitably raised by these studies. First, it is known that although the MS-2 serum contains Australia antigen this serum gives rise to a mild and now well characterized form of serum hepatitis. Second, the agent of serum hepatitis has not been finally characterized and the injection of heated and diluted whole serum containing Australia antigen is rather a crude way of inducing immunity. The serum may, of course, be regarded as an inactivated "vaccine", but some purified inactivated vaccines such as measles and respiratory syncytial virus have sensitized rather than protected the recipients. In terms of hepatitis this is an important problem because several workers have suggested that there are similarities between Australia antigen-associated hepatitis and type 3 hypersensitivity reactions in which the severity of the disease may depend on the balance between antigen and antibody (see review by A. J. Zuckerman, *Bull. Wld Hlth Org.*, **42**, 957; 1970). Finally, active immunization against serum hepatitis is likely to have only limited application, for example to children and adults in crowded institutions such as hospitals for the mentally handicapped and more so to certain groups at high risk to hepatitis such as patients and staff of renal dialysis units, transplant surgeons and perhaps some laboratory workers. The problem of active immunization against the much commoner epidemic form of hepatitis remains elusive.

APHIDS

A Sticky End

SOME species of wild potato seem to have a most effective mechanism for resisting attack by aphids—the glandular hairs on the leaves and stems discharge a gummy substance when damaged by the tarsi of an aphid; this exudate first of all restricts the insect's movements and further accumulations lead to the complete immobilization of the aphid and to its death. This mechanism is described by Dr R. W. Gibson of Long Ashton Research Station, Bristol, who has been experimenting with three species of wild potato, *Solanum polyadenium*, *S. tarijense* and *S. berthaultii*, and two aphid species, *Myzus persicae* and *Macrosiphum euphorbiae* (*Ann. Appl. Biol.*, **68**, 113; 1971).

In the three species of wild potato examined, the gummy exudate is produced from the tip of the glandular hair when an aphid mechanically ruptures the cell walls. In the intact hair, this substance seems to be a clear, water soluble liquid but on contact with air it changes to a dark brown or black insoluble sticky substance which is deposited on the aphid's limbs. Gibson found that not every contact of an aphid with a glandular hair resulted in the release of the exudate, but about 30 per cent of the aphids placed on any of the three species of wild potato became stuck to the plants by the substance within 24 hours. Starvation was probably the final cause of death; Gibson also noticed that the gummy substance occasionally found its way on to the labium of an aphid and this may also have prevented the aphids in his experiments from feeding.



Glandular hair on *Solanum berthaultii* of the type consisting of a four-celled head mounted on a stalk.

Although Gibson observed a wide variety of hairs on the leaves and stems of the three wild potatoes, the type with the four-lobed head (see figure) mounted on a stalk seems to be the principal, if not the only, source of the gummy material. These hairs are abundant on

Turning a Corner in Nucleic Acid Structural Studies

IN many ribonucleic acids, double helical base-paired regions are formed by a single polynucleotide chain folding back on itself. It is usually (and not unreasonably) assumed that these double helical regions have a structure similar to that determined by X-ray crystallography for the completely double helical molecules of reo virus and wound tumour virus RNA. But there have been no such impeccable guidelines for the conformation of the single stranded loops at the end of each double helix. Many of these loops, such as that containing the anticodon in transfer RNA, can be expected to have important roles in biological function and information on their structure is unquestionably of great significance. No doubt much will be revealed when the first single crystal analysis of a transfer RNA molecule is achieved. This happy event, however, still seems a little way off. In the interim attention has turned to the problem of turning a corner along a polynucleotide chain.

The information comes from the determination by Seeman *et al.* (see next Wednesday's *Nature New Biology*) of the crystal structure of the naturally occurring dinucleoside phosphate, uridine 3′, 5′ adenosine phosphate (UpA). There are, in fact, two molecules of the dinucleotide in each asymmetric unit in this crystal, thus providing at some modest additional effort to the authors two independent determinations of the structure of UpA. Although these two molecules have quite different overall conformations, Seeman *et al.* point out that these are attributable primarily to differences in orientation about only two single bonds—the two successive P-O bonds along the dinucleoside chain. The orientations found in one of the molecules are such that a polynucleotide chain containing a dinucleoside with such a conformation would turn a corner—actually through 180° in the space of one phospho-diester link. This structure observed for UpA seems to represent a particularly neat solution for the production of a sharp bend in a polynucleotide chain and it would be surprising if use is not made of this stereochemical possibility of some close variant of it in studies of the biological function of nucleic acid.

The interbase hydrogen bonding in UpA is also of considerable interest. Although base pairing is common in crystals of bases, it is relatively rare in crystals of nucleosides and nucleotides where hydrogen bonding of the bases to water, sugar hydroxyls and charged phosphate oxygens is frequently observed. This is a point which many builders of transfer RNA models seem not to have realized, because in most models tertiary structure is attributed to base pairing between single stranded regions of the clover leaf. In this context the structure of UpA is an exception to the rule because it does have base pairing. This, however, is not complementary pairing in which adenine–uracil pairs of the Watson–Crick type hold together two molecules of UpA, but is self-pairing between uracils and also between adenines.

all three wild species examined—they reach their greatest density on the stems—but are very scarce in the cultivated potato, *Solanum tuberosum*.

Could this resistance mechanism in wild potatoes be bred into the cultivated potato? Gibson provides no hint of the practical problems involved, but suggests that in theory this form of resistance in a strain could combine very successfully with resistance based on mechanisms which increase the restlessness of the aphids.

OCEANOGRAPHY

Changing Chemistry

from a Correspondent

IN connexion with the celebration of the 350th anniversary of the founding of Göteborg, the Nobel Foundation agreed to hold a symposium (the twentieth in the series) on the theme of the changing chemistry of the oceans with particular reference to the influence of man on the marine environment. This meeting took place at Aspenäsgarden near Göteborg on August 15–20.

The opening address was given by Professor Arne Tiselius (University of Uppsala), chairman of the Nobel Committee for Chemistry, and a Nobel prizewinner. He stressed the importance of marine science and requested that the delegates draw up a series of resolutions on aspects of marine pollution to be submitted for consideration at the United Nations conference on the human environment to be held in Stockholm next year.

Although many of the formal contributions, and much of the subsequent discussion, did revolve around various aspects of marine pollution, purely academic concepts were not neglected. The overall scope of the symposium included world-wide atmospheric and oceanic circulation patterns and their variability, trace gases in both the atmosphere and sea water, the chemistry of marine aerosols and surface films, bacterial activity in the oceans, the control of energy cycles in the sea, the impact of man on the major sedimentary cycles, the atmospheric transport of material to the oceans and the budget of trace solubles in sea water. The case histories of certain pollutants, such as mercury, chlorinated hydrocarbons (DDT and PCBs), phosphorus and hydrogen sulphide received special consideration.

Dr J. Ui (University of Tokyo) spoke of the dangers to health which may arise from the release of mercury into the natural waters of a densely populated area. He illustrated his talk with a harrowing film showing victims of the "Minimata" disease which was caused by consumption of fish contaminated by methylmercury formed bacterially from inorganic mercury present in wastes discharged into Minimata Bay, Japan.

It was his opinion, which was endorsed by other speakers, that mercury pollution, terrible though its consequences are, is only likely to be a local problem, because the amount of mercury reaching the sea as a whole is only a small fraction of that entering by geological processes. He considered that a much greater threat is posed by toxic chlorinated hydrocarbons such as DDT and polychlorinated biphenyls (PCBs) which enter the sea by way of the atmosphere, and are therefore distributed globally. These lipophilic compounds are concentrated by marine animals at many levels in the food chain.

Dr Ui's arguments were reinforced by analyses presented by Professor E. D. Goldberg (Scripps Institution of Oceanography) and Dr V. T. Bowen (Wood's Hole Oceanographic Institution) which showed that concentrations of the PCBs in marine animals from both the Pacific and the Atlantic were often many times higher than those of DDT; values as great as 300 p.p.b. (fresh weight) of PCBs have been recorded for zooplankton from the north Atlantic. It was suggested that concentrations of these chlorine-containing compounds are reaching the levels at which they may produce ecological effects, and that further increase may present a hazard to human health. It was the unanimous opinion of the symposium that restrictions on the use of these chemicals should be applied internationally.

Professor Goldberg discussed one of the most important concepts in contemporary environmental science—that man himself has now become an important geological force in his own right. He made a comparison between the fluxes of material introduced into the environment by man and those introduced by natural phenomena and concluded that man must now be considered a serious rival to nature.

Considerable time was spent on discussions about international measures which should be taken before it is too late to control and monitor the discharge of toxic, or potentially toxic, wastes into the sea. Emphasis was placed on the need for research into the ecological effects of the compounds concerned, and for studies of their routes through the marine food chain, and of the way in which they are degraded.

How *lac* Repressor Works

IN next Wednesday's *Nature New Biology*, Pastan and his colleagues report their latest manipulations of their cell free system which allows them to observe *in vitro* the regulated expression of the lactose operon (see *Nature New Biology*, **231**, 139; 1971). They have now focused their attention on the question of how the *lac* repressor prevents transcription, and have come to the conclusion that with the wild type *lac* operon the repressor does not prevent the actual binding of RNA polymerase to the promoter site but somehow prevents the bound polymerase from getting on with the job of transcription. But with a mutated *lac* operon, which carries a mutation in the promoter site that enhances the expression of the operon *in vitro* as well as *in vivo*, the repressor acts in part by preventing the binding of polymerase.

Pastan and his colleagues incubated wild type *lac* DNA with RNA polymerase in the presence of the cyclic AMP binding protein and cyclic AMP, which they believe somehow enhances the affinity of the polymerase for the promoter region. The polymerase as a result binds to the promoter and in the absence of repressor and the presence of the nucleoside triphosphates it can transcribe the operon. If, however, Pastan *et al.* incubate the DNA with repressor either before or after it is incubated with the polymerase, transcription is blocked. Moreover, if an inducer is added to the system, together with rifampicin to mop up any unbound polymerase, the repression is overcome and RNA is made. It is clear therefore that repressor and polymerase do not compete for the same binding site; each can bind independently of the other and the repressor must act by blocking a step after polymerase is bound. Wild type promoter and operator sites are thus not the same nor do they overlap.

When, however, Pastan *et al.* repeated these experiments with *lac* operon DNA carrying a promoter mutation, which enhances the expression of the operon, they found that the addition of repressor after polymerase had bound to the DNA only partially inhibited RNA synthesis. By contrast, when repressor is bound before polymerase is added repression is complete and there is no transcription. These and other observations lead Pastan and his associates to conclude that the polymerase and repressor partially compete for a site on this mutated DNA. They suggest that either the mutation has, by deletion, brought the wild type promoter and operator sites closer together or, and more likely perhaps, the mutation has generated a new promoter which is closer to the wild type operator site. It is clear, therefore, that the same repressor molecule can bring about repression in more than one way.

Mechanisms of Sound Localization

I. C. WHITFIELD

Neurocommunications Research Unit, University of Birmingham

In this shortened version of his Charnock Bradley Memorial Lecture delivered at the University of Edinburgh in November 1970, Dr Whitfield describes the mechanism whereby the differential intensity and time of arrival of a sound at the two ears allow the brain to determine the direction of arrival.

THEORIES of hearing have, from the earliest times, been overly concerned with frequency analysis, perhaps because of the classical interest in the relation between musical instruments and the physics of sound. Yet for survival in a hostile world, the role of the auditory system in identifying and locating the sounds made by friends and foes has undoubtedly been the more important one for most species. The early resonance ideas which concerned the cavities of the inner ear were indeed formulated more with the idea of providing an amplifying property than an analytic one, but as early as 1683 the Paris physician du Verney[1] proposed a resonance theory of frequency analysis, antedating Helmholtz by at least 150 years.

Theories of sound localization seem to have developed much later. Boring[2] has suggested that this was due to the philosophical climate of the early nineteenth century, which found the concept of auditory space difficult. Because of the ample evidence that the blind, for example, can find their way around using auditory cues, attempts to circumvent the difficulties induced by this philosophy were made by invoking tactile mechanisms as an explanation for sound localization.

By the middle of the century, experiment was beginning to take over, and with it the recognition that localization of sound depends on the differences between the stimulus conditions at the two ears[3]. An early attempt to control these differences was made by Alison[4], who in 1858 described experiments with a "differential stethophone" which could be used to lead sound from a common source independently to the two ears. He showed that if the sound was louder in one ear than the other, then all the sound seemed to be in that ear, irrespective of the actual situation of the source. He interpreted his results as supporting the idea that localization depends on an intensity difference between the two ears.

The philosopher Alexander Bain, writing in 1855 (ref. 5), had to admit that the possession of two ears played some role in localization, but still insisted that this was very crude and that sound localization depended largely on "experience".

The work of the next hundred years was principally devoted to validating the importance of binaural phenomena and to demonstrating their accuracy; yet there is now reason to believe that past experience in the interpretation of auditory signals may well play some part in localization, though hardly to the extent which Bain supposed.

Rayleigh[6] in 1877 investigated very thoroughly the localization of tones produced by tuning forks and firmly established that intensity difference was a powerful factor in assessing the direction of a sound source. He realized, of course, that although these differences in intensity could be produced at high frequencies (short wavelength) by the shadowing effect of the head, it was difficult to account (in the absence of head movement) for our ability to localize low frequencies where shadowing is negligible. He thought that phase might play a part, and investigated this possibility by leading the sounds of two slowly beating tuning forks into the two ears through long tubes. He was unable to get any impression of movement of a sound image as the phases slowly changed and so rejected the idea. It was not until some thirty years later[7] that he returned to the problem; he then reported that if the experiment were performed with the ends of the tubes separated from his ears by a short distance—instead of having the tubes actually in his ears—the impression of directional change was very definite.

In 1908 Mallock[8] studied the apparent direction of the sound of rifle fire when he stood at different positions close to the line of fire. He found that not only was the apparent direction of the sound source not that of the rifle itself, but that the position of the source varied with the position of the observer along the line from gun to target. He concluded that what he was localizing was the shock wave of the bullet, and that this localization depended on the differential time of arrival of the crack at the two ears. This seems to have been the first study of the influence of a pure time difference in localization rather than a phase difference.

The First World War stimulated much experimentation in sound localization, and the development of electronic methods in acoustics in the 1920s and 1930s extended and elaborated the studies without adding anything substantially new. Study of the physiological mechanisms involved had to await a further stage in electronic development and these date from the late 1940s onwards. Many of the mechanisms are, indeed, still not understood.

Phylogenetic Development

In looking at what little is known, it is best perhaps to start with a look at the internal ear. It is now well known that a sound (say a single tone) sets up a travelling wave along the basilar membrane which passes from the basal to the apical end, reaching a maximum amplitude at some point and then rapidly dying out. The distance it travels before peaking and dying out depends on the frequency—the lower the frequency the further along the cochlea is this point. If a click with a sharp leading edge is used as a stimulus, then the higher frequencies are gradually filtered out as the wave travels along and only the lowest components arrive at the apex.

During phylogenetic development of the ear, the basilar membrane seems to have become progressively elongated. It is less than 1 mm long in reptiles, 5 to 10 mm long in birds and 20 to 30 mm long in mammals. Parallel with this there seems to have been an increase in the high frequency response. Reptiles (and fishes) respond only up to 1 or 2 kHz, birds reach 5 to 10 kHz, but among mammals the response of the guinea pig extends to about 40 kHz and that of the cat to 70 kHz. Man is, of course, a rather poor mammal with an upper limit of 20 kHz. This extension of the high frequency response is of interest because of the importance of high frequencies for localization mechanisms.

The input data for the central nervous system—on the basis of which the source must be located—consist, of course, of impulses in the auditory nerves, and these impulses are in turn generated by mechanical movements of the hair cells induced by vibration of the cochlear partition of which they form a part. It seems that when the hairs of the hair cells are flexed in one direction there is an increased tendency for impulses to be initiated in the associated nerve, and a correspondingly reduced tendency for impulse initiation when they are flexed in the opposite direction. Certainly there is abundant evidence that nerve impulses tend to be discharged at a particular phase of a stimulating sinusoid[9], and we can see at once that the time difference hypothesis and the phase difference hypothesis are—as far as the nerve is concerned—much the same thing. We have presumably to look for some central nervous mechanism which can assess the time disparity (Δt) between corresponding impulses in the two auditory nerves. It is not, however, quite as simple as that because an action potential lasts about 1 ms and the maximum delay between the arrival of a click at the two ears, even in humans, is about 700 µs; in an animal such as the cat the delay is closer to 200 µs. We can in fact deal with delays much less than this, such as those which occur when the sound is only a little off centre. Experiments performed by delivering clicks through headphones to the two ears separately indicate that delays of 40 to 50 µs give an off-centre image. What is the mechanism? If we look at the rate of propagation of the travelling wave along the membrane we see that near the base it is travelling very rapidly[10]. To reach a point about 7 mm from the base takes only a few microseconds (this represents a position responding maximally to about 4 to 5 kHz). If we stimulate with a click containing high frequency components, then there is a barrage of impulses in the auditory nerve fibre array (several thousand fibres), which gives a statistical picture of the time of onset. For lower frequency pure tones we would expect localization to be worse, and this is, in fact, the case.

Comparing Information

How does the central nervous system deal with this time disparity information? Clearly, the information in the two auditory nerves has to be compared, and the first point, anatomically, at which this can happen is the superior olive, which receives connexions from both sides. The medial olivary nucleus has a remarkable structure; it consists[11] of bipolar cells with oppositely directed dendrites. The auditory input from one side makes contact with one set of these dendrites and that from the opposite side with the other set. The whole configuration is, of course, symmetrical with respect to the two sides of the brain stem. A number of workers, notably Hall[12], have recorded outputs from these cells while stimulating the two ears with paired clicks. The probability of a cell firing is found to be a function of the time disparity between the two signals. If the contralateral click is leading then the probability of a response is higher than if the ipsilateral click is leading; increasing the intensity of the clicks also raises the probability, of course. There are, however, two of these nuclei, one on each side, and we find that the ratio of the firing probabilities on the two sides is independent of the absolute intensity level. A difference of intensity between the two sides will affect this ratio, but this actually enhances the effect because the sound in the leading ear would normally be louder than that in the trailing ear if the stimuli came from a real single source.

The problem thus seems to be solved; some higher level of the nervous system has simply to assess the comparative outputs of the two medial olivary nuclei which vary with both time and intensity. When cats, for example, are deprived of both auditory cortices, they cannot localize sound (though they can respond to it—they are not deaf[13]); we note, too, that there are nerve cells in the cortex which respond only when the sound is on one side of the animal's head and not at all when it is on the other[14]. If we postulate that these are the cells which are (directly or indirectly) "looking at" the differential olivary output, it seems superficially as if we may have identified the mechanism. Again, however, there are difficulties.

The relative development of the nuclei of the olivary complex varies considerably from species to species[15]. Some animals—the bat, the hedgehog, the dolphin—apparently have no medial olive, whereas the cat and the dog have the medial and lateral olivary nuclei about equally developed. In monkeys and man it is the medial olive which predominates, though in no species is the lateral nucleus entirely absent. It is interesting that these differences are reflected in nomenclature; in the cat it is the medial nucleus which anatomists have labelled the accessory nucleus, but in man it is the lateral nucleus which is so named. It is tempting to note the apparent correlation of these histological differences with head size; a medial olive is associated with a large head size, for which Δt would be large, and not with a small head size, for which Δt would be smaller. Before coming to any conclusions, however, we must recall that more "visual" species have larger medial olives than less visual ones.

Masterton, Jane and Diamond[16] trained cats to distinguish between clicks presented by earphones to the right ear and to the left ear. They then presented the animals with paired clicks (right–left (RL) or left–right(LR)) in which one preceded the other by 500 µs. As expected, they found that a normal cat treats RL just like a single click on the right and LR like a single click on the left. They then divided the trapezoid body so that the cross-connexions between the two accessory olives were severed. The animals could no longer solve the RL and LR problem. They could, however, still solve the L v R problem, so that relative intensity cues were evidently available by a different route. The experimenters further showed that partial lesions of the lateral lemniscus were capable of dissociating the two paths so that the L v R problem and the LR v RL problem were no longer treated as equivalent, that is both problems could be learned but there was no transfer.

Localization and the Pinna

We thus see that the time/intensity analysis of the accessory olive is not the sole mechanism of localization. Indeed when we come to look at the matter more carefully we see that time is a very ambiguous clue. There are in fact complete surfaces (hyperbolae of revolution) with the same time delays. Because of the irregular head shape and the existence of the pinnae, however, the relative frequency attenuation varies greatly with the orientation of the source. Thus the waveform at one ear is quite different from that at the other ear if the sound is complex (as it usually is) and this difference may be unique for each position. Batteau[17,18] conducted many experiments on the role of the pinna in localization and showed how the received sound varies as the direction of the source is changed. Indeed this gives some indication of how we can locate sound even with one ear, though not very accurately. Such localization is, of course, not mechanical but depends on learning the patterns from past experience, just as we learn visual distance judgment from such things as atmospheric colour filtering. That there is some physical basis for the proposal is shown by the observation that artificial pinnae on microphones can be used to transmit the effect to a subject through earphones.

Now Rayleigh observed that when he had tubes in his ears leading in sounds he had no impression of direction, whereas when the ends of the tubes were an inch or so away he got the impression of direction very definitely. No doubt this is an example of the psychological effect of one clue cancelling the effect of another. If we perform on ourselves the experiment of delivering to our own ears through headphones clicks which are disparate in time—as in the experiments with the cat—we find that as the interval is altered, the sound certainly moves, but is lateralized rather than localized, that is to say it moves from side to side either inside your head or very close to it; it is not located out in space as is the case for a real source. It seems that the frequency spectrum distortion provided by the head and pinnae is an important factor in this effect. But it may not be a complete explanation because Toole[19] has recently shown that even real external sources in an anaechoic chamber may in certain circumstances produce images inside the head.

There is, indeed, another factor in localization which we have not yet considered, and which may in normal circumstances contribute to the exteriorization of the source. So far we have been regarding a sound as two signals reaching the two ears at two points in time. This may be true in a large flat unpopulated open space, but in most circumstances these signals are accompanied by later versions reflected from surrounding objects. As sound travels roughly one foot in one millisecond, the sound of a voice reflected from a room wall may reach the listener five to ten milliseconds later, and there are many of these reflexions. Of course we do not hear them separately, and all the sound appears to come from the primary source. It it not that the reflexions are suppressed; they add to the total loudness, so that one must speak much more loudly in the open air than in a room. This phenomenon is known as the precedence effect[20], and is used commercially in sound reinforcing systems. The difficulty of situating a public address loudspeaker near the person talking is that it feeds back into the microphone and causes a howl. If, however, the speech fed to the loudspeakers is delayed so that their sound reaches the ear a few milliseconds later than the direct voice then the loudspeakers can be placed near the audience at the back of the hall and yet all the sound appears to come from the direction of the speaker's mouth.

With a single source on the left, the sound reaches the right ear about 0.5 ms later than the left ear, If, however, there is a reflector 3 feet away on the right, a second sound will arrive at the right ear 6 ms later and at the left ear 6.5 ms later. The auditory nerves will certainly respond to all four signals and indeed they can be traced in gross responses right through the auditory pathway, though the second response may be rather smaller than the first. Although, as we have seen, the second signal pair adds to the intensity of the whole and even to the intelligibility of speech, it is nevertheless the first signal pair which predominantly determines the apparent location.

Function of the Cortex

We have recently[21,22] been investigating the role of the cortex in this phenomenon, as it seemed that the cortex played an important role in "out-thereness". First, we trained cats to go to a food box on the left when a loudspeaker behind that box was delivering a train of five tone pulses per second, and conversely to go to a food box on the right when a similar train of tone pulses came from a speaker on the right. In other words, they were trained to "go to" the sound. It is known from the experiments of Neff[13] that a cat with no auditory cortex cannot do this but, provided either the right or the left auditory area is intact, the animal can perform the discrimination just like a normal animal.

If we intersperse among these real rights and lefts some compound rights and lefts made by having the right pulse precede the left by 5 ms or vice versa, normal cats will treat these exactly as if they were real rights or lefts—they "transfer" immediately without further training. This, of course, is just what we would expect from the precedence effect we have been discussing. If, however, we take out one auditory cortex a difference emerges. We removed the right auditory cortex from half of the animals and the left auditory cortex from the other half. After the operation both sets of animals made no mistakes about stimuli from the right or left: we did not expect them to. But there is a clear difference between the response of the two groups to the compound stimuli (RL or LR). The right lesion cats perform correctly on a compound right (RL) but fail on a compound left (LR), whereas the left lesion cats can do the compound left but fail on the compound right. In other words, the contralateral auditory cortex seems essential for the correct interpretation of these compound signals. Some light on what actually occurs is given by other experiments in which uni-decorticate cats were trained to go left to a signal on the left, but to go right when signals were present on both right and left speakers without any fixed temporal relationship (to avoid the precedence effect the two trains were unsynchronized so that the left/right interval varied continuously). When these animals are exposed to compound signals the left lesion animals appear to be normal, they go left to a compound left (LR) and right to a compound right (RL) signal. But the right lesion cats go to the right whichever stimulus (LR or RL) occurs. With reversal training in which the animals are trained to go right to a single stimulus and left to the double stimulus it can be shown that the left lesion cats are not, in fact, normal, as they now break down and tend to go left to both RL and LR. These results suggest that it is the temporal order of the pair of stimuli which is lost when the cortex contralateral to the earlier member of the pair is damaged.

These observations may explain the difficulty which humans with one sided temporal lobe damage are reported to have in localizing sounds on the side contralateral to the damage, while having no difficulty with sounds on the ipsilateral side. Possibly the precedence effect breaks down in these circumstances, and reflexions cause confusion.

There are evidently comparatively simple and well understood mechanisms at the level of the brain stem which are capable of using the clues of differential intensity and differential time of arrival of a sound at the two ears to compute the direction from which it arrives, just as a range finder might. Some ambiguities in the result can obviously be resolved by moving the receivers (head movement) in the sound field, but only if the sound is repeated or continuous. In any real situation ambiguities arise not only because more than one position of the source can give rise to the same time differences but because of multiple reflexions. In the resolution of these ambiguities, the cortex seems to play an essential role, as it does in the assignment of position as well as direction to a source (what I have called "out-thereness"). It could well be asked whether "out-thereness" has any meaning except in so far as we are able to collate the auditory input at a cortical level with our motor experience of exploring the world around us.

[1] du Verney, J. G., *Traité de l'Organe de l'Ouie* (E. Michallet, Paris, 1683).

[2] Boring, E. G., *Sensation and Perception in the History of Experimental Psychology* (Appleton-Century Crofts, New York, 1942).

[3] Weber, E. H., *Ber. Sächs Ges. Wiss.*, **2**, 226 (1848).

[4] Alison, S. S., *Phil. Mag.*, **4**, Ser 16, 385 (1858).

[5] Bain, A., *The Senses and the Intellect* (Parker, London, 1855).

[6] Rayleigh, Lord, *Phil. Mag.*, **5**, Ser 3, 456 (1877).

[7] Rayleigh, Lord, *Phil. Mag.*, **6**, Ser 13, 214 (1907).

[8] Mallock, A., *Proc. Roy. Soc.*, A, **80**, 110 (1908).

[9] Rose, J. E., in *Frequency Analysis and Periodicity Detection in Hearing* (edit. by Plomp and Smoorenburg), 176 (Sijthoff, Leiden, 1970).

[10] Tasaki, I., Davis, H., and Legouix, J.-P., *J. Acoust. Soc. Amer.*, **24**, 502 (1952).

[11] Cajal, S. Ramon y, *Histologie du Système Nerveux de l'Homme et des Vertébrés* (A. Maloine, Paris, 1909).

[12] Hall, J. L., *J. Acoust. Soc. Amer.*, **37**, 814 (1965).

[13] Neff, W. D., Fisher, J. F., Diamond, I. T., and Yela, M., *J. Neurophysiol.*, **19**, 500 (1956).

[14] Whitfield, I. C., *The Auditory Pathway* (Arnold, London, 1967).

[15] Harrison, J. M., and Feldman, M. L., in *Contributions to Sensory Physiology*, **4** (Academic Press, New York, 1970).

[16] Masterton, B., Jane, J. A., and Diamond, I. T., *J. Neurophysiol.*, **30**, 341 (1967).

[17] Batteau, D. W., *US Naval Ordnance Test Station Report No. 3109* (1962).

[18] Batteau, D. W., in *Hearing Mechanisms in Vertebrates* (edit. by de Reuck, A. V. S., and Knight, J.) (Churchill, London, 1968).

[19] Toole, F. E., *J. Acoust. Soc. Amer.*, **48**, 943 (1970).

[20] Wallach, H., Newman, E. B., and Rosenzweig, M. R., *Amer. J. Psychol.*, **62**, 315 (1949).

[21] Cranford, J., Diamond, I. T., Ravizza, R., and Whitfield, I. C., *J. Physiol.*, **213**, 24 P (1971).

[22] Cranford, J., Ravizza, R., Diamond, I. T., and Whitfield, I. C., *Science*, **173**, 286 (1971).

Effect of Earthquakes on Deep-sea Sediments

T. J. G. FRANCIS
Institute of Geological Sciences, UKAEA Blacknest, Brimpton, Reading, Berkshire

It is suggested that sediments filling the oceanic trenches and transform faults suffer periodic liquefaction by earthquakes. Such a phenomenon could explain the presence of undeformed turbidite beds in highly tectonic zones and make them an ideal site for toxic waste disposal.

GEOLOGICAL and geophysical evidence from the mid-oceanic ridges generally supports the concepts of plate tectonics, but the interpretation of evidence from the areas where plates are colliding is much more obscure[1-9]. One baffling problem is the almost complete lack of deformation in the sediments found in oceanic trenches[10,11], the "sinks" of plate theory, where one plate is under-thrusting another and being consumed into the mantle. Relative motion between colliding plates may be several centimetres per year[8], and the boundary between them is marked by a steeply dipping earthquake zone, the Benioff zone, extending as much as 700 km into the mantle[12,13].

Several seismic reflexion profiles have now been made of the sediments filling oceanic trenches (Fig. 1), but there is no evidence for the accumulations of contorted sediments that were originally expected[10,11]. One possibility is that crumpled sediments lie beneath the overlying undeformed fill, but cannot be detected because the latter is acoustically absorptive[14]. But beneath flat-lying turbidite beds Scholl *et al.* have identified oceanic pelagic sediment, very similar in thickness and acoustic properties to that exposed to seaward of the trench and beneath that a rather well defined basement. What evidence there is for chaotic reflexion hyperbolae at the base of the continental slope in active trenches[15] can be explained by reflexions from sediments which have slumped down the continental slope[16] or from harder rock features of the slope itself. The two plates shown in Fig. 1 are moving together at about 6 cm yr^{-1} (ref. 8) and, if the oceanic plate is carrying the trench sediments along on its back like a conveyor belt, a column of sediment approximately 1 km thick is constantly being pushed up against the continental slope. In a million years 60 km of such a column—more sediment than is visible in the whole of Fig. 1—must be disposed of. It is difficult to believe that any geological process can do this so efficiently that what is left can hardly be observed on a seismic reflexion record.

But it has always been assumed that the trench sediment is behaving as a plastic solid, displaying much the same mechanical properties as those of a sediment core resting on the laboratory bench. The static and dynamic physical properties of unconsolidated sediments are, however, seldom the same. In particular many unconsolidated sediments display the phenomenon of thixotropy, whereby a body changes from a semi-solid, jelly-like state ("gel") to a liquid state ("sol") on being shaken and reverts back to the jelly-like state on standing. Thixotropic behaviour by the sediments filling oceanic trenches under the influence of earthquake vibrations could account for their lack of deformation.

Vibrational Environment of Epicentral Zone

Such vibrations could originate in the Benioff seismic zone, which crops out along the inner margin of oceanic trenches[12,13]. It is certain that much of the relative movement between colliding plates is accompanied by seismic activity. But fault movement can occur without earthquakes. For example, some sections of the San Andreas fault, separating the North Pacific and American plates, move with "fault creep", whereas others seem to be "locked" and strain is relieved by infrequent but severe earthquakes[17]. The locked stretches are thought to be the result of the curvature of the fault, that is, of increased forces holding the plates together. Because the Benioff zone involves underthrusting rather than strike-slip faulting, the forces normal to the zone must in general be greater than in the case of a transform fault. Thus the shallow faults of a

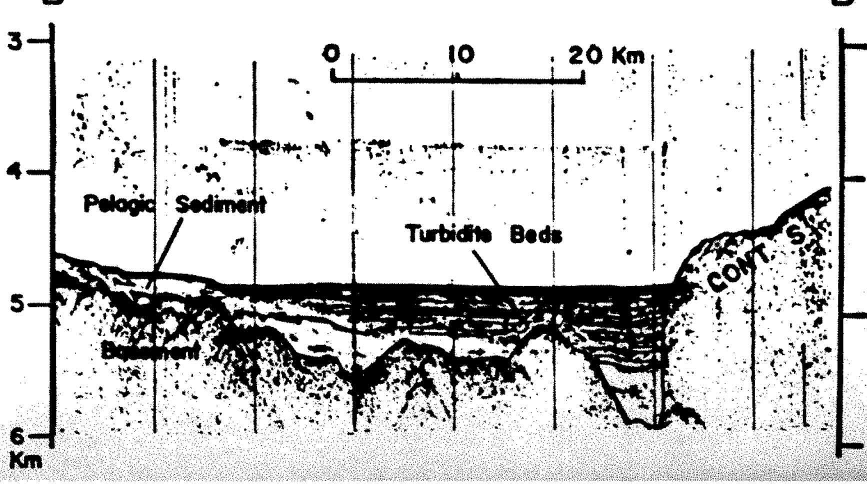


Fig. 1 Seismic reflexion profile taken by Scholl, van Huene and Ridlon[11] across the Peru–Chile trench, showing undeformed turbidite fill.

Benioff zone are probably of the locked variety. It seems plausible to conclude therefore that most, if not all, the relative movement between colliding plates is accompanied by earthquakes.

It is generally agreed that shallow earthquakes are produced by relative motion of the two sides of a fault surface[18]. Relative motion initiates at some point on the fault surface, then spreads outward on that surface at a finite velocity. Rupture velocities determined theoretically and experimentally give figures somewhat less than the shear velocity, or roughly half the compressional velocity, of the faulting material[18-22]. Thus P and S waves from the initial rupture will precede the active faulting everywhere except where it is initiated. At 5 km from the initial point, typical crustal rocks would experience approximately 1 s of vibration before the rupture starts. Near the source the vibration contains much high frequency energy (say, 2–100 Hz), most of which is attenuated by teleseismic distances. Moreover, the point at which rupturing starts is unlikely to be at the Earth's surface. Even where shallow earthquakes have resulted in surface faulting, it is usually thought that faulting started at some depth, possibly several kilometres[23]. Thus the hard rock surface at the epicentre itself will usually experience at least a second of vibration before any faulting arrives.

During the Parkfield, California, earthquake of June 1966 an array of accelerographs was in operation across the part of the San Andreas fault which ruptured[22-25]. The earthquake had a Richter magnitude of 5.6 and the USCGS reported $m_b = 5.3$. The fault ruptured over a length of 30 km, the rupture initiating at a depth of about 5 km. Surface faulting was visible over a similar distance to the fault length inferred from Love Wave spectra, but offsets of the fault were only a few centimetres. A maximum acceleration of $0.5g$ was recorded 200 feet from the fault trace, falling to about $0.1g$ at a distance of 15 km[24,25]. Much of the energy recorded in the accelerograms was at periods less than 1 s, and the shaking lasted for a few seconds. Although this earthquake is different in many respects from those occurring just beneath the ocean floor on the Benioff zone, these figures give a general idea of what to expect there from an earthquake of similar magnitude.

Housner[26] has shown that maximum ground accelerations decrease with distance from the fault, but that the rate of decrease is small over distances comparable with the dimensions of the fault. An earthquake such as the Parkfield one subjects a ground area of a few hundred square miles to maximum accelerations of more than $0.1g$. A major earthquake with Richter magnitude 7.5 would subject an area of no less than 14,000 square miles to such accelerations, and an area of 2,000 square miles to maximum accelerations of more than $0.3g$. The duration of strong shaking can exceed half a minute for major events.

Thixotropy of Unconsolidated Sediments

Boswell[27] has shown that, except for coarse clean sands and gravels, all unconsolidated sediments are thixotropic to some degree, and that thixotropy is augmented by an increase in the proportion of finely divided particles and of platy or acicular grains as compared with equidimensional particles. It is also usually increased by the presence of electrolytes such as those present in seawater as opposed to freshwater. Boswell found many sands to be thixotropic at porosities between 35% and 50%, muds and clays at porosities between 50% and 70%. The actual porosity range over which a given sediment exhibited thixotropy varied from a few per cent to 20%. Expressed in terms of the Atterberg limits of soil mechanics, all unconsolidated sediments are likely to be thixotropic near the liquid limit; the question is how far the thixotropic properties extend towards the plastic limit. Even at larger water contents than the liquid limit, thixotropy may be important in reducing the viscosity of sediment slurries.

Freundlich and Juliusburger have shown that thixotropy can account for some quicksands[28]. In particular, they demonstrated that a quicksand from Knott End, Fleetwood, was thixotropic due to the presence of only 2.1% of fine clay. Separation of the clay fraction suppressed the thixotropic behaviour, but it reappeared on remixing the clay with the residue. Sands neighbouring the quicksand contained only 0.3% clay and were not thixotropic.

In physical terms, thixotropy occurs when particles form a loosely packed "house-of-cards" structure[27-30]. Shearing stresses break the contacts between particles and thixotropy arises because time is required for the structure to reform. Particle shape is important because isometric particles tend to form close-packed structures whereas platy and acicular ones form open structures. Factors such as the concentration of electrolytes (affecting the electrostatic repulsive forces between particles) and the viscosity of the pore fluid are important because they control the time required for the structure to reform.

Physical Properties of Deep-sea Sediments

High porosity is perhaps the most striking characteristic of the unconsolidated sediments of the ocean floor[31,32]. Porosities in the region of 70% are the rule and only rarely does the presence of an increased sand fraction reduce the porosity below 60%. The pelagic sediments of abyssal hills and the clayey silts and silty clays of abyssal plain turbidites all have porosities in the region of 80%. Furthermore, porosity decreases slowly with depth. At the Preliminary Mohole site it fell only 5% over 168 m (ref. 33). The sound velocity gradient in unconsolidated sediments and the well established relationships between sound velocity and sediment porosity provide an indirect means of inferring porosity at depth. One can conclude that even at depths of 1 km below the ocean floor, the porosity of unconsolidated silt-clay sediments is probably in the region of 50%. The fact that high porosity extends to depth implies that a house-of-cards type of structure does also, with only gradual orientation of the sediment particles towards the structure of laminated siltstone or shale.

Porosity alone, however, is not a reliable guide to either the strength of an unconsolidated sediment or its closeness to the liquid limit. In general, the shear strength of deep-sea sediments is low, but the strength of sediments of similar porosity may vary by a factor of a hundred or more[32,34]. Hamilton has pointed out that both very high and very low rates of accumulation can lead to sediments with little decrease of porosity with depth[33]. When the rate of accumulation is very high, the sediment layer is thick and very weak, but has not had time to consolidate under its own overburden pressure[35]. When the rate of accumulation is very low (say, < 1 cm per 1,000 yr), the contacts between sediment particles are firmly cemented before the overburden pressure becomes appreciable. The house-of-cards structure is thus transformed into a welded three-dimensional network capable of supporting a considerable overburden and giving the sediment appreciable strength. This is the explanation given for the surprisingly high strength of the sediments from the Preliminary Mohole[33,36], and is probably generally true of deep-sea pelagic sediments.

Thus the properties of many sediments on the ocean floor are such that, given the right vibrational environment, thixotropy is likely to be an important phenomenon. Rapidly deposited turbidite beds are more likely to display thixotropy than slowly deposited pelagic clays. No quantitative information is available on the level of vibration needed to induce thixotropy, but there is a great deal of evidence that the strong motion of earthquakes is sufficient to disturb the inter-particle relationships of unconsolidated sediments.

Effects of Earthquake Strong Motion

Liquefaction of saturated, cohesionless sands and silts has been reported in several earthquakes[37], but the mechanism of

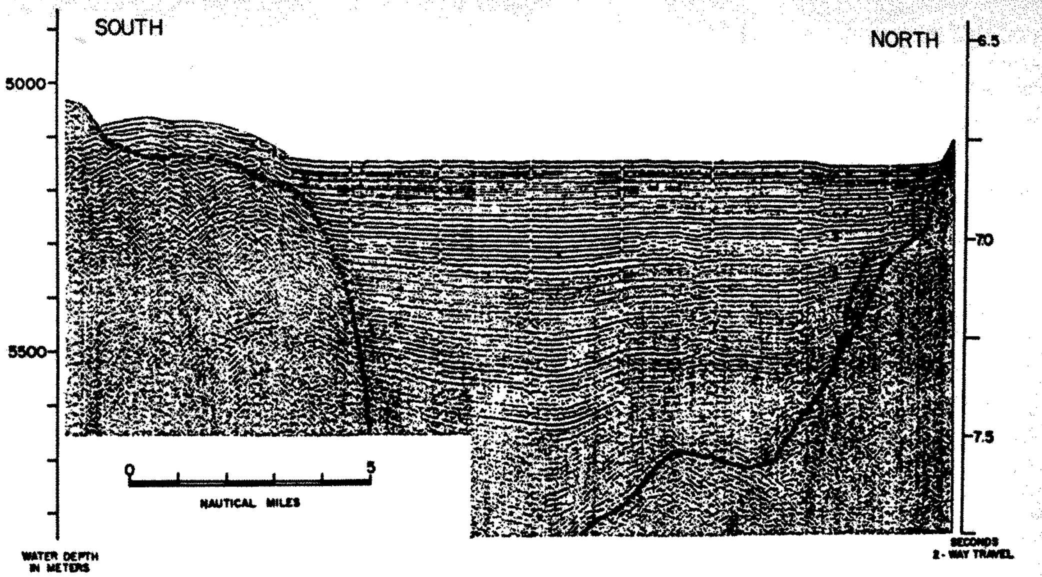


Fig. 2 Seismic reflexion profile taken by Van Andel, Corliss and Bowen[45] across the Vema Fracture valley, showing undeformed horizontally bedded sediments.

liquefaction when this occurs on land is generally thought to be moving pore water rather than thixotropy. Earthquake vibrations tend to shake cohesionless sands and silts into a more close-packed structure and thus increase the pore water pressure. The pore water flows upwards towards the surface and brings about liquefaction. The much smaller grain size of deep-sea sediment makes this mechanism less likely than thixotropy on the ocean floor, but the fact that it has been observed to have taken place on land demonstrates that the strong motion of earthquakes does disrupt the inter-particle relationships of unconsolidated sediments.

It is now well established that earthquakes provide a common triggering mechanism for submarine turbidity currents[38]. The classic example of this occurred off the Grand Banks of Newfoundland in 1929, when a magnitude 7.2 (Richter) earthquake[39] set a turbidity current in motion down the southern slope of the Banks. The velocity of the current has been inferred from the sequence of telegraph cable breaks[40] and its extent has been demonstrated by coring[41]. Sediment across 100–200 km of the continental slope was set in motion by this earthquake. The important point is that the level of earthquake vibration was sufficient to disturb the inter-particle relationships of the unconsolidated sediment throughout the epicentral area.

Liquefaction of Sediments in Oceanic Trenches

The various threads of the argument can now be drawn together to explain the absence of deformation observed in the sediments of oceanic trenches.

Throughout their existence, the turbidite beds of oceanic trenches will have experienced an unusual environment for marine sediments. Disturbed by the strong vibrations of frequent earthquakes, their inter-particle relationships will not have been static. Liquefaction, probably brought about by the phenomenon of thixotropy, will have periodically broken the contacts between particles and prevented their becoming permanently cemented.

In contrast, the pelagic sediments, approaching the trench on the oceanic plate, will have already acquired considerable strength as a result of inter-particle cementing before they reach the earthquake zone. The vibrational environment could therefore liquefy the turbidite beds but is unlikely to have such a drastic effect on the bulk of the pelagic sediments. Furthermore, since the initial ruptures of earthquakes generally lie at least a few kilometres below the base of the sediments, liquefaction of sedimentary formations could already occur before the appearance of faulting in the basement surface.

The brief and intermittent earthquake movements cannot impose shear stresses on the liquefied body of sediment and the latter will be effectively decoupled from the oceanic conveyor, just as the sea itself is. The situation is comparable with that of a bowl of water given a sharp tilt. The bowl moves, but the water level remains the same. Similarly the liquefied sedimentary pond in the trench retains its level and horizontal stratification as the oceanic plate slips by beneath. Moreover, although the cumulative movement of the oceanic plate, towards and underneath the continental plate, is quite rapid in geological terms, the actual shape of the sedimentary trap of the trench probably retains a rough dynamic equilibrium. Over a long period of time, several tens of millions of years, the relative velocities between plates will change and trenches come and go, But over a shorter time term, up to a few tens of millions of years, the relative velocities between the plates are probably quite constant. The general position of trenches would not vary although local adjustments of depth might occur. This might give rise to liquefied sediment redistributing itself along the trench. Such flows would not alter the character of the trench fill, but rather would tend to maintain its horizontal stratification and low strength.

Liquefaction of Sediments along Transform Faults

Besides the island arc/trench systems, the other prominent submarine earthquake zone follows the axis of the mid-oceanic ridges[42]. In general, of course, the crest of the mid-oceanic ridge is such a new feature that no appreciable sedimentary cover exists[43], but some fracture zones form troughs deep and extensive enough to trap turbidite sediments originating well away from the ridge itself. The Vema Fracture zone[44–47] in the equatorial Atlantic offsets the mid-Atlantic ridge by more than 300 km, is the locus of earthquake epicentres, and is filled by more than 1 km of horizontally stratified sediments, predominantly turbidites from the Demerara abyssal plain (Fig. 2). Interpretation of magnetic anomalies indicates that the north and south walls of the Vema transform fault are moving past each other at 2.8 cm yr^{-1} or, expressed differently, that the basement on the south side of the profile shown in Fig. 2 is about 20 million years older than that on the north side[7]. Nevertheless, the sedimentary fill observed is essentially undeformed.

Van Andel *et al.* originally interpreted the lack of deformation in the sediments of the Vema Fracture to indicate a long period of tectonic tranquillity, but were still convinced of the tectonic nature of the walls of the fracture[45]. A recent JOIDES drill hole coinciding with the profile of Fig. 2 has established an exceptionally high sedimentation rate in the Vema Fracture Valley, of the order of 1 m every 10^3 yr[47]. Even this rate, however, is but a small fraction of the rate at which the north and south walls of the transform fault are moving past each other and rapid sedimentation alone cannot account for the lack of deformation. Again the answer may lie in the effect of earthquakes on the physical properties of the sediment itself. The sediments filling the Vema Fracture have been shown to be rapidly deposited turbidites and consequently liable to thixotropy. Provided that movement on the Vema transform fault is largely seismic, the sedimentary fill in the fracture will be liquefied during movement of the walls and consequently will escape deformation and retain its horizontal stratification. It is quite reasonable to suppose that the largest earthquakes on the transform fault produce faulting throughout its 300 km length. In such an event the whole sedimentary fill of the transform part of the fracture and for many kilometres on either side would be liquefied.

Conclusions and Consequences

On a geological timescale liquefaction will be a frequent but transient occurrence, at any particular place averaging the order of seconds per year. Several months may go by without liquefaction, and at much longer intervals very large earthquakes will induce it for half a minute or more—a period comparable with their duration of strong shaking. Liquefaction implies a reduction in the viscosity of the sediment from that of a plastic solid (say, 10^4 poise) to that of a thick soupy liquid (say, 10 poise) and the loss of the plastic solid's yield strength.

Bostrom and Sherif have recently suggested that the tectonic sinks of the Earth's surface be used for waste disposal[48]. The occurrence of liquefaction in the turbidite beds of oceanic trenches would make this suggestion much more plausible. Furthermore, thickly sedimented transform fault valleys could be put to the same use. Waste material packaged to a density greater than that of the sediments would sink through the sediment whenever it liquefied. It is worth making an estimate of the rate at which a solid body would sink through the liquefied sediment.

Consider a sphere of diameter $d = 100$ cm and density $\rho_s = 3.0$ g cm^{-3} falling at a velocity U through a liquefied sediment of density $\rho_L = 1.5$ g cm^{-3} and viscosity $\nu = 10$ poise. The Reynolds number in such a case will be of the order of 10^3 so that the drag force the body experiences is independent of the viscosity of the liquid and is given by

$$F = \tfrac{1}{2} C_D U^2 S \rho_L$$

where S is the cross sectional area perpendicular to the direction of the motion and C_D is a dimensionless number called the drag coefficient. For a sphere $C_D \approx 0.4$ for Reynolds numbers in the range 10^3 to 10^5 (ref. 49). The terminal velocity of the sphere is given by

$$\frac{4}{3}\pi\left(\frac{d}{2}\right)^3 (\rho_s - \rho_L) g = \tfrac{1}{2} C_D U^2 \pi \left(\frac{d}{2}\right)^2 \rho_L$$

and therefore

$$U^2 = \frac{4}{3}\left(\frac{\rho_s - \rho_L}{\rho_L}\right)\frac{gd}{C_D}$$

Thus, with the values given, $U \approx 6$ m s^{-1}. Because the motion at these high Reynolds numbers is independent of the viscosity of the fluid, the velocity with which the sphere sinks through the sediment is only a factor of about $\sqrt{2}$ less than that at which it sinks through the sea. The duration of liquefaction has been tentatively estimated at s yr^{-1}, so that the average rate of sinking through the sediment of a body such as the sphere considered above might be about 20 m yr^{-1}. This rate far exceeds geological sedimentation rates and could be verified experimentally with simple apparatus in a few years.

That waste material dropped on the turbidite beds filling oceanic trenches and transform fault valleys would sink rapidly through the sediment is an attractive concept. Burial to a depth of a kilometre or more would occur in the human lifespan. As a site for the disposal of highly toxic chemical or radioactive wastes, the sedimented trenches and transform faults appear to be ideal. Such wastes have been dumped at sea for several years with remarkably little thought given to the consequences. Yet a major problem in the disposal of highly concentrated radioactive waste is the difficulty of ensuring that the package will last at least a few half-lives of the nuclides it contains. If natural geological processes exist which would allow such packages to bury themselves to a depth of a kilometre or so in sediment and this beneath seawater 5 km deep, such difficulties do not arise. A guaranteed lifetime of 50 yr for the package would suffice. And if leakage of the toxic waste then ensued, its effect even on the benthic environment would probably be undetectable.

I thank Dr H. I. S. Thirlaway and his colleagues for the facilities at Blacknest. This work was undertaken as part of the research programme of the Institute of Geological Sciences.

Received April 29; revised July 28, 1971.

[1] Hess, H. H., in *Petrologic Studies* (edit. by Engel, A. E. J., *et al.*), 599 (Geol. Soc. Amer., New York, 1962).
[2] Dietz, R. S., *Nature*, **190**, 854 (1961).
[3] Vine, F. J., and Matthews, D. H., *Nature*, **199**, 947 (1963).
[4] Wilson, J. T., *Nature*, **207**, 343 (1965).
[5] Vine, F. J., *Science*, **154**, 1405 (1966).
[6] McKenzie, D. P., and Parker, R. L., *Nature*, **216**, 1276 (1967).
[7] Morgan, W. J., *J. Geophys. Res.*, **73**, 1959 (1968).
[8] Le Pichon, X., *J. Geophys. Res.*, **73**, 3661 (1968).
[9] Sykes, L. R., *J. Geophys. Res.*, **72**, 2131 (1967).
[10] Ross, D. A., and Shor, jun., G. G., *J. Geophys. Res.*, **70**, 5551 (1965).
[11] Scholl, D. W., von Huene, R., and Ridlon, J. B., *Science*, **159**, 869 (1968).
[12] Sykes, L. R., *J. Geophys. Res.*, **71**, 2981 (1966).
[13] Isacks, B., Oliver, J., and Sykes, L. R., *J. Geophys. Res.*, **73**, 5855 (1968).
[14] Chase, R. L., and Bunce, E. T., *J. Geophys. Res.*, **74**, 1413 (1969).
[15] Ewing, M., Houtz, R., and Ewing, J., *J. Geophys. Res.*, **74**, 2477 (1969).
[16] Houtz, R., Ewing, J., Ewing, M., and Lonardi, A., *J. Geophys. Res.*, **72**, 4713 (1967).
[17] Pakiser, L. C., Eaton, J. P., Healy, J. H., and Raleigh, C. B., *Science*, **166**, 1467 (1969).
[18] Savage, J. C., *Bull. Seismol. Soc. Amer.*, **55**, 263 (1965).
[19] Yaffe, E. H., *Phil. Mag.*, **42**, 739 (1950).
[20] Press, F., Ben-Menahem, A., and Toksoz, M. N., *J. Geophys. Res.*, **66**, 3471 (1961).
[21] Mansinha, L., *Bull. Seismol. Soc. Amer.*, **54**, 369 (1964).
[22] Filson, J., and McEvilly, T. V., *Bull. Seismol. Soc. Amer.*, **57**, 1245 (1967).
[23] McEvilly, T. V., Bakun, W. H., and Casady, K. B., *Bull. Seismol. Soc. Amer.*, **57**, 1221 (1967).
[24] Cloud, W. K., and Perez, U., *Bull. Seismol. Soc. Amer.*, **57**, 1179 (1967).
[25] Housner, G. W., and Trifunac, M. D., *Bull. Seismol. Soc. Amer.*, **57**, 1193 (1967).
[26] Housner, G. W., in *Earthquake Engineering* (edit. by Wiegel, R. L.), 75 (Prentice-Hall, New York, 1970).
[27] Boswell, P. G. H., *Quart. J. Geol. Soc. Lond.*, **104**, 499 (1949).
[28] Freundlich, H., and Juliusburger, F., *Trans. Faraday Soc.*, **31**, 769 (1935).
[29] Fischer, E. K., *Colloidal Dispersions* (Wiley, 1950).
[30] Overbeek, J. Th. G., in *Colloid Science* (edit. by Kruyt, H. R.), **9** (Elsevier, 1952).
[31] Nafe, J. E., and Drake, C. L., in *The Sea* (edit. by Hill, M. N.), **3**, 794 (Interscience, New York, 1963).
[32] Hamilton, E. L., *J. Geophys. Res.*, **75**, 4423 (1970).
[33] Hamilton, E. L., *J. Geophys. Res.*, **69**, 4257 (1964).
[34] Horn, D. R., Horn, B. M., and Delach, M. N., *J. Geophys. Res.*, **73**, 1939 (1968).
[35] Fisk, H. N., and McClelland, B., *Bull. Geol. Soc. Amer.*, **70**, 1369 (1959).
[36] Moore, D. G., *J. Geophys. Res.*, **69**, 4271 (1964).
[37] Seed, H. B., in *Earthquake Engineering* (edit. by Wiegel, R. L.), 227 (Prentice-Hall, 1970).
[38] Heezen, B. C., in *The Sea* (edit. by Hill, M. N.), **3**, 742 (Interscience, 1963).
[39] Gutenberg, B., and Richter, C. F., *Seismicity of the Earth and Associated Phenomena* (Princeton University Press, 1949).

[40] Heezen, B. C., and Ewing, M., *Amer. J. Sci.*, **250**, 849 (1952).
[41] Heezen, B. C., Ericson, D. B., and Ewing, M., *Deep-Sea Res.*, **1**, 193 (1954).
[42] Heezen, B. C., and Ewing, M., in *The Sea* (edit. by Hill, M. N.), **3**, 388 (Interscience, 1963).
[43] Ewing, M., Ewing, J. I., and Talwani, M., *Bull. Geol. Soc. Amer.*, **75**, 17 (1964).
[44] Heezen, B. C., Gerard, R. D., and Tharp, M., *J. Geophys. Res.*, **69**, 733 (1964).
[45] Van Andel, Tj. H., Corliss, J. B., and Bowen, V. T., *J. Mar. Res.*, **25**, 343 (1967).
[46] Van Andel, Tj. H., Phillips, J. D., and Von Herzen, R. P., *Earth. Plan. Sci. Lett.*, **5**, 296 (1969).
[47] Von Herzen, R. P., Simmons, G., and Folinsbee, A., *J. Geophys. Res.*, **75**, 1973 (1970).
[48] Bostrom, R. C., and Sherif, M. A., *Nature*, **228**, 154 (1970).
[49] Goldstein, S., *Modern Developments in Fluid Dynamics*, 1 (Clarendon Press, Oxford, 1938).

Murine Leukaemia Virus Group-specific Antigen in the C-Type Virus-containing Human Cell Line, ESP-1

RAYMOND V. GILDEN

Flow Laboratories, Inc., Rockville, Maryland 20852

WADE P. PARKS, ROBERT J. HUEBNER & GEORGE J. TODARO

National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

The C-type virus particle in ESP-1 cells seems to be a murine virus.

IDENTIFICATION and classification of non-cytopathic viruses isolated from human tissues or human cell cultures require a combination of morphological, biological, biochemical and immunological tests. Because of their characteristic budding appearance at the plasma membrane, C-type leukaemia–sarcoma viruses are usually identified by morphological criteria[1]. Further classification as to species of origin can be accomplished immunologically. Mouse, hamster, cat and rat C-type viruses each contain an internal virion protein, the group-specific (gs) protein[2] which carries species-specific antigenic determinants[3–5]. Thus, C-type viruses of these species can be identified specifically by a number of different immunological tests. C-type particles have been described in a human cell line, ESP-1, derived from a patient with lymphoma, and the results indicated that this putative human virus has none of the known gs antigens[6]. We describe here immunological studies showing that the ESP-1 cells contain an antigenic determinant identical to the species-specific antigenic determinant of murine C-type viruses.

The ESP-1 cell line was obtained at the forty-first passage level through the courtesy of Drs Elizabeth Priori and Leon Dmochowski (M. D. Anderson Hospital and Tumor Institute, Houston, Texas). The original cultures were received as monolayer cultures and maintained in two different laboratories throughout these studies. Extreme care was taken to avoid contact with other C-type virus-infected cell cultures: this included use of isolated work space and maintenance of flasks in incubators containing only ESP-1 cultures. Media consisted of McCoy's plus 20% foetal calf serum or RPMI 1640 plus 10% heat inactivated foetal calf serum. Although the ESP-1 cell line is contaminated with mycoplasma, no antibiotics other than penicillin and streptomycin were used.

To determine the immunological relationship of the C-type virus in ESP-1 cells with the known mammalian C-type viruses, complement-fixation (CF), radioimmunoprecipitin inhibition (RIPI), and gel diffusion studies were performed. The following reagents gave consistent positive reactions in CF tests with 10–20% v/v ESP-1 cell homogenates: eight serum pools from rats bearing murine sarcoma virus (MSV) induced tumours which were selected for reactivity with the mouse gs antigen[7] and serum from two rabbits and three guinea-pigs immunized with the purified[8] gs antigen of mouse C-type viruses (MuLV). In contrast, no CF reactivity was obtained with guinea-pig antiserum prepared against purified feline (FeLV)[4], rat (RaLV), or hamster (HaLV)[9] gs antigens. As Table 1 shows, concen-

Table 1 Reactivity of ESP-1 with Representative Antisera directed against Mammalian C-Type Viruses as detected by CF and RIPI

Sera *	Test	Antigen †	Antigen titre or range ‡
Rats with MSV-induced tumours	CF	MuLV	16– 64
		ESP-1	2– 8
Guinea-pig anti-murine gs	CF	MuLV	16–128
		ESP-1	2– 8
	RIPI	MuLV	250
		ESP-1	10
Guinea-pig anti-feline gs	CF	FeLV	64–128
		ESP-1	< 1
Guinea-pig anti-hamster gs	CF	HaLV	8– 16
		ESP-1	< 1

* Sera were tested at 4 antibody units as defined by the reaction with purified gs antigens.

† Antigens were 10–20% (v/v) cell homogenates prepared from virus-releasing cell cultures. In all cases uninfected cell controls prepared in an identical manner failed to react with these sera.

‡ Expressed as the reciprocal of the highest antigen dilution showing 3+ or greater fixation of C′ (CF), or 50% or greater inhibition in the RIPI test.

trated ESP-1 cell homogenates had antigen titres eight to ten times smaller than routinely obtained with the homologous murine systems (murine leukaemia virus grown in rodent cells). The regularity with which positive reactions were obtained at different cell passage levels, with different sources of anti-murine gs sera and in different laboratories, strongly suggested cross-reactivity in ESP-1 cells with MuLV gs antigens. Equally important in indicating specificity for MuLV was the absence of reactivity in CF tests with ESP-1 cell extracts and antisera which detect feline and hamster gs antigens.

Further evidence that the ESP-1 antigen was cross-reactive with MuLV gs antigen was obtained by RIP inhibition (RIPI) tests. The double antibody precipitation method was used[10] with free and antibody-bound antigen being separated by centrifugation through sucrose gradients. This method permits a clear separation of free antigen (molecular weight ~25,000) which remains in the upper 10% of the gradient and antibody-bound antigen which is sedimented. MuLV gs antigen was labelled with I^{125} (5×10^5 c.p.m./µg protein) by the chloramine-T method[11] and only used after a final purification through SDS-polyacrylamide gels[9]. The precipitation tests were made in conditions in which a specific anti-MuLV gs rabbit serum was diluted to give 50% precipitation (serum dilution 1 : 5,000) of approximately 0.01 µg of labelled antigen. Only homogenates of cells infected with known strains of MuLV and the ESP-1 cell showed any measurable inhibition, while cells infected with hamster or feline C-type viruses and uninfected human, hamster and mouse cells gave negative results. ESP-1 antigen titres by RIPI were consistently twenty times smaller than comparably prepared MuLV antigens from murine cell cultures.

To establish fully the relationship of the immunological determinants of the antigen in ESP-1 cells with murine C-type virus gs antigen determinants, it was necessary to show precipitin lines of identity in immunodiffusion tests, using sera specific for the murine gs antigen. Because of the relatively low antigen concentration, 50–100% (v/v) ESP-1 cell concentrates or highly concentrated supernatant fluids were used. Figs. 1*A* and *B* show two examples of precipitin lines of identity between highly purified murine gs antigen and ESP-1 concentrates using different sets of reagents prepared and tested in different laboratories. Both rabbit and guinea-pig anti-MuLV gs sera have given lines of identity using several ESP-1 preparations and purified MuLV gs antigen. Although the identity reactions were most clearly seen when ESP-1 preparations were placed between positive MuLV antigens, definite identity reactions were also seen if a positive control was placed in only one adjacent well. The specificity of the reaction shown in Fig. 1*B* was indicated by the lack of curvature of the precipitin lines when control antigens were placed in well 6. The diagnostic antisera did not react with FeLV, RaLV, or HaLV antigens, nor did they react with uninfected human cells grown in the same media. In parallel tests using antibody specific for FeLV, RaLV and HaLV gs antigens, no reactions were obtained with any of the ESP-1 preparations. These data show that the ESP-1 cells possess an antigen with determinants identical to those detected on the group-specific antigen of the murine C-type viruses.

Mouse C-type leukaemia–sarcoma viruses have previously been shown to grow in human cells[12–15]. Further, mouse and cat C-type viruses which have been grown in human cells for long periods in culture retain their original species-specific gs antigen reactivity[16]. These facts, together with the data presented above, suggest that the C-type virus particle in ESP-1 cells is a murine virus. Immunological identity of mouse and human C-type virus gs antigens, although remote, remains an alternative explanation of the present data.

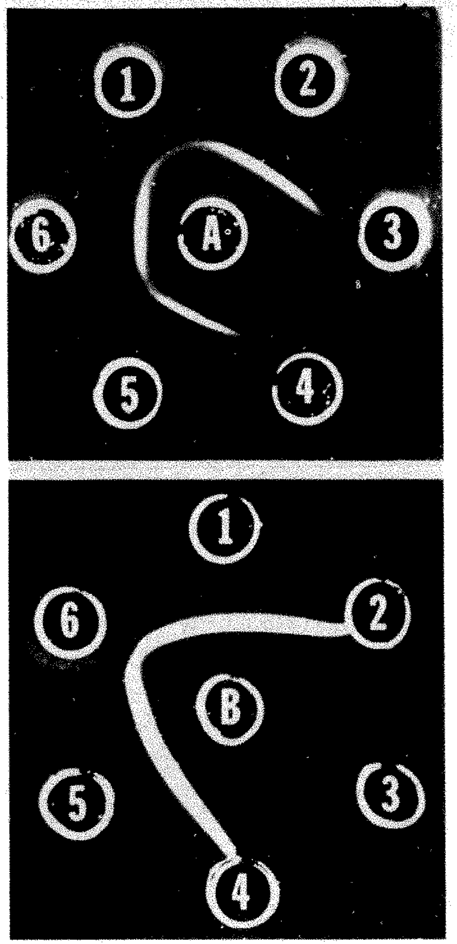


Fig. 1 Immunological identity of antigenic determinants in ESP-1 cells with MuLV gs antigen. Wells contained: Laboratory A, 1, human ESP-1 cells, 50% v/v; 2, MuLV-infected mouse cells (Kirsten strain); 3, human diploid fibroblast cells, 50% v/v; 4, FeLV (concentrated, ether disrupted); 5, MuLV-infected mouse cells (Rauscher strain); 6, purified MuLV gs; A, Rabbit 1 anti-MuLV gs. Laboratory B, 1, purified MuLV gs; 2, HaLV-infected hamster cells; 3, FeLV-infected cat cells; 4, RaLV-infected rat cells; 5, MuLV-infected rat cells; 6, ESP-1 concentrated supernatant fluid; B, rabbit 2 anti-MuLV gs.

This work was supported by contracts from the Special Virus Cancer Program of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. We thank Drs Cedric Long and Stephen Oroszlan and Dr Paul Price for their help, and David Bova, Martin White, Robert Toni, Evelyn Good, Charlotte Meyer and Lou Cooper for technical assistance.

Received August 1, 1971.

[1] Bernhard, W., *Cancer Res.*, **20**, 712 (1960).
[2] Huebner, R. J., *Proc. US Nat. Acad. Sci.*, **58**, 835 (1967).
[3] Gregoriades, A., and Old, L. J., *Virology*, **37**, 189 (1969).
[4] Oroszlan, S., Huebner, R. J., and Gilden, R. V., *Proc. US Nat. Acad. Sci.*, **68** (1971).
[5] Gilden, R. V., Oroszlan, S., and Huebner, R. J., *Virology*, **43**, 722 (1971).
[6] Priori, E. S., Dmochowski, L., Myers, B., and Wilbur, J. R., *Nature New Biology*, **232**, 61 (1971).
[7] Hartley, J. W., Rowe, W. P., Capps, W. I., and Huebner, R. J., *J. Virol.*, **3**, 126 (1969).
[8] Oroszlan, S., Fisher, C. L., Stanley, T. B., and Gilden, R. V., *J. Gen. Virol.*, **8**, 1 (1970).
[9] Oroszlan, S., Foreman, C., Kelloff, G., and Gilden, R. V., *Virology*, **43**, 665 (1971).
[10] Odell, W. D., Ross, G. T., and Rayford, P. L., *J. Clin. Invest.*, **46**, 248 (1967).
[11] Hunter, R., *Proc. Soc. Exp. Biol. Med.*, **133**, 989 (1970).
[12] Aaronson, S. A., and Todaro, G. J., *Nature*, **225**, 458 (1970).
[13] Boiron, M., Bernard, C., and Chuat, J. C., *Proc. Amer. Assoc. Cancer Res.*, **10**, 8 (1969).
[14] Wright, B. S., and Korol, W., *Cancer Res.*, **29**, 1886 (1969).
[15] Klement, V., Friedman, M. H., McAllister, R. M., Nelson-Rees, W. A., and Huebner, R. J., *J. Nat. Cancer Inst.*, **47**, 65 (1971).
[16] Hampar, B., Kelloff, G. J., Martos, L. M., Oroszlan, S., Gilden, R. V., and Walker, J. L., *Nature*, **228**, 857 (1970).

Virus-like Agents from Patients with Hodgkin's Disease

MAGDALENA EISINGER, SYLVIA M. FOX,
ETIENNE DE HARVEN, JUNE L. BIEDLER &
F. KINGSLEY SANDERS

Sloan-Kettering Institute for Cancer Research, 410 East 68th Street, New York, New York 10021

Cultured cells from patients with Hodgkin's disease underwent changes which seemed to be accompanied by the emergence of an agent of the herpes group.

THE aetiology of Hodgkin's disease is unknown. But the age incidence of the condition[1], the fact that an individual who has a close relative with this disease is three times more likely to contract it[2], and recent evidence of possible case-to-case transmission[3], all suggest that infective agents may be involved, and that these may be viruses, although other kinds of agents have been considered in the past[4].

Two difficulties have beset all attempts to implicate particular viruses as a cause of human tumours. Most such attempts involve tissue culture studies which must begin with biopsy specimens containing more than one cell type; thus, when cell lines have been successfully established in culture, it is uncertain whether these are indeed the malignant cells. Second, tumour cells seem to be especially favourable for the multiplication of viruses, and frequently harbour passengers which are not involved in the induction of malignancy. Hodgkin's disease presents an additional difficulty. Long recognized as a mixed neoplastic and inflammatory condition, the actual malignant cells have not been positively identified even in pathological specimens, although the classical Reed–Sternberg cells may be one such[6]. In this article we report that: (*a*) we have established from patients with Hodgkin's disease a number of long term cell cultures which we suspect may contain the malignant elements; (*b*) these cells, in culture, seem to release an RNA-containing agent into the supernatant medium; (*c*) certain of these cell cultures regularly undergo "transformation" in culture, with the appearance of a large number of rounded, free floating cells, certain populations of which become capable of independent growth in suspension; (*d*) such free floating cells release into the culture medium, in addition to the RNA agent already mentioned, a second agent containing DNA; (*e*) the latter has been tentatively identified as a member of the herpes group, though its reactions are typical of neither herpes simplex nor Epstein–Barr virus [EBV, HLV]. The relationship of these agents to either the aetiology or the pathological development of Hodgkin's disease is by no means clear.

Establishment of Cell Cultures

Early attempts to cultivate lymph node material from Hodgkin's disease using classical coverslip or plasma-clot methods[7] gave cultures of predominantly mixed cell type, which died out by the end of the second week or earlier. Subsequently, a long term, free floating cell line, consisting of several types of cell, was obtained by Ito *et al.*[8], and a similar one by Pontén[9]. In the latter case the free floating cells had arisen by "lymphoblastic transformation"[10] of an apparently random growing layer of fibroblast-like cells; the free floating cells could not be cultivated in the absence of a feeder layer.

The culture method we have used depends on the fact that biopsies of Hodgkin's disease lymph nodes contain many differentiated cells such as lymphocytes and macrophages, which may be capable of at most a few divisions in culture. It was assumed that the eventual death and disruption of such cells might release products inimical to the growth of undifferentiated cells potentially capable of giving rise to cell lines. This was consistent with our failure, as of others, to initiate cultures with five trypsinized lymph node biopsy specimens, trypsinization being notoriously destructive of cells of the lymphoid series. The method finally adopted was a modified Trowell-type organ culture[11]. Fragments of lymph node 0.5–1 mm in diameter were placed directly on stainless steel grids, two to four pieces per grid in organ culture dishes[12]; the medium was Eagle's MEM + 15% foetal calf and 10% pretested human serum. The pieces remained on the grids for at least 3–4 weeks, during which time they continued to shed cells onto the surface of the dish below. As soon as the latter became covered by a confluent layer of cells (mostly round cells plus fibroblasts), the grid was transferred to a new dish, such transfers being repeated at intervals until cells were no longer deposited. The pieces of node were then transferred without trypsinization to 60 mm Petri dishes plus 5 ml. of medium; the shed cells could rarely be transferred more than a few times, and were discarded.

In all cases cells now grew out from the node fragments which were very large, pleomorphic and thinly spread, with a markedly reticular cytoplasm, many lysosomes and sometimes eosinophilic inclusions and will henceforward be termed attached cells. They were mostly mononucleated, though sometimes binucleated, with prominent nucleoli varying in number from one to four (Fig. 1). They grew individually and showed neither a fibroblastic nor an epithelial pattern of growth. When confluence was achieved, the cells in the layer were randomly oriented and could now be passaged following trypsinization. They retained their distinctive morphology for at least seven or eight passages (7–8 weeks), and cultures stored at −78° C at earlier passage levels gave rise to attached cells when revived.

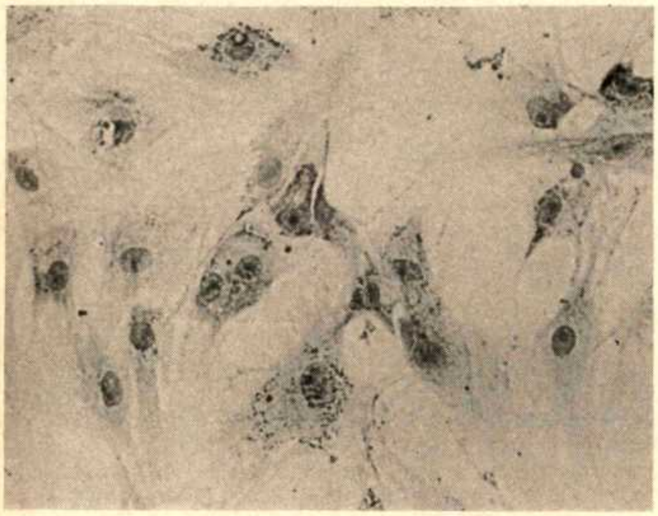


Fig. 1 Photomicrograph of cells from attached cell line (SK-HN-5b). Large, randomly arranged cells with one or two nuclei and prominent nucleoli. Reticulated cytoplasm with lysosomes (×192).

The chromosomes of the attached cell lines derived from five patients of both sexes had a model number of forty-six, the diploid number for man. In one of these five (a male) about two-thirds of the 104 metaphases counted were hypodiploid (forty-five) owing to the absence of the *Y* chromosome. Thirty-eight of fifty fully analysed karyotypes were normal, and among the twelve remaining aberrant karyotypes there were no consistent structural or numerical abnormalities. Thin sections of such cells, examined with the electron microscope, showed nuclei with dispersed euchromatin and large nucleoli. Occasional perichromatin granules were noted, very close to a thin rim of marginated heterochromatin. The cytoplasm was extremely rich in organelles of all types, an endoplasmic region being frequently distinguishable from peripheral ectoplasm. There were very many dilated rough endoplasmic reticulum cisternae containing proteinaceous material of moderate electron density, and several foci of Golgi structures. The ectoplasm showed many fewer organelles, occasional mitochondria and lysosomes being scattered among bundles of microfilaments. Structures that could be unequivocally identified as virus particles have not been seen so far.

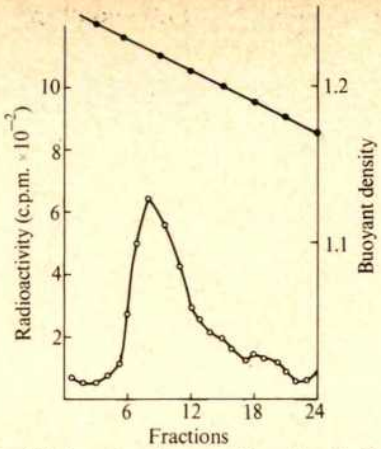


Fig. 2 Equilibrium density centrifugation in 20–58% sucrose of pelleted ^{3}H-uridine-labelled material from supernatant fluid of attached cell line SK-HN-CH.N, labelled as described in the text (16 h, 27,000 r.p.m., SW.27 rotor, 5° C). ●—●, Buoyant density; ○—○, radioactivity (c.p.m.).

Table 1 Attempts to Establish Cell Strains from Lymph Nodes, Skin, or Tumour of Patients with Hodgkin's Disease

Subject	Cultured organ	Age at biopsy (yr)	Sex	Time on grid (weeks)	Tissue in culture (weeks)	No. of cell generations	Cell strain
Hodgkin's patient 1	Lymph node	14	♂	7	27	34	SK-HN-1
Hodgkin's patient 2	Lymph node	8	♀	5	36	48	SK-HN-CH.H
	Lung tumour	9	♀	6	34	92	SK-HT-CH.H
	Skin	9	♀	2	19	70	SK-HS-CH.H
Hodgkin's patient 3	Lymph node	22	♀	3	17	40	SK-HN-3
	Spleen	22	♀	5	30	30	SK-HSp-3
Hodgkin's patient 4	Lymph	23	♂	4	17	28	SK-HN-4
Hodgkin's patient 5	Lymph	52	♂	6	10	18	SK-HN-5a
				16	7	12	SK-HN-5b
					24	180	SK-HNF-5a
					20	150	SK-HNF-5b
	Skin	52	♂	6	16	30	SK-HS-5
Hodgkin's patient 6	Lymph	15	♂	6	16	26	SK-HN-6
Hodgkin's patient 7	Lymph	23	♂	5	17	32	SK-HN-7
Hodgkin's patient 8	Lymph	14	♂	7	17	14	SK-HN-8

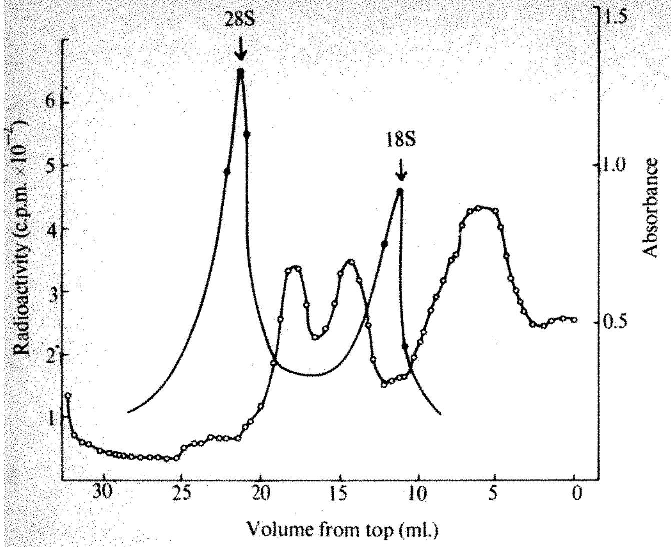


Fig. 3 Velocity sedimentation in sucrose (15–30%) of ^{3}H-RNA from the isopycnic labelled band at 1.20–1.22 liberated with SDS after dialysis to remove sucrose. 18S and 28S RNA markers from whole rat liver ribosomes (14 h, 25,000 r.p.m., SW.27 rotor, 10° C). ●—●, Abundance at 260 nm; ○—○, radioactivity (c.p.m.).

Using the above technique, it was possible to derive attached cell cultures of the same type from ten patients. In the case of eight of them (Table 1) they have now been carried for up to forty-eight cell generations. The other two were stored frozen at an early passage level; the cells, however, had a similar morphology, and none had reached "crisis"[13] before storage. In contrast, cells cultured from two normal lymph nodes went into "crisis" and failed to become established (Table 2). In two Hodgkin's cases (patients 2 and 5, Table 1) skin biopsies also gave rise to cell strains (SK–HS–CH.H, SK–HS–5), whose cells were not typical skin fibroblasts but resembled the attached cells obtained from the lymph nodes of the same individuals. One of these patients had clinical evidence of skin infiltration by tumour cells (patient 5). That the attached cells cultured by our technique may indeed be malignant was also suggested by preliminary heterotransplantation experiments in which 10^6 cells of the culture line obtained from patient 6 (SK–HN–6, Table 1) were inoculated into the cheek pouches of cortisonized weanling Syrian hamsters. Six out of twelve such inoculations gave rise to small tumours which began to regress after 1–2 weeks; 10^6 cells inoculated into the dorsal region of each of six newborn Syrian hamsters did not give rise to tumours. Cells from the skin of a normal individual gave typical fibroblast cultures which went through crisis and died out. An exception was the skin from the mother of patient 2 (Table 2), which did give rise to a continuously propagable line of fibroblasts. Patient 2, however, was homozygous for the Chediak-Higashi syndrome[14], and her mother by definition a heterozygote; it is not known what influence this rare genetic anomaly may have on the ability of fibroblasts to propagate indefinitely in culture.

Table 2 Attempts to Establish Cell Strains from Lymph Nodes and Skin of Individuals without Hodgkin's Disease

Subject	Cultured organ	Sex	Time on grid (weeks)	Tissue in culture (weeks)	No. of cell generations
Control patient 1	Lymph	♂	4	8*	14
Control patient 2	Lymph	♀	5	12*	12
Control mother of Hodgkin's patient 2	Skin	♀	2	20	70
Control patient 3 (normal)	Skin	♀	2	20*	28

* Cell strain went through "crisis" at this time and expired.

Release of Agent containing RNA

To establish whether the attached cell lines could be releasing anything resembling a virus, a search was made in the tissue culture media for material excreted by the cells which might have biophysical properties like those of viruses[15]. 5-^{3}H-uridine was incorporated in the medium at a concentration of 1 µCi/ml. Cells were labelled in log phase, 24 h after splitting semiconfluent cultures, and allowed to grow in labelled medium for 4 days. The medium was then removed (minimum volume per experiment = 300 ml.), clarified by centrifugation at 6,000 r.p.m. for 30 min at 5° C. It was then filtered through a 22 nm 'Millipore' membrane filter and pelleted at 15,000 r.p.m. for 2 h at 5° C in an SW.27 rotor. The supernatant was discarded and the pellet resuspended in three drops of Tris-EDTA buffer (0.01 M Tris, 0.001 M Tris, 0.0001 M EDTA, 0.1 M NaCl, *p*H 7.2), and layered over a 15–58% w/w sucrose gradient. The gradient was then centrifuged to equilibrium, and the gradient fractions collected and assayed for radioactivity. A typical result is shown in Fig. 2. A marked peak of radioactivity banded at a sucrose concentration corresponding to a density of 1.20–1.22. Two of our attached cell lines have been studied in this manner, and have consistently given the same result (six experiments).

Five other human and one hamster cell line were similarly labelled and treated to serve as controls. Cells from (1) a neuroblastoma (SK–N–SH), (2) a breast carcinoma (SKBR–3), (3) a continuous "floating" cell line (SK–LN) from a normal lymph node, (4) strain WI-38 embryonic lung fibroblasts, (5) the FL continuous line of amnion cells, all failed to show any uridine-labelled peak when similarly treated. (6) B34 hamster cells, a suspended cell culture line that continuously releases hamster sarcoma virus[16], served as a positive control; when labelled in the same way it gave a peak sedimenting at a density of 1.14–1.15. After labelling the attached cell lines in a similar manner with ^{3}H-thymidine, no peak was found.

It was then necessary to establish that the ^{3}H label in the 1.20–1.22 sucrose density peak was in fact in RNA, and that, if so, the RNA was not of cellular (for example, ribosomal) origin. Fig. 3 shows the result of a sedimenting ^{3}H-labelled RNA extracted from the 1.20–1.22 band on a sucrose velocity gradient at the same time as ribosomal markers. None of the three bands of labelled macromolecular RNA coincided with the 28S and 18S bands of the marker. The RNA is thus clearly not derived from ribosomes. It is, however, undoubtedly RNA, since treatment of the material with RNAase eliminated the peaks, all radioactivity now sedimenting at the top of the gradient.

There remained a trivial explanation that the ^{3}H-uridine peak was due to mycoplasma contamination; this, however, was ruled out by means of experiments to be described later.

The breadth of the labelled RNA peak (Fig. 2), however, indicates that the isopycnic band is still markedly heterogeneous. This is confirmed by electron microscopy of the material in the band. A range of particulate material is present, among which it has not yet been possible to identify any virions of characteristic morphology.

"Blastoid Transformation"

Cultures from the lymph nodes of two of the patients listed in Table 1 (patients 5 and 2) eventually underwent a further change. On continued passage the growth rate declined to a low level, and the cultures eventually became static for 2–3 weeks, though without showing a cytopathic effect. At this stage local foci of piled up cells appeared, consisting of highly basophilic "blastoid" round cells. At their first appearance the latter seemed to be "nested" within the cytoplasm of an attached cell, for they had a clear halo around them, which could be enhanced by the use of phase-contrast illumination (Fig. 4*a*). This did not seem to be the result of phagocytosis, because debris or degenerating cells were not found in this situation; moreover, many of the rounded cells could be seen to be in mitosis, while still within the cytoplasm of the reticular cells (Fig. 4*b*). At an intermediate stage of "transformation", practically every reticular cell could be seen to have one or more round cells within it. The question remains open as to whether the round cells actually arise within the reticular cells, or enter the latter's cytoplasm to complete their development.

This process of "blastoid transformation" continued for 4–5 weeks, when the cultures from one patient (Table 1, No. 5) had become entirely composed of free floating cells (blastoid cells) (Fig. 5) with large variations in cell size and number of nuclei; at the same time the growth rate increased approximately eight-fold, considerably exceeding that of the parent culture. Transformation occurred on two different occasions with separate samples from this same patient, each at about the same time in the history of the resultant cell line. On the first occasion transformation occurred after lymph node fragments had remained for 1 month on the grid, followed by nine transfers as monolayer cultures. The second time the fragments were on the grid for 4 months, followed by six monolayer passages. A single stock of reticular cells from one of these cell lines before transformation was kept stored at −78° C; on four later occasions, when cells from this stock were thawed out and subsequently propagated, complete transformation into blastoid cells took place. Cells from a second patient (2) also gave rise to blastoid cells of the type described, but in this case they could only be propagated by using a feeder layer, and thus resembled those of Pontén[9]. At the time of writing, attached cell lines from three more patients (6, 7 and 8) are beginning to undergo similar changes.

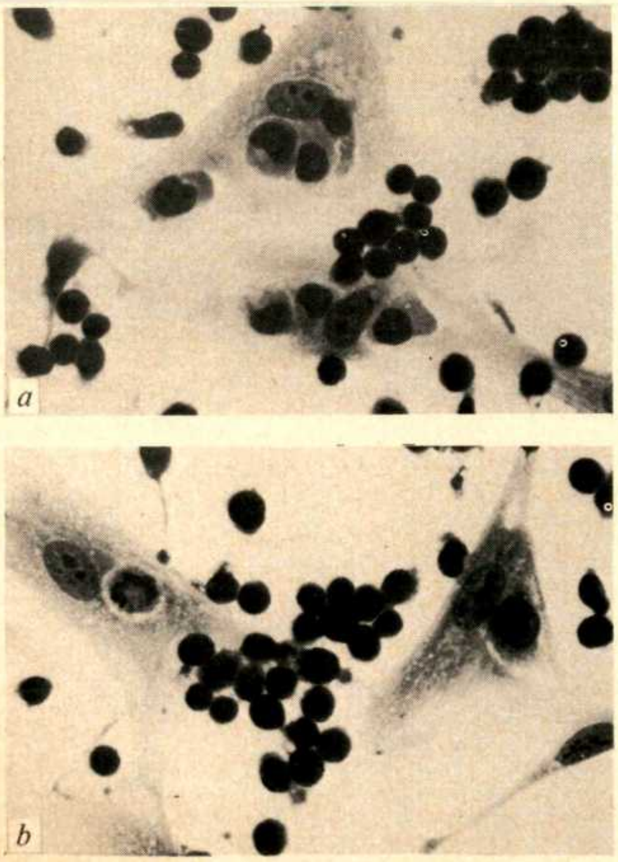


Fig. 4 *a*, "Blastoid transformation" intermediate stage (×384). Note cells nested within the cytoplasm of settled cells; multinucleate giant cell. *b*, Further advanced "blastoid transformation" (×384). Note group of cells in mitosis.

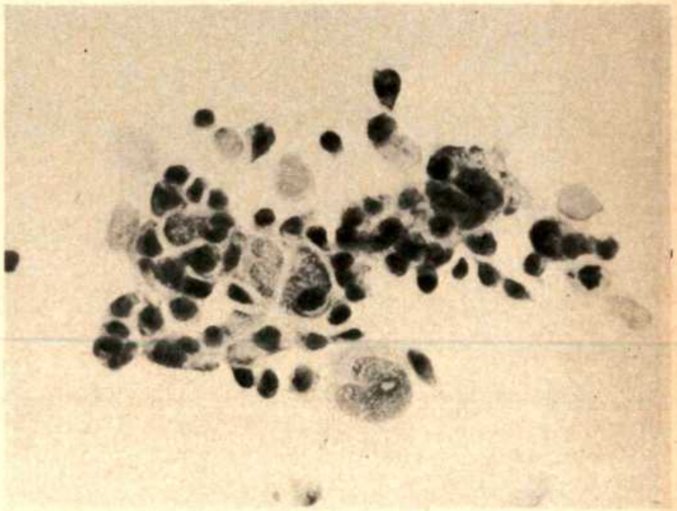


Fig. 5 Cells from suspended culture of transformed cells. Note large variations in cell size and nuclei (×375).

At least three types of blastoid cells could be distinguished (Fig. 5): (*a*) small, highly basophilic cells with a large round nucleus; (*b*) medium sized, less basophilic, sometimes vacuolated cells, with a large nucleus containing multiple irregularly shaped nucleoli; (*c*) multinucleated giant cells with many nucleoli. The floating cells generally also had forty-six chromosomes although two out of forty-one cells from the floating cell line derived from patient 5 also lacked the *Y* chromosome; a notable feature of these cells was the elevated frequency of enhanced secondary constrictions of chromosomes 1, 9 and 16.

The blastoid cells differed strikingly in ultrastructure from the attached cells. Their nuclei showed more condensed heterochromatin and larger numbers of perichromatin granules, and throughout their cytoplasm mitochondria with a dense matrix and conspicuous "mitochondrial ribosomes" could be seen. No boundary was apparent between ectoplasm and endoplasm, and the microfilaments, so conspicuous in the reticular cell lines, were barely visible. Moreover, the endoplasmic reticulum was poorly developed, and its cisternae contained no accumulated secretion product. Most ribosomes appeared as "free" monosomes, while in the reticular cells most of them were attached to membranes in typical "polysome" patterns. Clumps of blastoid cells showed many examples of close contact of their plasmalemma, giving the appearance of tight junctions. Thus while the reticular cells have some of the ultrastructural features of histiocytes, the floating blastoid cells resemble blastoid elements of the haemopoietic system.

Release of Agent containing DNA

Labelling experiments were also performed with one of the suspended cell lines that arose following the "blastoid" transformation just described (patient 5). The technique was the same as that used with the settled cell lines. 5-^{3}H-uridine still became incorporated into material banding isopycnically on sucrose at 1.20–1.22 (Fig. 6). But when ^{3}H-thymidine instead of 5-^{3}H-uridine was used, a strongly labelled DNA-containing peak was consistently found banding at the same position in the gradient (1.20–1.22; Fig. 7). At the outset, it was suspected that both the DNA and RNA-containing bands might be due to concurrent mycoplasma contamination, and bacteriological examination revealed that this culture line was indeed infected with mycoplasma. The following evidence, however, makes it unlikely that mycoplasma was responsible for this band.

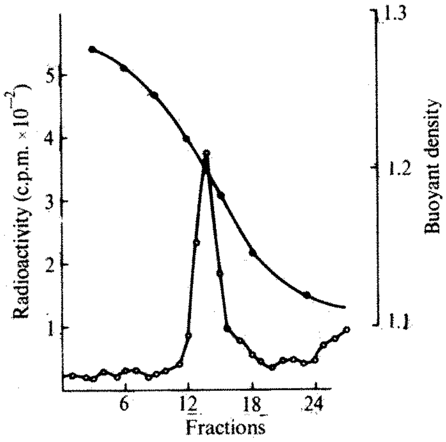


Fig. 6 Equilibrium density centrifugation in 20–58% w/w sucrose of pelleted ^{3}H-uridine-labelled material from supernatant fluid of "transformed" suspended cell culture SK-HNF-5b (16 h, 27,000 r.p.m., SW.27, 5° C). Symbols as in Fig. 2.

(*a*) Mycoplasma, isolated from the culture line itself, grown up in broth and concentrated by centrifugation, banded on sucrose at an equivalent density of 1.24–1.26, many fractions further down the gradient than the labelled band mentioned above. (*b*) Cultures of FL amnion cells and WI-38 fibroblasts were deliberately infected with a high level of this same mycoplasma, and then labelled with ^{3}H-uridine in the manner described. The tissue culture fluids were then processed in the same way as before, and the concentrates banded isopycnically in sucrose. No labelled peak was seen. (*c*) The mycoplasma-contaminated "blastoid" cell line was treated by Dr J. Fogh until there was no longer any evidence of mycoplasma. ^{3}H-thymidine labelling was undertaken, this time in another laboratory known to be free from mycoplasma, and the fluids examined. A ^{3}H-labelled peak at 1.20–1.22 was again present, of greater size than before. (*d*) Electron microscope examination of thin sections of the pelleted supernatant revealed the presence of double-enveloped nucleocapsids typical of the herpesvirus group. (*e*) There was also immunological evidence—to be discussed in the next section—of the presence of herpesvirus antigens, both in the cells and in the material from the sucrose band.

Serology of DNA containing Agent

Serological studies of the SK-HNF-5 cell line are being conducted in collaboration with Miss Gayla Geering and Drs Germaine Trempe, T. Takahashi and Lloyd Old. As expected from the morphological evidence of the presence of an associated herpesvirus, herpesvirus gs antigen has been demonstrated in the pelleted virus. Immunodiffusion and immunofluorescence studies have failed to show EB virus-related antigen in the SK-HNF-5 cell line; however, a new antigen has been found in the cell line by immunodiffusion, and precipitating antibody to this antigen is found in the sera of a high proportion of normal human subjects.

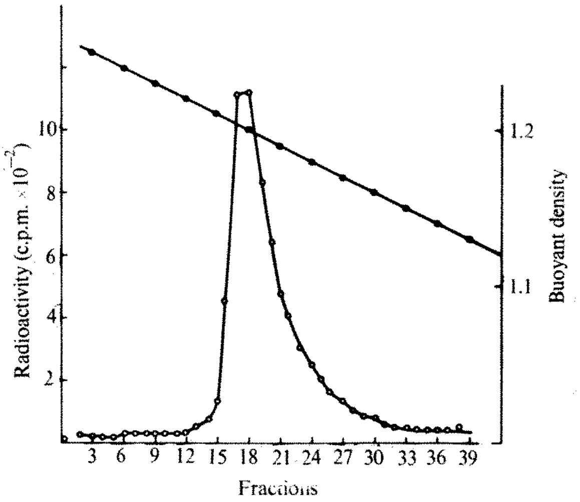


Fig. 7 Equilibrium density centrifugation in 20–58% w/w sucrose of pelleted ^{3}H-thymidine-labelled material from supernatant fluid of "transformed" suspended cell culture SK-HNF-5b (16 h, 27,000 r.p.m., SW.27 rotor, 5° C). Symbols as in Fig. 2.

Our findings suggest that the process of "blastoid transformation" in this case may have been accompanied by the emergence of an agent of the herpes group. Further experiments are in progress to discover whether a similar phenomenon occurred during the other instances of transformation reported.

This work was supported in part by a grant from the National Cancer Institute. In addition to those mentioned we thank Dr Charles W. Young for clinical material; Dr Jørgen Fogh for mycoplasma studies: Dr Mathilde Krum for help with chromosome studies and histology; Miss Barbara Dziuban, Mr Edward Bittar and Miss Barbara A. Spengler for technical assistance; and Mrs Emma M. Tessier and Miss Patricia Potenza for secretarial assistance.

Received August 2, 1971.

[1] McMahon, B., *Cancer Res.*, **26**, 1189 (1966).
[2] Razis, D. V., Diamond, H. D., and Craver, L. F., *Amer. J. Med. Sci.*, **238**, 327 (1959).
[3] Vianna, N. J., Greenwald, P., and Davie, J. N. P., *Lancet*, **i**, 1209 (1971).
[4] Craver, L. F., in *Hodgkin's Disease* (edit. by Molander, D. W., and Pack, G. T.), 3 (Thomas, Springfield, Illinois, 1968).
[5] Higgins, G. K., in *Hodgkin's Disease* (edit. by Molander, D. W., and Pack, G. T.), 20 (Thomas, Springfield, Illinois, 1968).
[6] Lukes, R. J., and Butler, J. J., *Cancer Res.*, **26**, 1063 (1966).
[7] Grand, C. G., *Cancer Res.*, **9**, 183 (1949).
[8] Ito, Y., Shiratori, O., Kurita, S., Takahashi, T., Kurita, Y., and Ota, K., *J. Nat. Cancer Inst.*, **41**, 1367 (1968).
[9] Pontén, J., *Intern. J. Cancer*, **2**, 311 (1967).
[10] Benyesh-Melnick, M., Fernbach, D. J., and Lewis, R. T., *J. Nat. Cancer Inst.*, **31**, 1311 (1963).
[11] Jensen, F. C., Gwatkin, R. B. L., and Biggers, J. D., *Exp. Cell. Res.*, **34**, 440 (1964).
[12] Merchant, D. J., Lahn, R. H., and Murphy, O., *Handbook of Cell and Organ Culture* (Burgess, Minneapolis, 1960).
[13] Girardi, A., Jensen, F., and Koprowski, H., *J. Cell Comp. Physiol.*, **65**, 69 (1965).
[14] Tan, C., Etcubanas, E., Lieberman, P., Isenberg, H., King, O., and Murphy, M. L., *Amer. J. Dis. Child.*, **121**, 135 (1971).
[15] Todaro, G. J., Zeve, V., and Aaronson, S. A., *Nature*, **226**, 1047 (1970).
[16] Nowinski, R. C., Old, L. J., O'Donnell, P. V., and Sanders, F. K., *Nature New Biology*, **230**, 282 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Eclipsing Binary Model of Cygnus XR-1

AGRAWAL *et al.*[1] present an interesting observation of the variable X-ray source Cygnus XR-1 at energies above 20 keV. This communication points out that their observations may be consistently interpreted in terms of the eclipsing binary model for the source that was originally outlined in an article by myself[2] (article 1).

Agrawal *et al.* observed the source in nineteen consecutive periods of approximately 5 min from 0002 to 0315 UT, on April 6, 1971. The mean count rate in the 22.5–99 keV energy range for periods 1 to 12 was 260 counts per min (although non-random variations in the count rate appear to be present) and there was "no significant variation" in this source count rate for the following four periods (13 to 16). The three succeeding periods, however (17 to 19), show "considerably lower values" of source count rate; the authors infer from these results the fact that the source "decreased from the mean value corresponding to (periods) 1–12 by a factor of ~2. The observations (between periods 16 and 17) are consecutive in time and therefore the fall in intensity has taken place within a few minutes".

According to the eclipsing binary model outlined in article 1, Cygnus XR-1 is composed of a source of small angular extent (either a dense star or a small, optically thin region associated with it) having a spectrum similar to that of a black body at $kT=12.5$ keV and a non-X-radiating companion which eclipses it at intervals of 2.9850 d. The system is surrounded by an X-radiating plasma the photon flux of which between 1 and 100 keV can be approximated by a power law spectrum the exponent of which is -1.7. Using an epoch of (primary) minimum derived in article 1 of JD 2 439 382.71 [which is uncertain to something like 1 h], a minimum would thus be predicted to occur at JD 2 441 047.88. Because the eclipse minimum was shown in article 1 to last at least 0.66 day but less than 0.80 day, this would predict an occultation of the black body source sometime between 2330 and 0115 UT on April 5–6, 1971. If we accept the discrepancy of 1 h as being caused by the uncertainty in the epoch of primary minimum, it is possible to interpret the observations of Agrawal *et al.* as being caused by the immersion of the black body source behind its non X-radiating companion. To support this interpretation we note first that the variation occurs in the right direction, that is, a decrease in flux before the time of minimum, second that the flux decrease in the 22–99 keV range is of the amount (~1/2 the maximum flux observed from Cygnus XR-1) ascribed to the black body source in the eclipsing model, that is, it is the amount which, added to the state of minimum flux defined in article 1, would produce the state of maximum flux. Third, no effect was seen over the same time period in the energy region 99–154 keV by Agrawal *et al.*[1]. Using the data in their Table 1, the 19 observations had a χ^2 of 19.5; 39.2% of all random distributions are more random than this for 18 degrees of freedom. Thus, the observation in this energy region is consistent with that expected from the random arrival of photons from a non-varying source, which in the eclipsing binary model would be identified with the X-radiating plasma surrounding the system. The black body source intensity above 100 keV, of course, is negligible with $kT=12.5$ keV. Fourth, the total immersion of the black body takes place over a short time period, that is, over a period of minutes. This is the expected time of occultation of an object of a few kilometres diameter at a distance of ~1 kpc in an orbit of period 3 days about a solar size object. If the flux difference between the states of maximum and minimum flux is taken to be $\sim 1.5\times10^{-8}$ ergs cm^{-2} s, then the distance of the 1.5×10^{8} K black body producing this flux is 0.9 R kpc, where R is the radius of the black body in kilometres. The irregularity in the flux immediately before immersion is perhaps related to the line of sight passing through a region near a distorted stellar atmosphere the boundary of which is not sharp in the sense of the solar limb.

Since the publication of article 1, three other observations of Cyg XR-1 have been made in the region above 20 keV. Weber and Reinert (private communication) observed the source on JD 2 440 352.87 and obtained a spectrum consistent with a state of minimum flux. The nearest minimum predicted in article 1 was at JD 2 440 352.83, consistent with the observation. Haymes and Harnden[3] observed the source between JD 2 440 377.78 and 378.07; their resultant, high quality spectrum is nearly coincident with their previous measurement of the object in the state of maximum flux. The nearest minimum predicted in article 1 occurs at JD 2 440 376.71, 1.22 days before, and so is consistent with the observed state. Agrawal *et al.*[4] also observed Cyg XR-1 between JD 2 440 328.36 and 328.47; the spectrum they derived would be classified as "uncertain" (with regard to the level of flux) according to the criteria outlined in article 1. Because the nearest minimum is predicted to occur at JD 2 440 328.96, this observation must have occurred at a time of maximum intensity.

Observation of Cyg XR-1 from the satellite Explorer 42 (ref. 5) in the 2.4 to 6.9 keV region indicated that approximately 25% of the flux exhibits short time variability. Although no eclipse phenomena seem to be present in this energy region (see article 1), this observation clearly indicates the multiple source nature of the object. The observation of the variable source Cen X-3 from the same satellite[6] seems to indicate the same type of pulsation observed from Cyg XR-1 in this energy range. Since Cen X-3 is best fitted by a black body spectrum of 3.3×10^{7} K, it is tempting to interpret the smooth variation in flux level in Fig. 1 of ref. 6 as the immersion of an eclipsing binary system. The lower temperature of the black body and the less intense continuous radiation relative to it make the variability more evident for this source in the satellite's lower energy range of observation.

The eclipsing binary model for the variability of Cyg XR-1 as presented in article 1 thus still seems to be consistent with all observations of the object so far.

JOSEPH F. DOLAN

Warner and Swasey Observatory,
Case Western Reserve University,
Cleveland, Ohio

Received August 9, 1971.

[1] Agrawal, P. C., Gokhale, G. S., Iyengar, V. S., Kunte, P. K., Manchanda, R. K., and Sreekantan, B. V., *Nature*, **232**, 38 (1971).
[2] Dolan, J. F., *Space Sci. Rev.*, **10**, 830 (1970).
[3] Haymes, R. C., and Harnden, jun., F. R., *Astrophys. J.*, **159**, 1111 (1970).
[4] Agrawal, P. C., Biswas, S., Gokhale, G. W., Iyengar, V. S., Kunte, P. K., Manchanda, R. K., and Sreekantan, B. V., *Astrophys. Space Sci.*, **10**, 500 (1971).
[5] Oda, M., Gorenstein, P., Gursky, H., Kellogg, E., Schreier, E., Tananbaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **166**, L1 (1971).
[6] Giacconi, R., Gursky, H., Kellogg, E., Schreier, E., and Tananbaum, H., *Astrophys. J. Lett.*, **167**, L67 (1971).

Optical Identification of Cygnus X-1

THE star HD 226868 is coincident, within a 3″ error, with a faint and possibly variable radio source which itself is coincident, though with a large uncertainty, with the Cygnus X-1 pulsating X-ray star[1].

Table 1 Data on HD 226868 (= BD 34° 3815)

$V=8.89$; $(B-V)=+0.85$; $(U-B)=-0.24$ (Hiltner[3])		
S_p	$=$BOIb	(Morgan, Code and Whitford[2]; RGO)
$V_r(*)$	$=-12$ km s^{-1}	(Seyfert and Popper[8])
$V_r(K)$	$=-24$ km s^{-1}	
	$=-13\pm7$ km s^{-1}	(RGO)
Equivalent width of K line		
	$=0.3$ Å	(Seyfert and Popper[8])
	$=0.6\pm0.1$ Å	(RGO)
Central depth of diffuse interstellar line (4430 Å)		
	$=9\pm2\%$	(RGO)
Q	$=-0.89$ mags	
$E_{(B-V)}$	$=1.07$ mags	
m_0-M	$=11.5$ mags	
d	$=2.0\pm0.5$ kpc	

Table 1 lists information about HD 226868 obtained from the literature and from spectrograms taken by observers at the Royal Greenwich Observatory (RGO) with the image tube and photographic spectrographs attached to the 98 inch telescope; the measurements were made between July 26 and August 4, 1971. The RGO spectra were at dispersions of 60, 70 and 180 Å mm^{-1} over the spectral range 3600 to 5200 Å. The star appears to be a normal BOIb supergiant as classified by Morgan *et al.*[2]. (The visibility of the high Balmer lines in HD 226868 is slightly less than the visibility of those in a comparison star HD 192422, BO.5Ib, which tends to decrease its luminosity towards II.)

The allocation of the spectral type BOIb is supported by the photoelectric colours of Hiltner[3] which give

$$Q=(U-B)-0.72(B-V)-0.05(B-V)E_{B-V}= -0.89$$

agreeing precisely with the standard value[4].

The spectroscopic distance modulus of HD 226868, calculated using $M_v= -5.8$ (ref. 5) and the value, $R=3.0$, for the ratio of total to selective absorption in this region[6], is 11.5 mags, corresponding to a distance of 2.0 kpc. The chief uncertainty in the distance modulus arises from cosmic dispersion in the absolute magnitude of the spectral type, and corresponds to an error in the distance of ±0.5 kpc. The intensities of the interstellar K-line and 4430 Å diffuse band are consistent with the amount of reddening and the distance to the star. The kinematic distances of the star and K-line absorption are liable to large uncertainty because the star is near the null points of the galactic velocity field, but they are also consistent with the spectroscopic distance modulus.

The distance of 2.0 kpc which we ascribe to the star is, apparently, inconsistent with the upper limit of 1.0 kpc placed on the distance of Cygnus X-1 by Gursky *et al.*[7] from the low energy turn-off in its X-ray spectrum due to photoelectric absorption in the interstellar medium. But because of the tentative identification of the supergiant with Cyg X-1 and the uncertainties expressed by Gursky *et al.* about the accuracy of their method, we feel it premature to attempt to save the phenomenon. If the double coincidence of position between the Cyg X-1 source, the radio star and the supergiant is too strong to be by chance, the X-ray source may be a companion to the supergiant, rather than identical to it. If the X-ray source has an optical absolute magnitude of +4, like the Crab Nebula pulsar, its optical luminosity is 10^{-4} times that of the supergiant. A distance of 2.0 kpc would imply that the observed power output in the X-ray region (0.5 to 100 keV) would be more than 10^{37} erg s^{-1} and comparable with the Crab Nebula.

PAUL MURDIN
B. LOUISE WEBSTER

Royal Greenwich Observatory,
Herstmonceux Castle,
Hailsham, Sussex

Received August 12, 1971.

[1] Braes, L. L. E., and Miley, G. K., *Nature*, **232**, 246 (1971).
[2] Morgan, W. W., Code, A. D., and Whitford, A. E., *Astrophys. J. Supp.*, **2**, 41 (1955).
[3] Hiltner, W. A., *Astrophys. J. Supp.*, **2**, 389 (1956).
[4] Johnson, H. L., *Lowell Obs. Bull.*, **4**, 37 (1958).
[5] Blaauw, A., in *Basic Astronomical Data* (edit. by Strand, K. A.) (University of Chicago Press, 1963).
[6] Johnson, H. L., in *Nebulae and Interstellar Matter* (edit. by Middlehurst, B.) (University of Chicago Press, 1968).
[7] Gursky, H., Gorenstein, P., Kerr, F. J., and Grayzeck, E. J., *Astrophys. J. Lett.*, **167**, L15 (1971).
[8] Seyfert, C., and Popper, D. M., *Astrophys. J.*, **93**, 461 (1941).

Possible Black Hole in Beta Lyrae

β LYRAE is one of the most controversial and puzzling of binary systems[1]. The central issue concerns the absolute masses of the two components. The one-spectrum radial-velocity curve does not reveal what those masses are, but any pair of masses satisfying its mass function make the system remarkable. Table 1 gives pairs of masses which satisfy the observed 8.5 $M_{\odot}$ mass function[2]. Here $q=M_{\rm BRT}/M_{\rm FNT}$, and the entries in the table are the minimum masses allowable—that is, the orbital inclination angle is assumed to be 90°. Early investigators, faced with the absence of the secondary component in the spectrograms at all phases, assumed that the components of the system obey a mass-luminosity law and favoured a mass ratio considerably larger than unity. This, however, results in very large masses, especially for the B8 primary. Three arguments have since been put forward which make the choice $q>1$ dubious. First, Sahade[3] has called attention to the behaviour of the unusual emission features present in the spectrograms, noting that, should they be associated with the secondary star, a value for the mass ratio considerably less than unity results. Second, a similar small value comes from a distance determination by Abt[4], which was based on β Lyrae's co-motion with few stars. As these were found to be ordinary main sequence objects, the absolute magnitude of β Lyrae was determined to be $M_v = -3.4$, which agrees with the spectroscopic luminosity class. So modest a luminosity can hardly be associated with such a massive primary component as indicated for $q > 1$. Finally, Woolf[5] has used Abt's distance in combination with an assumed brightness temperature to find the absolute area of the primary star and thus its absolute "radius". Further, assuming that β Lyrae A fills its Roche lobe, this "radius" is compared to the observed value of $a_1 \sin i$ to obtain the mass ratio of the system, again giving $q < 1$.

Table 1 Minimum Absolute Masses which Satisfy the Observed Mass Function

$M_{\rm BRT}/M_{\rm FNT}$	$M_{\rm FNT}$ (solar masses)	$M_{\rm BRT(B8)}$ (solar masses)
0	8.5	0
1/4	13.3	3.3
1/2	19.2	9.6
1/1	34.0	34.0
2/1	76.5	153.0

It is difficult to accept such a small mass ratio for the system, however, because the secondary must then be extraordinarily underluminous for its mass. Nevertheless, I shall show that this is a necessary consequence of the present observational evidence.

Although spectroscopy does not reveal the mass ratio of β Lyrae's components, the photometry of the system provides vital information through the photometric effect of tidal and rotational distortion. The aim of this article is to use this effect to determine the mass ratio by the method of fitting theoretical light curves to the observations throughout the phase interval between eclipses. The principle is straightforward, yet the method has not had widespread application. One reason is that the mass ratio is only one of several parameters which influence the out-of-eclipse variation and thus it is usually weakly determined. Furthermore, it has been difficult until recently to carry out calculations on the light changes of stars which display such severe photometric distortion as β Lyrae. The calculations presented here are adapted from a program for computing monochromatic, complete light curves of close binaries in which all the distortion and mutual irradiation effects are considered. The computer program and its application are presented in detail elsewhere[6].

I shall show that β Lyrae is a most interesting exception in which the photometric technique can be applied to the determination of the mass ratio. Two factors are responsible for this. First, the primary (more luminous) component is in contact with its Roche lobe, a fact made evident by the pattern of gas-streaming in the system, pointed out by Struve[1] and also by Sahade[3]. Thus its relative size is uniquely determined by any assumed value of q. Second, this component is the only one which contributes significantly to the observed light variation between eclipses. The secondary may thus be neglected in the theoretical calculations, reducing considerably the number of free parameters, and elevating the mass ratio to the role of the dominant parameter. Because the neglect of the faint component is important to the application of the method, its justification will be considered in some detail, using information from the well known spectroscopic data and the recently published light curve of Larsson-Leander[7], which resulted from the 1959 IAU International Campaign on β Lyrae.

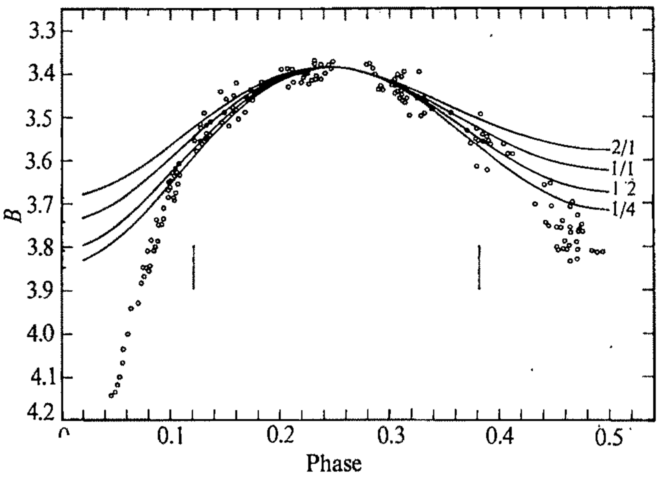


Fig. 1 The B light curve of Larsson-Leander with the theoretical curves as solid lines, given for labelled values of the mass ratio $M_{\rm BRT(B8)}/M_{\rm FNT(INV)}$. Other required parameters for the calculation are given in the text. The primary eclipse egress is shown and observations in the range of phases from 0.5 to 0.89 have been reflected about phase 0.5.

The principal feature of the spectroscopy of this system is the total absence of an ordinary absorption spectrum attributable to a secondary component, even at mid-primary eclipse. At this phase, the light level drops to 48% of its value at the quarter phases. As the absence of one member of a binary from spectrograms is commonly taken to indicate that a factor of at least six separates the luminosity of the stars, it may be concluded that the secondary is responsible for no more than 8% of the light of the system. (The $B-V$ colour curve is quite flat so that this statement is true for either yellow or blue light.) Now consider the light curve shown in Fig. 1. It is noteworthy that the phase of external tangency, marking the end of eclipse, is visible clearly at a phase of 0.11, from which it can be seen that the amplitude of the eclipse effects reaches 0.20 magnitudes. A secondary with 8%, or less, of the system's light cannot be responsible for such changes. In fact, careful consideration shows that what the observations do reveal, with considerable fidelity, is the tidal and rotational distortion of the primary component alone. This can be seen by correcting the observations for the presence of a supposed 8% secondary, allowing, for purposes which will become evident, that it is somewhere in the distortion range between a perfectly spherical and a fully distorted or contact star. Treating the spherical case first, it is merely necessary to subtract 8% light from the observations at all phases. This results, on a magnitude scale, in a slight increase in the amplitude of the out-of-eclipse light variation amounting to 0.015 magnitudes. As the secondary is allowed to be more distorted, it is found that the corrected data approach more closely the original observations.

The theoretical calculations thus give the light variation of one contact component only, as a function of phase. The computations depend on just four parameters, in addition to the mass ratio. As the mass ratio sets the size of the contact primary in this case, the inclination angle is the only other geometrical parameter required and it has been assumed to be 90°. The coefficient of limb darkening was taken as 0.60, somewhat larger than theory predicts. The gravity-darkening law was that for an atmosphere in radiative equilibrium (von Zeipel's law). An effective temperature of 11,000 K was assumed, but the calculations are not sensitive to this parameter. The choice of 90° inclination, radiative equilibrium, and a coefficient of limb darkening larger than expected, as well as the neglect of a possible light contribution by the secondary, all insure the greatest light variation possible for any given mass ratio. This means than an upper limit for the mass ratio $M_{\rm BRT}/M_{\rm FNT}$ is found here. The results of the theoretical calculations for selected values of the mass ratio are shown as solid curves in Fig. 1. It is evident that the primary has the minor share of the system's mass, and that very likely $q \simeq 1/2$ is a realistic upper limit. If the secondary happens to be spherical, and contributes the maximum amount, 8%, to the light of the system, then it is conceivable that the mass ratio is a factor of two smaller. A significantly smaller mass ratio would also be appropriate if the primary could somehow have a convective atmosphere. It is also conceivable that the system's inclination angle differs so significantly from 90° that further reduction would be needed. Thus it is seen that the primary must not have more than ~35% of the mass of the system.

For $q = 1/2$, the absolute masses resulting for the primary and secondary are approximately 10 $M_{\odot}$ and 20 $M_{\odot}$ respectively. The mass of the primary is in good agreement with that determined by Abt from the slightly evolved position of β Lyrae A on the colour-luminosity diagram, but perhaps the evolutionary state of the star is also a function of its duplicity and the star may not be in equilibrium. It is now virtually certain that the secondary must be a most remarkable object, at least four magnitudes underluminous for its mass. Such a large value for its mass excludes the possibility of its being a white dwarf or neutron star, but it may be a black hole, or perhaps some previously unknown stellar state.

The photometric data furnish no information that the secondary is a collapsed object. In fact, there is clear indication of a geometrical eclipse in which about 1/3 of the primary is hidden, so the eclipsing body must be substantial in size, yet it must be within its Roche critical surface, if the primary is at its own lobe. This is because the principal eclipse is over at a phase of 0.11, whereas the eclipse of two contact components would continue until a phase of 0.16 regardless of the mass ratio[8]. It is quite likely that the agent responsible for the primary eclipse is a disk, the existence of which is given strong credibility by the polarimetric work of Rucinski[9]. There also certainly appears to be a light loss due to eclipse at the secondary minimum. This loss does not seem to be due to the eclipse of the light of the primary reflected from the disk because then the reflexion effect of the system would have to be quite pronounced, which is not at all the case, so it is conceivable that the secondary itself is actually eclipsed. It would probably be meaningless to use the eclipse light loss at the two minima to infer the surface brightness ratio of the two components, because there exists the likely contribution of the disk to the principal minimum. Despite these difficulties in interpretation of the complete light curve of this star, the results for the mass ratio obtained here cannot be vitiated unless one is prepared to believe that the light variation between eclipses has some unknown and mysterious source. In view of the strong evidence for the primary being at its Roche lobe and also dominating the light of the system, the basis for such an idea is totally lacking.

On the contrary, the evidence is conclusive for the presence of a massive, underluminous object in the system of β Lyrae and we should probably look to it for an explanation of the remaining problems of the light curve.

An important item of evidence furnished by the identification of a black hole in a close binary system is that the collapse must have taken place without much mass loss because the system was not disrupted; 20 $M_{\odot}$ must be approximately the mass of the pre-collapse object. It can also be suggested that the disk about the secondary was created by a mass-shedding process during collapse. As the secondary is invisible because of its small size, there is no reason to consider that the disk is an opaque, star-hiding body[10], having a considerable fraction of the mass of the system[5]. The stability of such a ring would be questionable, so it is probable that only a small fraction of the mass of the collapsing object was lost to the disk.

The importance of this system for understanding the nature of massive, underluminous stars is great, and it is necessary to encourage all pertinent observations. Finally, it is ironic to note that β Lyrae, the prototype of all β Lyrae systems, is not a β Lyrae system.

EDWARD J. DEVINNEY, JUN.

Department of Astronomy,
University of South Florida,
Tampa

Received May 4; revised August 16, 1971.

[1] Struve, O., *Pub. Astron. Soc. Pacific*, **70**, 5 (1958).
[2] Batten, A. H., *Sixth Cat. Orb. El. Spect. Bin. Systems, Pub. Dom. Astrophys. Obs.*, **13**, No. 8, 66 (1967).
[3] Sahade, J., Huang, S.-S., Struve, O., and Zebergs, V., *Trans. Amer. Phil. Soc., N.S.*, **49**, 1 (1959).
[4] Abt, H. A., Jeffers, H. M., Gibson, J., and Sandage, A. R., *Astrophys. J.*, **135**, 429 (1962).
[5] Woolf, N. J., *Astrophys. J.*, **141**, 155 (1965).
[6] Wilson, R. E., and Devinney, jun., E. J., *Astrophys. J.* (in the press).
[7] Larsson-Leander, G., *Arkiv för Astron.*, **5**, 253 (1969).
[8] Kopal, Z., *Close Binary Systems*, 143 (J. Wiley and Son, New York, 1959).
[9] Rucinski, S. M., *Acta Astronomica*, **16**, 127 (1966).
[10] Huang, S.-S., *Astrophys. J.*, **138**, 354 (1963).

Compact Radio Sources in the Galactic Nucleus

SMALL-SCALE radio structure within Sgr A, the source identified with the galactic centre, is of great interest because of the recent discovery of similar structure in the infrared[1] and because of current speculation on black holes in the galactic nucleus[2]. In this communication we describe new observations

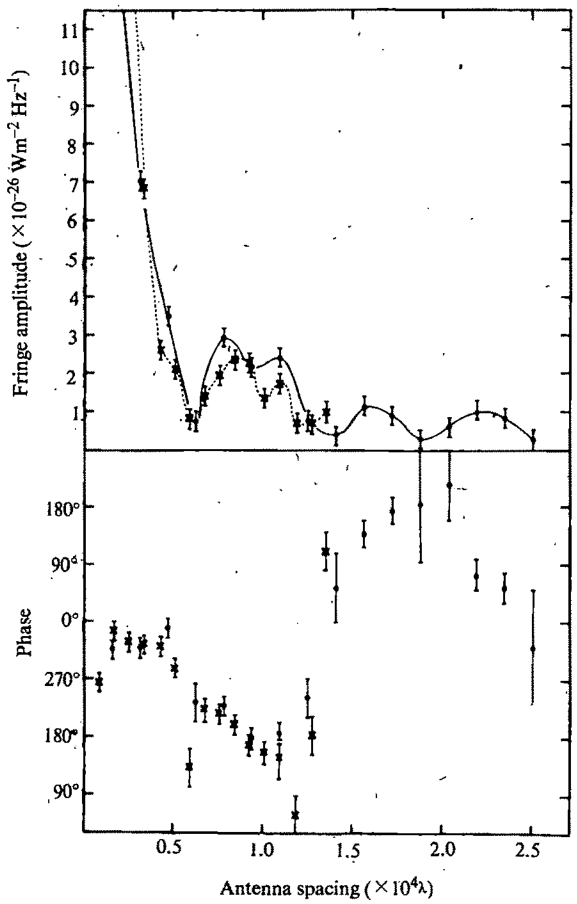


Fig. 1 Fringe amplitude and phase for Sgr A at transit at (×) 2.7 and (●) 5 GHz.

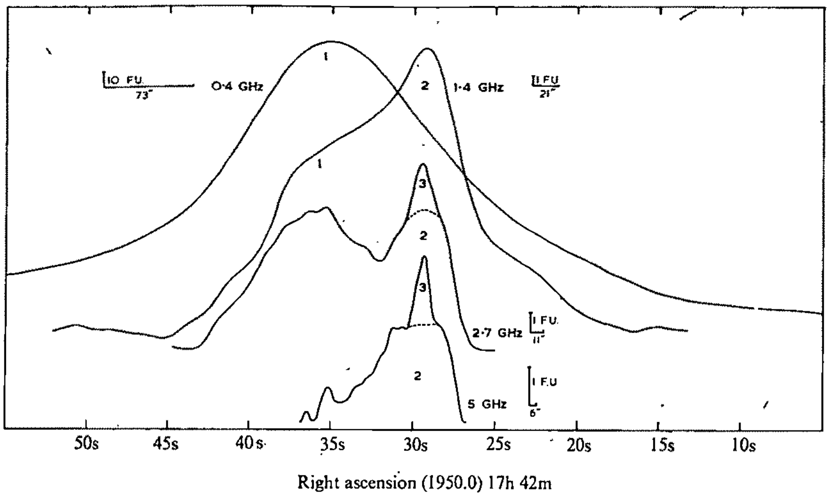


Fig. 2 Strip brightness distributions in right ascension for Sgr A at transit at 0.4, 1.4, 2.7 and 5 GHz. Horizontal bar indicates beamwidth, vertical bar indicates intensity of a point source. The components are numbered as in Table 1.

of Sgr A with the Cambridge 1 mile telescope at frequencies of 2.7 and 5 GHz. The beamwidths in right ascension were respectively 11 and 6 arcsec, the best of these being the highest resolution yet applied to the galactic centre. The observations suggest a three component model for Sgr A, and confirm the existence of radio structure on a scale of 10 arcsec. The relation of these compact radio sources to the infrared emission is also examined.

The observations were made between January and March 1971 at centre frequencies of 2.6970 and 4.9938 GHz, with sixteen antenna spacings along an east–west baseline. At each frequency the system temperature was about 1,000 K and the infrared bandwidth was 4 MHz. All observations were made during ±1 h of meridian transit, with the antennas pointing 8 arcmin north of the true declination of the source to allow for atmospheric refraction. The primary source for calibration of phase and intensity was 3C 147, which was assumed to have flux densities of 13.0 and 8.2 flux units at 2.7 and 5 GHz respectively. The data were averaged for intervals of 15 min, for an r.m.s. sensitivity of 0.3 flux units in the fringe amplitude. In our experiment, the telescope produced a principal response whose half-width in right ascension was 11 and 6 arcsec at 2.7 and 5 GHz, with first grating lobes separated from the main beam by 4.1 and 2.2 arcmin in right ascension respectively.

Previous observations of this type[3–5] have provided curves of fringe visibility and phase for Sgr A up to baselines of 2000 λ at frequencies ~1 GHz. The maximum baseline of the present observations is 25,000 λ at 5 GHz. The fringe visibility and phase from the new observations are shown in Fig. 1 for the galactic centre at transit. At spacings <4000 λ the curves show the tail of the response to the main part of Sgr A, which has an angular diameter ~3 arcmin. This is the part of the visibility function that has appeared in previous interferometric observations of the source. At spacings >4000 λ, the new visibility function shows the presence of fine structure in the source at even the longest spacing. The existence of fine structure was originally recognized by Ekers and Lynden-Bell (private communication) who used the interferometer at the Owens Valley Radio Observatory.

Before Fourier inversion of the observations, the fringe amplitudes were multiplied by a Gaussian weighting function which fell to a value of 30% at the largest spacing; this weighting function reduced sidelobes to 5% or less. The synthesized fan beam was centred on declination −28° 59′ 30″, and the beamwidth in declination was 16 and 9 arcmin at 2.7 and 5 GHz respectively.

Table 1 Model Proposed for Sgr A

Component name	r.a. 1950	dec. 1950	Halfwidth in r.a.	Flux density (× 10^{-26} W m^{-2} Hz^{-1})			
				0.4	1.4	2.7	5.0 GHz
1 Sgr A East	17 h 42 m 36 s ±1 s	−28° 59′ 00″ ±60″	150″ ±30″	250±50	160±30	>80	—
2 Sgr A West	17 h 42 m 29.3 s ±0.3 s	−28° 59′ 15″ ±50″	45″ ± 5″	—	30± 5	25±5	25±5
3 Compact structure*	17 h 42 m 29.3 s ±0.1 s	−28° 59′ 20″ ±30″	10″ ± 2″	—	—	3±1	3±1

* Only the strongest feature has been tabulated.

The resulting brightness distributions are shown in Fig. 2. The new data at 2.7 and 5 GHz are shown together with earlier scans obtained at 0.408 and 1.407 GHz with sixteen spacings of the same telescope[6]. The profiles clearly show the shift in position of the peak of the source as noted by other observers[6–9] and also show the existence of a prominent small diameter source at higher frequencies. The profile at 5 GHz, however, does not show structure of low spatial frequency (that is, of angular size >2 arcmin) that may be present, because of the limited number of antenna spacings used.

Fourier transforms were also made of the data taken at local hour angles ±00 h 45 m, which correspond to position angles ±6° across the source. A comparison of the three scans gives information on the declination of the various components and suggests a three component model of Sgr A which is summarized in Table 1. We emphasize, however, the uncertainties in this model which are a consequence of our limited coverage in the Fourier transform plane. In particular, there seem to be additional components of size <10 arcsec and intensity <1 flux unit, which we have not tabulated. Among these are the peaks at r.a. 17 h 42 m 35 s, and other peaks or ridges to the east and west of the 3 flux unit source at 17 h 42 m 29.3 s.

We interpret the radio spectra of the components in Table 1 as follows. The 2.5 arcmin source at r.a. 17 h 42 m 36 s (Sgr A East) is probably non-thermal because: (i) it emits most of the flux, and the spectral index $\alpha < -0.25$ ($S \propto \nu^{\alpha}$) derived from lunar occultations[10] refers chiefly to this component. The predominance of this source at 0.2 to 0.4 GHz explains the increase in diameter of Sgr A with decreasing frequency and also its position shift to the east; (ii) H109α recombination line emission is at least an order of magnitude less than one would expect from a thermal source of this flux density[11]; (iii) for a thermal source of this size and intensity to be optically thin at 200 MHz (ref. 10), the electron temperature would have to be $>10^5$ K.

Both sources in the complex at 17 h 42 m 29.3 s, consisting of Sgr A West and the compact structure with diameter ~10 arcsec, appear to have flat spectra. At present we cannot distinguish between a thermal and non-thermal origin for the sources in this region. If they are thermal, then their linear sizes and flux densities are comparable with those of compact H II regions[12]. The sources would become optically thick below 1 GHz and this would help to explain the position shift at low frequencies. If, on the other hand, these sources are non-thermal, they must simply have a flatter spectrum than Sgr A East. The same effects of diameter increase and position shift would then be observed in lunar occultations. On a synchrotron interpretation, the compact component would be similar to the bright radio knots seen in Cas A (ref. 13) and would have a minimum energy of 10^{46} erg.

On the basis of existing measurements, the group of infrared sources prominent at 5 to 20 μm (ref. 1) is displaced by about 10 to 15 arcsec to the east of our component 3. None of the localized sources at these infrared wavelengths coincides with any of the radio components. This may be similar to the situation in some H II regions, for example, the Orion Nebula, where the IR nebula[14] seen at 10 to 20 μm does not coincide with the radio peak.

As the small components seen in Sgr A at both radio and infrared wavelengths have diameters of the order of a light year or less, it may be possible to detect intensity variations on a timescale of a few months. We also suggest that the source be monitored for interplanetary scintillation, which might reveal even smaller radio components in Sgr A.

We thank Drs R. D. Ekers, D. Lynden-Bell, F. J. Low and G. H. Rieke for informing us of their results before publication and G. H. Macdonald for permission to refer to unpublished work. D. D. was supported by a Frank Knox fellowship and A. H. M. M. by an SRC studentship.

D. DOWNES
A. H. M. MARTIN

Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
Cambridge

Received August 12, 1971.

[1] Rieke, G. H., and Low, F. J., *Nature* (in the press).
[2] Lynden-Bell, D., and Rees, M. J., *Mon. Not. Roy. Astron. Soc.*, **152**, 461 (1971).
[3] Lequeux, J., *Ann. d'Astrophys.*, **25**, 221 (1962).
[4] Moffet, A. T., and Maltby, P., *Astrophys. J. Suppl. Ser.*, **7**, 93 (1962).
[5] Fomalont, E. B., *Astrophys. J. Supp. Ser.*, **15**, 203 (1968).
[6] Macdonald, G. H., thesis, Univ. Cambridge (1968).
[7] Little, A. G., *Symposium on Radio and Optical Studies of the Galaxy*, 113 (Mount Stromlo Observatory, 1966).
[8] Thompson, A. R., Riddle, A. C., and Lang, K. R., *Astrophys. Lett.*, **3**, 49 (1969).
[9] Lipovka, N. M., *Astron. Zh. (USSR)*, **48**, 260 (1971).
[10] Maxwell, A., and Taylor, J. H., *Astrophys. Lett.*, **2**, 191 (1968).
[11] Reifenstein, E. C., Wilson, T. L., Burke, B. F., Mezger, P. G., and Altenhoff, W. J., *Astron. Astrophys.*, **4**, 357 (1970).
[12] Wynn-Williams, C. G., *Mon. Not. Roy. Astron. Soc.*, **151**, 397 (1971).
[13] Rosenberg, I., *Mon. Not. Roy. Astron. Soc.*, **151**, 109 (1970).
[14] Kleinmann, D. E., and Low, F. J., *Astrophys. J.*, **149**, L1 (1967).

Reduction of Dinitrogen in Aqueous Solution

NITROGENASE, which contains iron, molybdenum and thiol groups, operates in an aqueous environment. Many attempts have been made to produce chemical models for nitrogenase based on the above knowledge and recently Schrauzer and co-workers have described dinitrogen-reducing systems of this type[1,2] but they use high pressures of dinitrogen (135 atm) and obtain only 3 to 5 μmol. of ammonia from mmol quantities of reagents[2] (see Table 1, example 1).

When the levels of reduction are very low and other nitrogen-containing ligands are present, it is essential to use $^{15}N_2$ to confirm the reduction of dinitrogen to ammonia. We have therefore tested with $^{15}N_2$ two of Schrauzer's systems and other systems which we have developed. Our quantitative

Table 1 Dinitrogen Reducing Systems

	Reagents * † (mmol × 10^3) and reaction times	Approximate yield of $^{15}NH_3$ ‡ (mmol × 10^3)
(1)	1-Thioglycerol (2,500); Na_2MoO_4 (5,000); $FeSO_4$ (100); $NaBH_4$ (6,600); 120 h, $p^{14}N_2 = 135$ atm	3 to 5§
(2)	Cysteine (20); Na_2MoO_4 (40); $FeSO_4$ (0.8); $NaBH_4$ (290); 60 h	3
(3)	Cysteine (20); Na_2MoO_4 (40); $NaBH_4$ (290); 60 h	0
(4)	2-AET (60); Na_2MoO_4 (60); $FeSO_4$ (1); $NaBH_4$ (390); 12 h	90
(5)	BSA (40,000)¶; Na_2MoO_4 (20); $FeSO_4$ (0.8); $NaBH_4$ (290); 60 h	5
(6)	BSA (50,000)¶; $FeSO_4$ (20); $NaBH_4$ (290); 60 h	3
(7)	2-AET (20); Na_2MoO_4 (40); $FeSO_4$ (0.8); $NaBH_4$ (290); 60 h, pCO=0.5 atm	0‖
(8)	2-AET (20); Na_2MoO_4 (20); $FeSO_4$ (0.8); $NaBH_4$ (290); $H_2NC(CH_2OH_3)$ (100); 60 h	0
(9)	V^{2+} (100); Mg^{2+} (5,000); KOH (56,000); 0.2 h; 70°	4**
(10)	Mo^{3+} (40); Mg^{2+} (180); Ti^{3+} (300); KOH (12,800); 24 h	3††

* $p^{15}N_2 \sim 1$ atm; 30 ml. gas. † Final volume of aqueous solution ~1.5 ml. ‡ Mass spectral analysis of derived $^{15}N_2$. § Data from ref. 2, product is $^{14}NH_3$. ‖ Compare ref. 2, where pCO of 0.1 atm does not inhibit. ¶ Milligram quantity × 10^3. ** Reaction product is $^{15}N_2H_4$. †† Solvent methanol/water (5 : 1).

2-AET = 2-Aminoethanethiol; BSA = bovine serum albumin.

results, some of which are negative, are given in Table 1, examples 2 to 10.

Schrauzer's system contains cysteine, iron, molybdenum and $NaBH_4$ (ref. 1) and undoubtedly gives traces of $^{15}NH_3$ from $^{15}N_2$ even at 1 atm (example 2), but not in the absence of iron (example 3). Working along parallel lines we have used 2-aminoethanethiol and sodium borohydride to develop a system which gives greatly increased yields of ammonia at pressures of 1 atm and reaction times of a few hours, that is, approaching the natural conditions (example 4).

High yields of ammonia are only obtained when iron is used together with molybdenum, but we have been able to replace the thiol by bovine serum albumin (a thiol-containing protein) and to obtain low yields of ammonia even when iron is the only added metal (compare examples 5 and 6); here molybdenum may be present at a trace level but added molybdenum alone is not effective. Low levels of dinitrogen reduction using iron complexes have been reported, but no experimental details have been given[3].

High concentration of carbon monoxide inhibits our system (example 7) as does *tris*-hydroxymethylaminomethane—a buffer substance often used in the preparation of active extracts from the natural system (example 8)—presumably because it sequesters the iron from its active role.

We have also confirmed the results of Shilov and co-workers[4] that $^{15}N_2$ is reduced to hydrazine-$^{15}N_2$ in strongly alkaline solution with molybdenum or vanadium as a catalyst (examples 9 and 10). There is thus no doubt that dinitrogen is reduced in these aqueous systems and further modification may yield useful systems for the catalytic activation of dinitrogen.

R. E. E. HILL
R. L. RICHARDS

Agricultural Research Council,
Unit of Nitrogen Fixation,
University of Sussex,
Brighton BN1 9QJ

Received August 13, 1971.

[1] Schrauzer, G. N., and Schlesinger, G., *J. Amer. Chem. Soc.*, **92**, 1808 (1970).
[2] Schrauzer, G. N., Schlesinger, G., and Doemeny, P. A., *J. Amer. Chem. Soc.*, **93**, 1803 (1971).
[3] Newton, W. E., Corbin, J. L., Schneider, P. W., and Bulen, W. A., *J. Amer. Chem. Soc.*, **93**, 268 (1971).
[4] Shilov, A., Denisov, N., Efimov, O., Shuvalov, N., Shuvalova, N., and Shilova, A., *Nature*, **231**, 460 (1971).

Occurrence of Biogenic Siliceous Sediments in the Atlantic Ocean

ALTHOUGH the deposition of biogenic siliceous sediments is not widespread in the modern Atlantic and Caribbean, calcareous siliceous or siliceous sediments (including cherts) are recorded at various intervals in the Mesozoic[1-7] and Tertiary sequences[1-5,7-10] of both areas. On the basis of the available data it appears that the deposition of these sediments was widespread in these areas during the Lower Tertiary and possibly the Mesozoic, and more restricted during the Upper Tertiary. In this article I describe and attempt to account for the distribution of these sediments.

There are unfortunately few data concerning either the stratigraphical or the geographical distribution of Mesozoic biogenic siliceous sediments. The discovery of radiolarian oozes and cherts at eleven localities investigated by the Deep Sea Drilling Project suggests that these sediments may have been deposited intermittently over a wide area during the Upper Jurassic[1,5,6] and Upper Cretaceous (Cenomanian to Campanian)[2-4,8].

The distribution of biogenic siliceous sediments is better documented for the Lower Tertiary (see Fig. 1). Eocene siliceous oozes and/or cherts are recorded at several widely separated localities, and in the North Atlantic the prominent and widespread reflector horizon A can at least in part be identified with Middle Eocene chert beds.

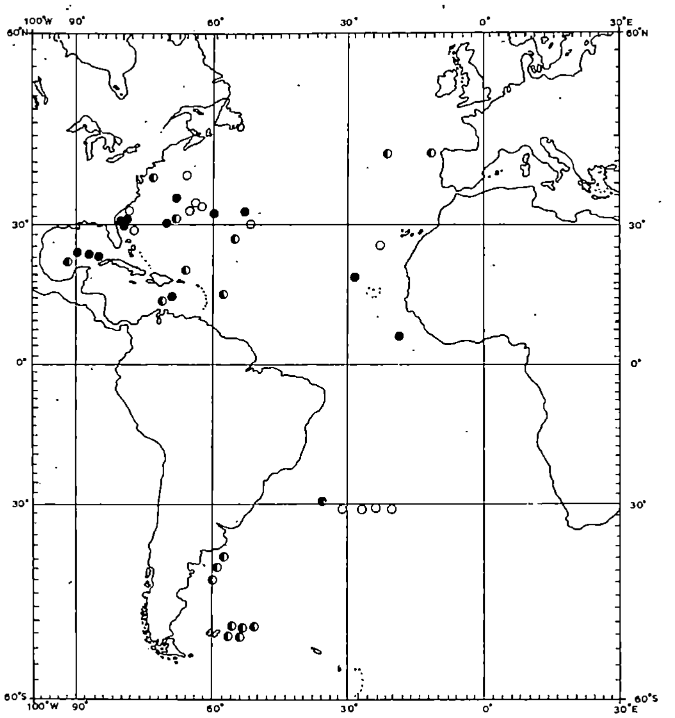


Fig. 1 The distribution of Eocene sediments in the Atlantic: filled circles represent chert, half-filled circles represent unconsolidated siliceous or calcareous siliceous ooze, and open circles represent calcareous ooze.

As a consequence of ocean floor spreading, the present location of the Palaeogene siliceous sediments does not conform with their position of deposition. Their distribution in the North Atlantic and Caribbean is evidently also unrelated to the present hydrography, biology and productivity of these areas. Fig. 2 shows the position of the Palaeogene localities plotted on Francheteau's[11] reconstruction of the Eocene Atlantic. On this chart two areas of biogenic siliceous sedimentation are defined: a southern zone situated along the western margin of the Argentine Basin, and a central equatorial zone. By analogy with the Recent oceans both areas were regions of upwelling characterized by a high production of plankton, and reflect the positions of silica belts established for the surface suspension during the Palaeogene. The southern area still lies within the major belt of silica accumulation which is associated with the Antarctic Convergence and West Wind Drift. Data presented by Saito and Funnell[12] show that this has been an area of opaline siliceous sedimentation since the Cretaceous. Although upwelling occurs at the equator in the Atlantic, the equatorial zone has no modern counterpart. In the Pacific Ocean, however, radiolarian ooze accumulates within a zone extending from 20° N to 20° S (ref. 13), which is associated with the equatorial current system. According to information published by Riedel[14] and Riedel and Funnell[15], this zone was already established during the Lower Tertiary.

The variation between the pelagic sediments associated with the Atlantic and Pacific equatorial current systems may be due to differences in their surface productivity which are caused by the higher concentration of phosphates, nitrates and silicates in the Pacific[16]. The low concentration of these nutrients in the equatorial Atlantic is directly related to the exchange of water between the North and South Atlantic, which results in their removal via the south flowing deep water of the North Atlantic[16]. On the basis of evidence obtained for Upper Cretaceous and Tertiary deep water circulation in the North Atlantic by Leg II of the Deep Sea Drilling

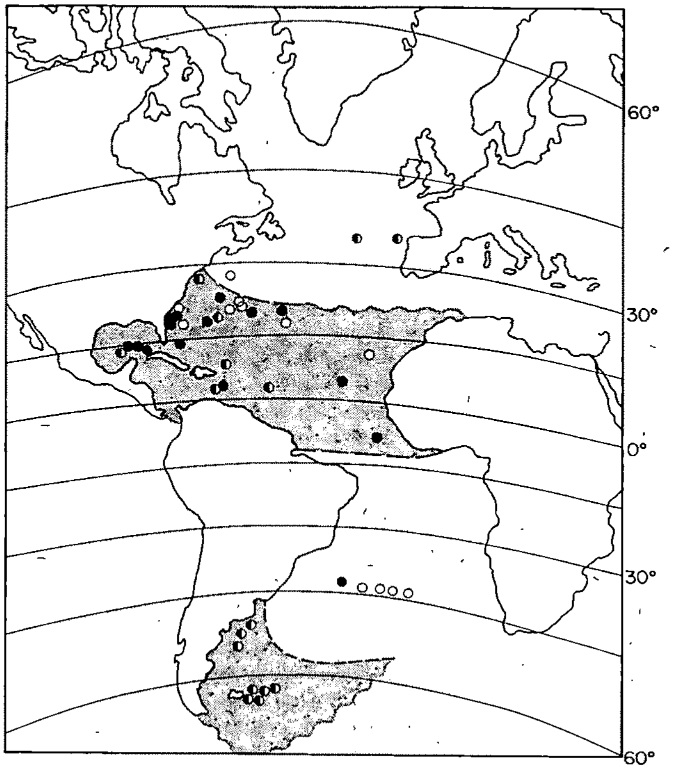


Fig. 2 The potential distribution of biogenic siliceous sediments in the Eocene Atlantic and Caribbean.

Project[5], it appears that a similar process may have operated in the Lower Tertiary Atlantic. Consequently it is unlikely that an equatorial zone of biogenic siliceous sedimentation would have developed in the Palaeogene Atlantic unless there was an influx of water rich in nutrients into this area from an outside source. Such an influx could conceivably have been achieved by the flow of Pacific equatorial water across the Isthmus of Panama via the east flowing equatorial surface countercurrent and subsurface Cromwell current (see Fig. 3). As the continuity of the present countercurrents is apparently seasonal in either ocean[17], the bulk of this flow was probably effected by the Cromwell current. At present this current is approximately 300 km wide and is symmetrical about the equator[17,18]. It occupies depths between 50 and 200 m and has a total volume transport of about 40×16^6 m^3 s^{-1} (ref. 18). A depth greater than 200 m was therefore necessary to facilitate the passage of this current across the Isthmus of Panama during the Lower Tertiary.

The distribution of calcareous and siliceous or calcareous-siliceous sediments on the Palaeogene ocean floor can be explained in terms of the circulation pattern proposed in Fig. 3. In this model the accumulation of calcareous ooze is confined essentially to areas of low productivity at the centre of the gyres. Siliceous or calcareous siliceous sedimentation occurred in regions of high productivity associated with areas of upwelling, or at the gyre margins. As in the modern oceans the accumulation of opaline silica in the bottom sediments can be attributed to the absence of a critical depth for its preservation, and to the resistance of radiolarians, silicoflagellates and the more robust species of diatoms to solution in the water column[13]. Also the increased production of carbon dioxide in sediments which underlie the fertile areas of oceans and the concomitant depression in the pH of the sea water[19,20] result in a decrease in the solubility of opaline silica and enhance the preservation of siliceous skeletal material[21] for its duration at the sediment–water interface. From the data presented in Figs. 2 and 3 it is apparent that the formation of the Eocene cherts in the North Atlantic is closely associated with the deposition of opaline silica in the Palaeogene equatorial high productivity zone. In terms of the modern Atlantic the information contained in these charts suggests that the equatorial cherts should not be encountered south of 6° N and north of 36° N.

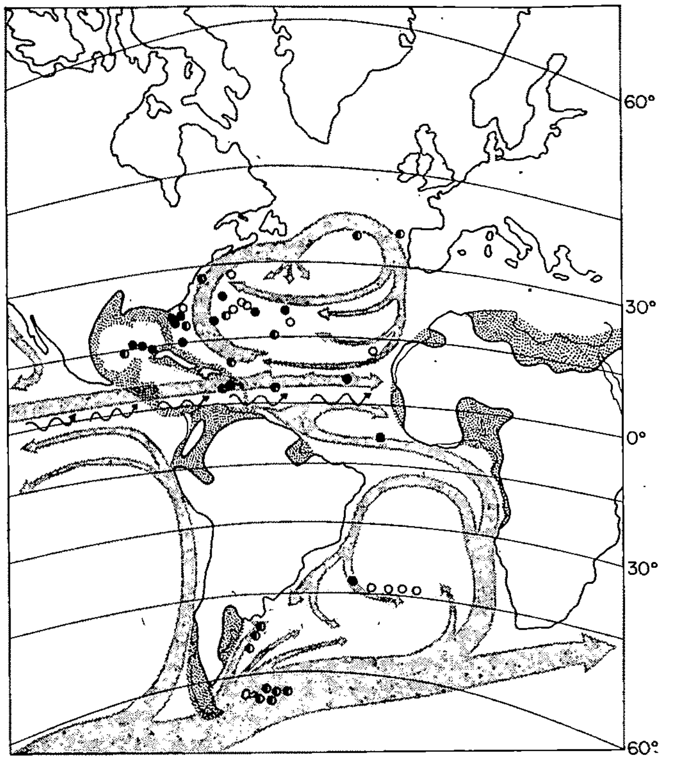


Fig. 3 Proposed circulation pattern for the Eocene Atlantic. The broad arrows represent surface currents, the sinuous arrow the Cromwell or equatorial undercurrent. The stippled regions on the continents represent shelf areas.

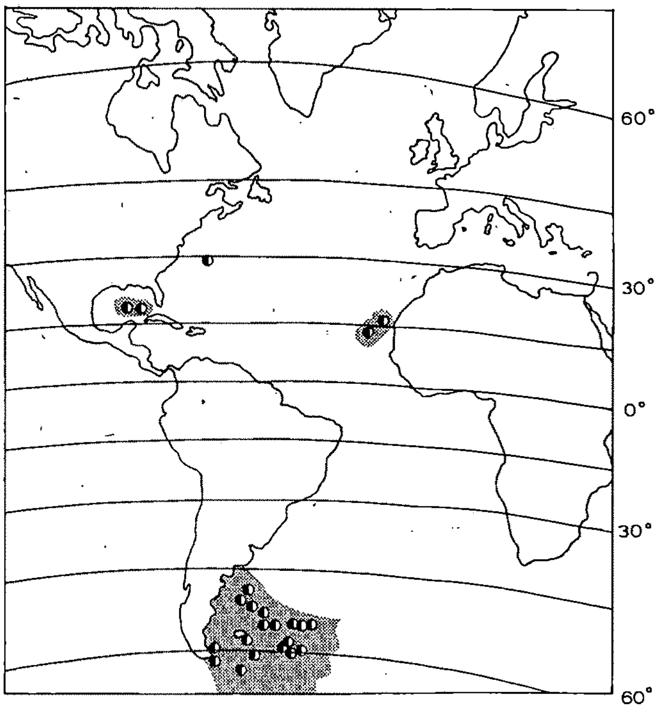


Fig. 4 The distribution of biogenic siliceous sediments in the Miocene Atlantic and Caribbean. The areas of primary production are stippled.

The equatorial zone of siliceous sedimentation had ceased to exist by the Miocene (see Fig. 4). The results of drilling and piston coring in the Atlantic and Caribbean[4,5,7,12] show that during this epoch the deposition of opaline silica occurred primarily in areas of upwelling along the western margin of the Argentine Basin and along the west coast of Africa. In the Gulf of Mexico radiolaria which may have been derived from the Pacific flourished in what is still a region of high biological productivity[22] and contributed to the formation of calcareous siliceous oozes. The distribution of biogenic siliceous sediments in the Miocene Atlantic suggests a pattern of surface and deep water circulation similar to the Recent.

This research was supported by NERC. I thank Dr Jean Francheteau for permission to publish his reconstructions of the Eocene and Miocene Atlantic, Dr John Harvey, Dr P. Liss (UEA) and Dr W. W. Hay (Illinois) for discussions, and Miss L. Bloom and Mr L. Lewis for help.

A. T. S. RAMSAY

School of Environmental Sciences,
University of East Anglia,
Norwich, and
Department of Geology,
University of Illinois,
Urbana-Champaign

Received June 25; revised August 13, 1971.

[1] Ewing, M., *et al., Initial Reports of the Deep Sea Drilling Project*, 1 (US Government Printing Office, Washington, 1970).
[2] Peterson, M. N. A., *et al., Initial Reports of the Deep Sea Drilling Project*, 2 (US Government Printing Office, Washington, 1970).
[3] Maxwell, E. A., *et al., Initial Reports of the Deep Sea Drilling Project*, 3 (US Government Printing Office, Washington, 1970).
[4] Worzel, J. L., *et al., Geotimes*, **15**, No. 6, 11 (1970).
[5] Ewing, J., *et al., Geotimes*, **15**, No. 7, 14 (1970).
[6] Ryan, B. E., *et al., Summary of Deep Sea Drilling Project, Leg 13* (1970).
[7] Hayes, D. E., *et al., Summary of Deep Sea Drilling Project, Leg 14* (1970).
[8] Bader, R. G., *et al., Geotimes*, **14**, No. 6, 14 (1969).
[9] Schlee, J., and Gerard, R., *Cruise Report and Preliminary Core Leg MV Caldrill I, 17 April to 17 May, 1965* (1965).
[10] Ramsay, A. T. S., *Reports of the Second Planktonic Conference* (in the press).
[11] Francheteau, J., in *Palaeomagnetism and Plate Tectonics* (thesis, Univ. California, 1970).
[12] Saito, T., and Funnell, B. M., in *The Sea*, **4** (Interscience, in the press).
[13] Lisitzin, A. P., in *The Micropalaeontology of Oceans*, 173 (Cambridge University Press, 1971).
[14] Riedel, W. R., in *The Micropalaeontology of Oceans*, 567 (Cambridge University Press, 1971).
[15] Riedel, W. R., and Funnell, B. M., *Quart. J. Geol. Soc. London*, **120**, 305 (1964).
[16] Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., *The Oceans, Their Physics, Chemistry and General Biology* (Prentice Hall Inc., 1963).
[17] Knauss, J. A., in *The Sea*, **2**, 235 (John Wiley, 1963).
[18] Neumann, G., in *Ocean Currents* (Elsevier, Amsterdam, 1968).
[19] Berger, W. H., *Deep Sea Res.*, **15**, 31 (1968).
[20] Berger, W. H., and Parker, F. L., *Science*, **168**, 1345 (1970).
[21] Tappan, H., and Loeblich, A. R., in *Symp. on Palynology of the Late Cretaceous and Early Tertiary, Geol. Soc. Amer. Spec. Paper*, **127**, 247 (1971).
[22] Bognadov, D. V., Sokolov, V. A., and Khromov, N. S., *Oceanology*, **8**, 371 (1970).

First Diagnostic Carboniferous Fossils from New Zealand

THE first New Zealand fossils indicative of Carboniferous age have been found in rocks from the South Canterbury region of the South Island. They are conodonts, accompanied by rare fish scales, and were obtained from the marble of the Kakahu inlier in a Tertiary area 10 miles south-west of Geraldine[1]: fossil sample locality No. S102/607, grid ref. 627815 (latitude 44° 09′ S, longitude 171° 04′ 30″ E).

The fossils were recovered from residues obtained by digestion of marble samples in acetic acid. The conodonts include some taxa which are known only from rocks of Pennsylvanian age, that is they are equivalent to Upper Carboniferous in North American terminology. The conodonts belong chiefly to the following genera, the time ranges of which as given by Hass[2] are appended: *Idiognathodus* Lower Pennsylvanian to Upper Pennsylvanian; *Cavusgnathus* Upper Mississippian to Lower Permian; *Gnathodus** Uppermost Devonian to Middle Permian; *Gondolella* Middle Pennsylvanian to Upper Triassic; *Hindeodella*? Lower Silurian to Upper Triassic; and *Ligonodina* Middle Ordovician to Middle Triassic.

The conodonts are opaque and whitish to dark grey in colour; many are distorted, no doubt as a result of the low grade regional metamorphism (chlorite 2 subzone) of the containing rocks. In spite of the distortion most specimens are generically determinable.

Specimens of *Idiognathodus* and *Cavusgnathus* are about equally common in the fauna as recovered. The remaining genera are represented by far fewer specimens; among these are specimens of *Gnathodus bassleri* (Harris and Hollingsworth) and *G.* cf. *roundyi* Gunnell. *Gondolella* is represented by a few incomplete specimens, mostly with unornamented platforms.

Ranges of represented genera indicate an upper Middle Pennsylvanian or lower Upper Pennsylvanian age for the Kakahu fauna. *Idiognathodus* seems to be absent from both the youngest and oldest Pennsylvanian faunas of North America; an abundance of *Idiognathodus*, as in the Kakahu fauna, is reported to be a characteristic of the Desmoinesian Stage (that is, upper Middle Pennsylvanian). *Gondolella* first appears in the Desmoinesian. On the other hand *Gnathodus bassleri* has been regarded by Lane[5] as characteristic of the Lower Pennsylvanian (Morrowan), but in Nevada it is reported by Webster[6] to range through the Derryan (that is, lower Middle Pennsylvanian). Further taxonomic work is necessary to resolve finally the question of the age of the Kakahu fauna within the Pennsylvanian.

Only indeterminate pelmatozoan remains and doubtful radiolaria had previously been recorded from the Kakahu rocks[7]; their age was therefore in doubt and suggested ages have included Lower Palaeozoic (Ordovician?)[8], early Palaeozoic[9] and late Permian, Lower Kazanian[10]. Discussing the upper Palaeozoic and lower Mesozoic rocks of South Canterbury and North Otago, Gair (in Waterhouse[11]) suggested that the chlorite 2 subzone rocks of the Kakahu district could be "at least of Lower Permian or Carboniferous age". Gair subsequently included[12] the Kakahu Marble in the Haast Schist Group which he noted as grading into sediments of Permian and Triassic age belonging to the Torlesse Group. Wright mentioned[13] having processed Kakahu limestone for conodonts in the course of his work on Ordovician conodonts, but his samples proved barren.

The significance of the Kakahu conodonts lies chiefly in the implications stemming from their Upper Carboniferous age. As the first diagnostic Carboniferous fossils found in New Zealand they fill a gap in the country's fossil record which has been considered somewhat anomalous[14]. In the light of the broad regional setting of the Kakahu inlier the conodonts indicate that accumulation in the New Zealand Geosyncline began at least as early as the Pennsylvanian, thus supporting the suggestion of Fleming[15], among others, regarding the time of initiation of sedimentation in this trough. Furthermore, the conditions of accumulation at least locally within or adjacent to the trough were suitable for the deposition of unusually pure limestones totalling some hundreds of feet in thickness. Conversely, the conodonts disprove the Lower Palaeozoic age of the Kakahu rocks and thus also the suggestion that rocks of this age outcrop east of the median tectonic line.

* Range extended by recent work of Scott and Collinson[3] and Bender and Stoppel[4].

Survival of determinable conodonts in the chlorite 2 subzone of the Kakahu inlier points to the possibility of their presence in rocks of similar metamorphic grade elsewhere; this observation could prove significant in establishing the age of parts of the Torlesse and Haast Schist Groups.

The Kakahu conodonts are the most southerly occurrence of these problematical fossils known to us. The nearest known Pennsylvanian conodonts are from south-eastern Queensland, Australia[16].

We thank G. Mansergh of the NZ Geological Survey for information regarding the Kakahu limestones and for supplying the sample which first yielded conodonts, and M. Reid, Geology Department, University of Canterbury, for processing it. Mr D. R. Gregg kindly assisted with relevant literature. The research was supported by a University of Canterbury Grants Committee research grant.

D. G. JENKINS

Geology Department,
University of Canterbury,
Christchurch

T. B. H. JENKINS

Geology and Geophysics Department,
University of Sydney

Received February 9, 1971.

[1] Wellman, W. H., *Bull. Geol. Surv. NZ*, 50 (1953).

[2] Hass, W. H., in *Treatise on Invertebrate Paleontology* Part W (edit. by Moore, R. C.), 3 (Geol. Soc. Amer., and Univ. Kansas Press, 1962).

[3] Scott, A. J., and Collinson, C. W., *Kansas Geol. Soc. Twenty-sixth Ann. Field Conf. Guide-bk.*, 110 (1961).

[4] Bender, H., and Stoppel, D., *Geol. Jb.*, **82**, 331 (1965).

[5] Lane, H. R., *J. Paleont.*, **41**, 920 (1967).

[6] Webster, G. D., *Univ. Calif. Publ. Geol. Sci.*, **79**, 1 (1969).

[7] Campbell, J. D., and Warren, G., *Trans. Roy. Soc. NZ* (*Geol.*), 3, 99 (1965).

[8] Wellman, H. W., *Bull. NZ Dep. Sci. Ind. Res.*, 121 (1956).

[9] Grindley, G. W., *et al.*, *Bull. Geol. Surv. NZ*, 66 (1959).

[10] Waterhouse, J. B., *Trans. Roy. Soc. NZ* (*Geol.*), **5**, 161 (1967).

[11] Waterhouse, J. B., *Bull. Geol. Surv. NZ*, 72 (1964).

[12] Gair, H. S., notes accompanying *Geological Map of New Zealand Sheet 20, Mt. Cook* (first ed., 1968).

[13] Wright, A. J., *Nature*, **218**, 664 (1968).

[14] Brown, D. A., *et al.*, *The Geological Evolution of Australia and New Zealand* (Pergamon Press, Oxford, 1968).

[15] Fleming, C. A., *Quart. J. Geol. Soc. Lond.*, **125** 125 (1970).

[16] Palmieri, V., *Publ. Geol. Surv. Qd.*, 341 (Paleont. Pap. 17) (1969).

BIOLOGICAL SCIENCES

Functional Organization of Genetic Material as a Product of Molecular Evolution

MOLECULAR evolution consists of a sequence of events in which originally rare molecular mutants (DNA changes) spread into the species. Two important classes of mutations are nucleotide (or amino-acid) replacement and gene duplication.

From comparative studies of the protein sequences of related organisms, we now have some information on the rate of amino-acid substitution in evolution. The rate is different from protein to protein, but for each particular molecule, such as haemoglobin α or cytochrome c, it is remarkably uniform per year in diverse lines over geological times[1]. This constitutes very strong evidence for the hypothesis[1-3] that most nucleotide substitutions in evolution are the result of random fixation of selectively neutral or near neutral mutants. Furthermore, the rate at which neutral mutant genes are substituted in the population per generation is equal to the rate of occurrence of such mutants per gamete[5].

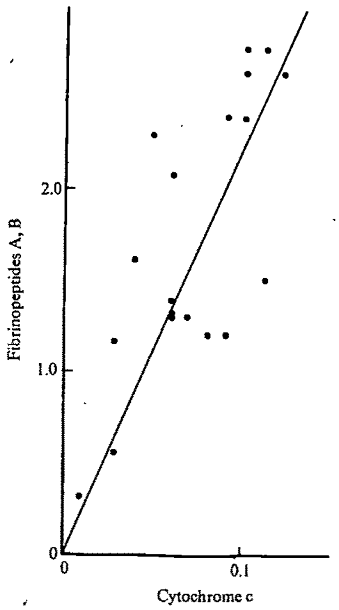


Fig. 1 The regression of the rate of amino-acid substitution of fibrinopeptides (actually their rapidly evolving parts) on that of cytochrome c. In each coordinate, the number of different amino-acid sites between two homologous proteins is expressed in terms of $-\log_e(1-p_d)$ where p_d is the fraction of differing sites. Dots represent twenty-one observed values (of which two sets of values coincide) from paired comparisons involving seven organisms (human, rhesus monkey, rabbit, dog, horse, pig and kangaroo). The estimated regression coefficient is 22.4.

There are significant differences in evolutionary rates among proteins. The highest rate (fibrinopeptides) is some 1,500 times greater than the lowest rate (histones)[4]. This means that the rate depends on the functional requirement of the molecule (refs. 1 and 3 and unpublished work of M. K. and T. O.). The greater the chance that the mutations are deleterious, the lower the evolutionary rate. The uniformity of evolutionary rate for each molecule implies that the fraction of neutral mutations among all mutations in each cistron remains constant per year.

The average rate of amino-acid substitutions estimated using nine proteins is about 1.6×10^{-9}/amino-acid site/yr[3]. If the difference of evolutionary rates among proteins is due to the difference in functional requirement rather than the difference in their mutation rate, we should expect that for any cistron the frequency of mutation at the time of occurrence is equal to that of the fibrinopeptides showing the highest substitution rate. This allows us to estimate the true mutation rate at the molecular level.

From the atlas of Dayhoff[6] we sampled seven organisms for which the complete amino-acid sequences of fibrinopeptides A, B and cytochrome c are given, from which to calculate the regression of substitution rate of the fibrinopeptides on that of cytochrome c for which the rate of substitution is well known. We therefore chose the rapidly evolving parts corresponding to the amino-acid positions four to seven in fibrinopeptide A and nine to twenty in fibrinopeptide B (alignment 9 of Dayhoff[6]). The remaining amino-acid positions not only evolve much more slowly but also show some non-randomness with respect to the pattern of substitution. We have therefore excluded these slowly evolving parts from our calculation. Fig. 1 illustrates the regression based on all the pairs of comparisons between seven organisms (human, rhesus monkey, rabbit, dog, horse, pig and kangaroo) with observed values shown by dots. The abscissa gives the number of substitutions in terms of $-\log(1-p_d)$ for cytochrome c while the ordinate gives that for fibrinopeptides, where p_d is the fraction of different amino-acids. (For the rationale of using such a logarithmic scale, see ref. 1.) The regression is about 22.4, suggesting that the true mutation rate is 22.4 times the substitution rate of cytochrome c, which is about 0.37×10^{-9}/amino-acid site/yr in mammalian evolution. The resulting mutation rate becomes

$$0.37 \times 10^{-9} \times 22.4 \approx 8.3 \times 10^{-9}$$

per amino-acid site/yr. This is roughly five times the average rate of substitutions of the nine proteins and agrees with the conclusion of Corbin and Uzzell[7], and with the conventional mutation rate per locus per generation of $10^{-5} \sim 10^{-6}$ if the average cistron codes for the protein of several hundred amino-acids long and if the average generation time is not much different from 1 yr.

If a large fraction of mutations are deleterious as discussed above, every higher organism must suffer a heavy genetic load. According to the Haldane–Muller principle[5] the mutation load of equilibrium populations is equal to one to two times the total deleterious mutation rate depending on the degree of dominance of mutations[8]. Recent investigations in *Drosophila* have shown that the detrimental mutations have a fairly high degree of dominance[9,10]. The mutation load must therefore be nearly twice the total mutation rate, although epistatic (synergistic) interaction in fitness among the loci may reduce the load somewhat[10,11].

Various species of mammals have about the same amount of DNA. The human haploid genome consists of about 3×10^9 nucleotide sites[12,13], equivalent to 10^9 amino-acid sites: thus the total mutation rate due to base replacement per genome per year amounts to

$$8.3 \times 10^{-9} \times 10^9 \approx 8$$

If the mutation rate is strictly proportional to chronological time, the rate per generation is probably twenty times this figure in man but half as large in mouse. In the main course of mammalian evolution, the average generation time was probably $2 \sim 3$ yr, so we tentatively equate 1 yr with one generation in mammals. If most of these mutations are deleterious, any mammalian species must suffer an intolerable mutation load: Muller argued[14] that the total rate for detrimental mutations in man is at most 0.5 per generation. What does such great discrepancy signify? We conclude that a large part of DNA in the genome is not essential for the life of the organisms in that base substitutions have no effects[3]. If we compare the above estimate of the total mutation rate with Muller's maximum detrimental mutation rate we are led to the conclusion that only 0.5/8 or 6% of the total DNA are as important in function as the cistrons such as those coding for cytochrome c or haemoglobins. If the estimated total mutation rate per generation is twenty times that per year and the total detrimental mutation rate is 1.0 (taking into account the "viability polygenes" of Mukai)[15], then the fraction becomes 0.6%. Although we conclude, with Muller[16], that the total number of "genes" in man is about 3×10^4, whereas he assumes a large gene size (corresponding to 30,000 amino-acids), we assume that the genes are much smaller in size (corresponding to several hundred amino-acids on average) but a large fraction of DNA is not "informational".

Mammalian DNA is about 1,000 times larger than bacterial DNA, so that the genetic material duplicated some ten times on average ($2^{10} = 1{,}024$) in the course of evolution from a unicellular organism to the mammals[17]. After each fixation of duplication in the species, irrespective of whether the duplicated part is a whole chromosome or a tiny fraction, the duplicated genes must have differentiated from the original genes through nucleotide substitution. It is possible that in this process many mutations which would have been deleterious before duplication become harmless (selectively neutral) if they occurred after duplication, for one set of genes provides the essential function of the organism. The originally deleterious mutants will spread into the population by random drift, so that there will be degeneration in many duplicated genes. The possibility of DNA degeneration after gene duplication has been pointed out by Ohno *et al.*[17] and Nei[15]. The probability of such degenerated parts acquiring new functions might be quite small. Some duplicated genes will acquire new functions, establishing themselves as important genes of the organism. But mutation is a random event, so that the chance that duplicated genes will acquire a new function must be much smaller than that they will become inert. Thus, we should expect that higher organisms have much non-informational (inert) DNA in their genome.

In this case, the rate of nucleotide substitution must be very rapid within those parts, for all the mutations are neutral. We estimate this rate approximately as $1/3 \times 8.3 \times 10^{-9}$/yr/nucleotide site, which is one-third of the estimated rate per amino-acid sites in rapidly evolving portions of the fibrinopeptides. We further multiply this by 1.2 to give total nucleotide substitution rate by taking account of the synonymous mutations[19]. The resulting rate is 0.33×10^{-8}/yr/nucleotide site. This is about five times as high as the rate estimated from the average proteins.

Recent investigations using DNA hybridization techniques indicate that the differentiation of DNA in mammalian evolution is very rapid. Laird *et al.*[20] estimated that the rate of DNA evolution is about three times as fast in artiodactyls and thirty times in rodents as the corresponding rate inferred from haemoglobins. Walker[21] estimated that the differentiation of DNA of rodents is fifteen times as fast as that of known proteins. It is possible that the evolutionary rate of total DNA is more nearly proportional to generations rather than to chronological time. More recently, Kohn[22], who has summarized these results, emphasizes that the substitution rate in DNA of mammalian genome is proportional to generations. He concludes that the number of nucleotide substitutions in non-repeated DNA is roughly five per genome per generation, and the total rate of substitutions is probably two to four times greater. It is interesting that his figure is roughly equal to our estimate, that is, eight substitutions per genome per year.

We thank Dr Kazutoshi Mayeda for reading our manuscript and correcting the English.

TOMOKO OHTA
MOTOO KIMURA

National Institute of Genetics,
Mishima,
Shizuoka-ken, 411

Received December 2, 1970; revised March 17, 1971.

[1] Kimura, M., *Proc. US Nat. Acad. Sci.*, **63**, 1181 (1969).
[2] Kimura, M., *Nature*, **217**, 624 (1968).
[3] King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).
[4] McLaughlin, P. J., and Dayhoff, M. O., in *Atlas of Protein Sequence and Structure 1969* (edit. by Dayhoff, M. O.), 39 (National Biomedical Research Foundation, Silver Spring, Maryland, 1969).
[5] Crow, J. F., and Kimura, M., *An Introduction to Population Genetics Theory* (Harper and Row, New York, 1970).
[6] Dayhoff, M. O., *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Maryland, 1969).
[7] Corbin, K. W., and Uzzell, T., *Amer. Nat.*, **104**, 37 (1970).
[8] Kimura, M., *Jap. J. Genet.*, **37** (Suppl.), 179 (1961).
[9] Crow, J. F., in *Population Biology and Evolution* (edit. by Lewontin, R.), 71 (Syracuse University Press, New York, 1968).
[10] Mukai, T., *Genetics*, **61**, 749 (1969).
[11] Kimura, M., and Maruyama, T., *Genetics*, **54**, 1337 (1966).
[12] Muller, H. J., *Bull. Amer. Math. Soc.*, **64**, 137 (1958).
[13] Vogel, F., *Nature*, **201**, 847 (1964).
[14] Muller, H. J., *Amer. J. Human Genet.*, **2**, 111 (1950).
[15] Mukai, T., *Genetics*, **50**, 1 (1964).
[16] Muller, H. J., in *Heritage from Mendel* (edit. by Brink, R. A.), 419 (University of Wisconsin Press, Madison, 1967).
[17] Nei, M., *Nature*, **221**, 40 (1969).
[18] Ohno, S., Wolf, U., and Atkin, N. B., *Hereditas*, **59**, 169 (1968).
[19] Kimura, M., *Genet. Res.*, **11**, 247 (1968).
[20] Laird, C. D., McConaughy, B. L., and McCarthy, B. J., *Nature*, **224**, 149 (1969).
[21] Walker, P. M. B., *Nature*, **219**, 228 (1968).
[22] Kohn, D. E., *Quart. Rev. Biophys.*, **3** (3), 327 (1970).

Toxicity of the Photoisomers of Cyclodiene Insecticides to Freshwater Animals

SUNLIGHT and ultraviolet light convert aldrin, dieldrin and heptachlor to their corresponding isomers, photoaldrin, photodieldrin and photoheptachlor respectively, which are more toxic to mosquito larvae and housefly adults[1-3]. In these insects the onset of toxic symptoms is much faster with the photoisomers than the parent compounds and has been assumed to be due to their rapid oxidative dehydrochlorination to lipophilic ketones[3,4]. The effect of photoisomerization of isodrin is opposite to that seen with other photoisomers. Photoisodrin, because of its rapid detoxification, is far less toxic than isodrin to these two insects[4,5].

Table 1 Toxicity of Cyclodienes and their Photoisomers to Some Freshwater Animals

Insecticide	LD$_{50}$ (p.p.m.)* Bluegills	Minnows	*Asillus*	Mosquitoes†
Aldrin	0.26	—	0.08	0.003
Photoaldrin	0.09	—	0.04	0.0005
Dieldrin	0.17	0.024	—	0.006
Photodieldrin	0.03	0.010	—	0.003
Heptachlor	—	0.013	0.10	0.005
Photoheptachlor	—	0.008	0.06	0.002
Isodrin	0.012	—	—	0.019
Photoisodrin	0.025	—	—	0.058
Endrin	0.010	—	—	0.017

* Mortalities recorded after 24 h.

† Data from refs. 1–3 for *Aedes aegypti* larvae.

The toxicity of all these photoisomers to freshwater animals which constitute food chains has not been reported. Because cyclodiene insecticides have been used extensively, the hazards of their persisting residues may be magnified by concentration of the lipophilic metabolites of their photoconversion products in food chains (Georgacakis and Khan, unpublished work). We report here the results of a preliminary investigation on the toxicity of these photoisomers to some freshwater animals.

We used animals collected from natural conditions or purchased from local suppliers. The fish were acclimated at 65° F in an environmental room for at least 5 days in mixtures of habitat water and distilled water in the following respective proportions: 2 : 1, 1 : 2, 1 : 8, and distilled water only. The distilled water was aged at this temperature for about 2 weeks with constant aeration (oxygen concentration 10 p.p.m.). Fish were exposed in 1 pint mason jars containing 200 ml. of constantly aerated water. Each jar contained no more than four fish, tadpoles or crayfish and about five replicates (twenty animals) were used for each concentration. Microcrustaceans were exposed in 25 ml. of water in plastic Petri dishes (five to ten animals per dish) with at least two replicates. Each experiment was repeated at least twice and the average values of the two used to determine LC$_{50}$ or LT$_{50}$ values[4]. Assays were performed at 65° F using the distilled water in which the fish were acclimated. Six graded concentrations (logarithmic dosages) between predetermined dosages which caused zero and 100% mortality were used to determine LC$_{50}$ values.

Table 1 shows LC$_{50}$ values of the cyclodienes and their photoisomers to fish, crustacea and insect. To the fish isodrin and endrin are the most toxic insecticides. Photoisodrin is about two times less toxic than isodrin to all these animals. Photoaldrin, photodieldrin and photoheptachlor are more toxic than their parent compounds aldrin, dieldrin and heptachlor, respectively. Photoaldrin seems to be specially toxic to mosquito larvae[1]. The onset of toxic symptoms leading to death is much faster with photoaldrin, photodieldrin and photoheptachlor than their parent compounds as judged by LT$_{50}$ values for a number of aquatic animals (Table 2). Again, photoisodrin seems to be less toxic than isodrin or endrin; tadpoles, fish, isopods and *Daphnia* are more sensitive than *Gammarus*, crayfish and plannarians. Toxicity of photodieldrin has been reported for only a few animals[6-8], where it has been found to be two to four times more toxic to rats, mice, guinea-pigs and pigeons, equally toxic to beagles and less toxic than dieldrin to the domestic fowl. To the freshwater animals tested here, however, photoaldrin, photodieldrin and photoheptachlor are more toxic than their parent compounds. Photoisodrin is less toxic than isodrin or endrin. Because the first three photoisomers can be converted to more toxic lipophilic ketones[3,4], the toxicity of these photoisomers produced by sunlight[1-3,8] or by microorganisms[9] may be magnified by the biological concentration[10] of lipophilic ketones in animal food chains. The possible hazards of such a magnification are obvious, and research on the analysis of these photoisomers and their ketones in the environment, especially in the Great Lakes region, is badly needed.

Table 2 Toxicity of Cyclodienes and their Photoisomers to Various Freshwater Animals

Animal*	LT$_{50}$: min of continuous exposure Aldrin	Photoaldrin	Dieldrin	Photodieldrin	Endrin	Photoisodrin
Crustacea						
Freshwater flea, *Daphnia pulex* (1)	290	205	280	220	180	380
Freshwater isopod, *Asillus* spp (1)	160	75	80	50	35	55
Freshwater amphipod, *Gammarus* spp (1)	2,200	1,200	2,200	180	150	350
Freshwater crayfish, *Cambarus* spp (1.8)	1,140	390	1,000	200	200	1,000
Planaria						
Freshwater flatworm *Dugensia* spp (1)	—	—	400	300	300	800
Amphibia						
Tadpoles (1)	—	—	50	30	—	—
Fish						
Guppies, *Gambia affinis* (1)	—	—	90	35	—	130
Minnows, *Pimephalus promelas* (0.7)	—	70	100	90	55	130
Bluegills, *Lepomis macrochirus*	>360†	100†	>360†	70†	360‡	440‡

* Values in parentheses show the concentration to which the animals were exposed.

† Concentration was 0.24 p.p.m.

‡ Concentration was 0.2 p.p.m.

This work was supported by grants from the National Science Foundation to Professor E. B. Hadley and from the University of Illinois at Chicago Circle Research Board. We thank Professor Hadley for facilities, and Mr W. P. Braker and Mr R. Lent for fish.

E. GEORGACAKIS
M. A. Q. KHAN

*Department of Biological Sciences,
University of Illinois, Chicago Circle,
Chicago, Illinois*

Received February 9; revised May 7, 1971.

[1] Sutherland, D. J., and Rosen, J. D., *Mosquito News*, **28**, 1955 (1968).
[2] Rosen, J. D., Sutherland, D. J., and Khan, M. A. Q., *J. Agric. Food Chem.*, **17**, 404 (1969).
[3] Khan, M. A. Q., Rosen, J. D., and Sutherland, D. J., *Science*, **164**, 318 (1969).
[4] Khan, M. A. Q., Sutherland, D. J., Rosen, J. D., and Carey, W. F., *J. Econ. Entomol.*, **63**, 470 (1970).
[5] Khan, M. A. Q., Chang, J. L., Sutherland, D. J., Rosen, J. D., and Kamal, A., *J. Econ. Entomol.*, **63**, 1807 (1970).
[6] Brown, V. K. H., Robinson, J., and Ricardson, A., *Food Cosmet. Toxicol.*, **5**, 771 (1967).
[7] Rosen, J. D., and Sutherland, D. J., *Bull. Environ. Contamin. Toxicol.*, **2**, 1 (1967).
[8] Henderson, G. I., and Crosby, D. G., *J. Agric. Food Chem.*, **15**, 888 (1967).
[9] Matsumura, F., Patil, K. C., and Boush, G. M., *Science*, **170**, 1208 (1970).
[10] Woodwell, G. M., Wurster, C. F., and Issacson, P. A., *Science*, **156**, 821 (1967).

Drug Dependence and Phenotypic Masking in *E. coli*

STREPTOMYCIN dependent (Sm^{D}) strains of *E. coli* L1 (which has an amber mutation in the ornithine transcarbamylase gene which is streptomycin suppressible) will grow when subcultured on Sm agar (500 μg/ml.) or on 3% EtOH agar, but not on paromomycin agar (Pm) or kanamycin (Km) agar, or any mixture of two of the drugs named (Table 1)[1]. Paromomycin dependent (Pm^{D}) mutants selected on Pm agar (200 μg/ml.), however, would grow on subculture to Pm or EtOH agar, EtOH+Pm, EtOH+Km or Pm+Km drug agars; but would not grow on Sm or Km agar, or on any other drug agar which contained streptomycin. These obvious phenotypic differences between Sm^{D} and Pm^{D} mutants were eliminated if the strains were first grown on EtOH agar and then subcultured to the different drug agars, when both types of dependent mutant were found to grow on EtOH or Sm or Pm agar, or on EtOH+Pm or Pm+Km agar (Table 1). Gorini *et al.*[1] considered the latter to be the true phenotype of the strains (which after growth on EtOH agar were called Drug^{D}) which had previously been masked by Sm or Pm which had so altered the ribosomes as to mask the true properties of the cells. They called the phenomenon "phenotypic masking".

We have investigated whether strains of *E. coli* other than L1 also exhibited phenotypic masking, and if so whether the same phenotypic pattern was eventually expressed by Sm^{D} and Pm^{D} mutants of different origin. Further, because ethanol and the aminoglycosides have no chemical similarity, we were particularly interested to test whether "the mutants are equally dependent either on ethanol or streptomycin or paromomycin . . ."[1].

Initial experiments were performed with *E. coli* SD, a streptomycin dependent strain, and *E. coli* BQPD, a paromomycin dependent strain selected by plating a sensitive strain (*E. coli* BQ) on 200 μg paromomycin sulphate/ml. nutrient agar. When these strains were plated on various drug agar plates, as Gorini *et al.*[1] had done, we obtained the growth pattern given in Table 1. Unlike that of the Harvard group, our streptomycin dependent strain grew on Sm+EtOH agar, as did both the streptomycin and paromomycin dependent strains after conversion to Drug^{D} strains by growth in alcohol agar. Our result for the Sm^{D} strain SD in regard to Sm+EtOH seems quite consistent with the behaviour of both our and Gorini's Pm^{D} strains which grow on Pm+EtOH agar. Again, although derived from different parent strains, after "unmasking" both SD and PD give growth on Sm+EtOH, whereas the Gorini Drug^{D} strains did not. Our Pm^{D} strain also differed in not growing on Km+EtOH.

Table 1 Phenotypic Characterization of Streptomycin and Paromomycin Dependent Strains of *E. coli*

Drug in plating medium	Drug provided for growth before plating					
	Streptomycin strain		Paromomycin strain		Ethanol Drug^{D} strain	
	*L1 Sm^{D}	SD	*L1 Pm^{D}	PD	*(L1 Sm^{D} or Pm^{D})	(SD or PD)
None	±	±	±	±	0	0
EtOH	+	+	+	+	+	+
Sm	+	+	0	0	+	+
Sm+EtOH	0	+	0	0	0	+
Sm+Pm	0	0	0	0	0	0
Sm+Km	0	0	0	0	0	0
Pm	0	0	+	+	+	+
Pm+EtOH	0	0	+	+	+	+
Pm+Km	0	0	+	+	+	+
Km	0	0	0	0	0	0
Km+EtOH	0	0	+	0	0	0

Concentrations of drugs whenever used: ethanol, 3%; Sm, 500 μg/ml.; Pm, 200 μg/ml.; Km, 100 μg/ml.

+, Growth after 24 h; 0, no growth even after 48 h; ±, some growth after 48 h, incubation at 37° C.

* The first column of each pair records the results of Gorini *et al.*[1].

When strains are transplanted directly from Sm media to Pm media or vice versa, no growth is obtained, nor can Drug^{D} strains of either derivation be grown on mixtures of Sm and Pm, which are always lethal.

To study the ability of the different drugs to support growth, we assumed that our Drug^{D} strains were of the same genotype (which seems likely, for they exhibited identical phenotype to these drugs irrespective of origin) and evaluated the viability of Drug^{D} SD and Drug^{D} PD on various drug agars. For these experiments the strains were first grown on 3% EtOH agar for 48 h, colonies were transferred to 3% EtOH nutrient broth and further incubated at 37° C for 21 h to give liquid cultures of Drug^{D} SD and Drug^{D} PD. Ten-fold dilution series were made in nutrient broth and 0.2 ml. spread/plate (duplicate) on each of the various drug agars listed in Table 2, where the results are given.

The parent cultures were grown in EtOH broth from small inocula, so it was assumed that the maximum survival would be obtained on EtOH agar and these counts were taken to represent the true viabilities of the cultures (100% survival). For these strains Sm agar is nearly as good a recovery medium as is EtOH agar (Table 2). On the other hand, Pm agar or mixtures of Pm+EtOH or Pm+Km agar and Sm+EtOH agar supported the survival and outgrowth of about 10^{-4} to 10^{-5} less cells, while all the other drug agars tried did not permit growth of the Drug^{D} strains.

It seems clear from this and similar experiments with other drug dependent strains of *Escherichia*, *Staphylococcus*, *Proteus* and *Bacillus*, to be reported, that Drug^{D} mutants do not form "a single class in which their dependence is equally satisfied by several drugs"[1]. Differences were also obvious in colony size and morphology: the typical coliform colony was obtained on Sm agar but atypical (small and "frosted") colonies were obtained on Pm and EtOH agar.

We have confirmed the finding that Sm/Pm mixtures are only lethal if both drugs are present in concentrations at, or above, the minimum concentrations required to kill the parent sensitive strain. It seems from this that the minimum inhibitory concentration of an aminoglycoside antibiotic is the external concentration of drug required to "saturate" the internal ribosome complement of the particular strain in the prevailing environmental conditions, cation concentration in particular. Assuming that "saturation" is brought about by drug molecules actually attaching to specific sites on the ribosomes[2-4], then the addition of a second aminoglycoside would lead to "double saturation" and "double kill" in the case of a sensitive strain. A strain resistant to one, but not the other, would still be killed by the drug saturating the ribosomes (assuming that resistance in this case is by non-attachment, the specific site being unavailable).

Table 2 Survival of Drug^D Dependent Mutants (after Growth in 3% Ethanol) on Transfer to Various Drug Agar Media

Drug in plating medium	Survival (%) (taking survival on EtOH agar as 100%)	
	Drug^D SD	Drug^D PD
None	0*	0*
EtOH	100	100
Sm	80	73
Sm+EtOH	0.03	0.004
Pm	0.02	0.004
Pm+EtOH	0.03	0.002
Pm+Km	0.02	0.004

No survivors were obtained on any of the following drug agars: Sm+Pm; Sm+Km; Sm+Nm; Pm+Nm; Km; Km+EtOH; Km+Nm; Nm; Nm+EtOH.

The strains SD and PD were grown in 3% ethanol broth at 37° C for 21 h before a decimal dilution series of each was made (in drug-free broth) to 10^{-6}. Aliquots (0.2 ml.) of each dilution from 10^{-1} to 10^{-6} were plated in duplicate on each type of agar or drug agar listed (total 192 plates per strain) and colonies counted after 48 h of growth at 37° C. The concentrations of drugs in the plates were: ethanol, 3%; Sm, 100 μg/ml.; Pm, 50 μg/ml.; Km, 100 μg/ml.; Nm, 50 μg/ml.

* Some very tiny colonies were visible after 48 h of incubation.

Why then should Sm/Pm mixtures kill the dependent strains which can grow (indeed depend for their growth) on either streptomycin or paromomycin? It seems likely that there are two separate sites on the ribosome for the attachment of these drugs, for if there were a single site there would be straightforward competition for attachment and whichever became attached growth would result. It is now well known that Sm attaches to the P_{10} protein of the 30S subunit[5]. We suggest that Pm also attaches to the P_{10} protein close to the Sm site so that, when both Pm and Sm are attached, the complex so formed prevents either drug from producing the message-correction required for survival of dependent strains, e.g. suppression of the amber mutation in the OTC message of the conditionally Sm dependent strain of Gorini and Kataja[6]. This hypothesis would also explain why Gorini, Rosset and Zimmermann[1] found "by transduction mediated by bacteriophage P1 . . . that the entire complex phenotype described above is transferred in one step from a Drug^D donor to a wild type recipient on selecting for resistance to any one of the drugs to which the donor is resistant or dependent".

Louis B. Quesnel
Peter York
Veronica M. Skinner

Department of Bacteriology and Virology,
University of Manchester,
Manchester M13 9PL

Received July 24, 1970; revised May 24, 1971.

[1] Gorini, L., Rosset, R., and Zimmermann, R. A., *Science*, **157**, 1314 (1967).
[2] Davies, J. E., *Proc. US Nat. Acad. Sci.*, **51**, 659 (1964).
[3] Cox, E. C., White, J. R., and Flaks, J. G., *Proc. US Nat. Acad. Sci.*, **51**, 703 (1964).
[4] Davies, J. E., Gilbert, W., and Gorini, L., *Proc. US Nat. Acad. Sci.*, **51**, 883 (1964).
[5] Ozaki, M., Mizushima, S., and Nomura, M., *Nature*, **222**, 333 (1969).
[6] Gorini, L., and Kataja, E., *Proc. US Nat. Acad. Sci.*, **51**, 487 (1964).

Cell Division after Laser Microirradiation of Mitotic Chromosomes

Mitotic chromosomes are now being studied by laser microirradiation which involves removing small areas (0.5–2 μm long) of either DNA or protein[1]. The cells are then observed in tissue culture or fixed and stained cytochemically. By these means, it has been possible to examine nucleolus production and function by selectively altering the nucleolar organizer region of chromosomes[2]. This type of investigation requires only that the cell completes mitosis and survives in interphase long enough for nucleolus formation and function to be assayed. Although much information can be gained from this type of short term experiment, there would be more scope for investigation if irradiated cells could be cultivated for long periods of time, and shown to be capable of undergoing subsequent mitoses.

Until recently it was only possible to surmise that irradiated cells could undergo additional mitoses. In previous studies of chromosome microirradiation we used primary cultures of salamander lung epithelium. These cells remain flat during mitosis permitting precise microirradiation of desired chromosome regions. By treating the cells with acridine orange solution (1–0.01 μg/ml.) for 5 min it was possible to sensitize the chromosomal DNA to intense visible laser light[3]. The cells remained viable, reforming nucleoli and nuclear membranes. We have observed many cells for as long as 3 weeks in culture, and two reached the next metaphase, although neither of them completed division. This type of investigation was particularly difficult because the time between mitosis in these cells may be several weeks. In addition, since it is a primary culture, many cells will not even go through another division.

Because of the inherent difficulties of working with primary cultures of a cell type with an erratic mitotic rate, we looked for cells with more desirous features. We wanted cells with a rapid mitotic rate, which remained flat during mitosis so that the chromosomes were continually visible, and which had relatively few large chromosomes. We found these features in an established line of cells of the rat kangaroo (*Potorous tridactylis*)[4], which have eleven chromosomes, varying from 1 to 10 μm long, a mitotic doubling time of 24 h and, like many marsupial cells, do not round up during mitosis.

We have investigated whether these cells could divide after laser-irradiation of the chromosomes. The procedures of microirradiation were similar to those described before[2,3], except that 0.001 μg/ml. of acridine orange was used instead of 0.01 μg/ml. Rat kangaroo cells seemed to respond to a slightly lower dye plus laser treatment than do the salamander cells. After two pulses of the laser with a cumulative imposed energy in the focal spot of 84 μJ, chromosomal alterations similar to those described before were produced[1-3]. The anaphase cell in Fig. 1 had been irradiated in this manner, followed by time lapse cinematography. One chromosome of each of the two groups was irradiated (the arrow indicates the lesion in one anaphase group). The additional photographs in this figure are abstracts from time lapse footage of both of the cells formed from the above anaphase cell. Twenty-six hours after irradiation, both cells underwent normal mitosis, apparently passing through G1, S, and G2, for chromosome duplication, nucleolus disappearance and spindle formation, all occurred in the usual time.

This experiment should dispel doubts that cells with microbeam irradiated chromosomes can continue to divide. It

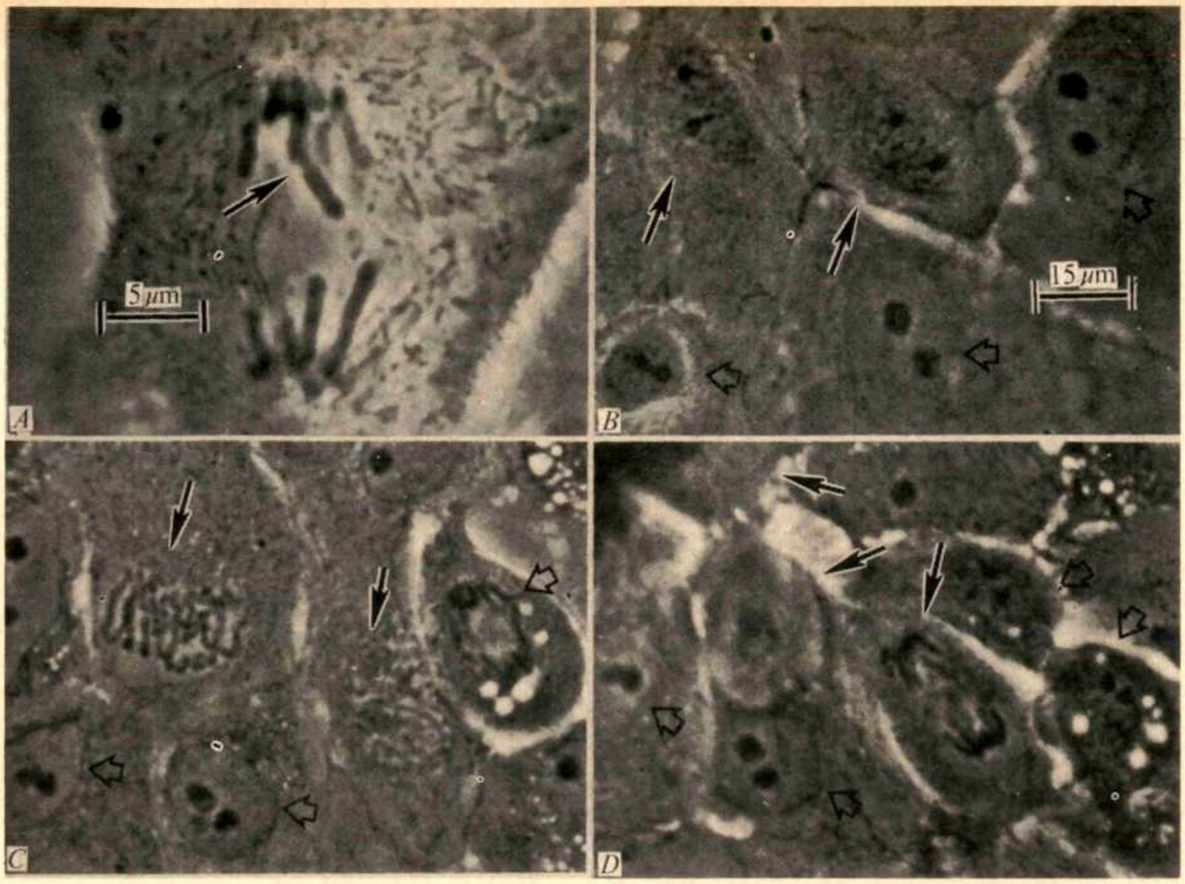


Fig. 1 Mitosis 26 h after laser microirradiation of chromosomes. *A*, Chromosome arm immediately after irradiation; the arrow indicates the altered region. *B*, 1 h after irradiation; closed arrows indicate the two daughter cells, open arrows indicate cells adjacent to irradiated cells. *C*, 25 h after irradiation; the two irradiated cells are indicated by closed arrows, one is in the middle prophase, and the other is in early prophase, one adjacent cell (open arrow) is in late anaphase. *D*, 26 h after irradiation; one of the irradiated cells has completed mitosis (closed arrows point to the two cells formed from this division), and the other irradiated cell is in late anaphase (closed arrow). Illustrations *B*, *C* and *D* are abstracts from a 16 mm time lapse film.

certainly seems feasible to establish clones from cells irradiated in this fashion. Yet the fate of the altered DNA segment of the chromosome remains to be established. It seems unlikely that the normal nucleotide sequences could be re-established in such a large segment of altered DNA.

This work was supported by the US National Science Foundation and the National Institutes of Health.

MICHAEL W. BERNS
WANNY K. CHENG
GAYLE HOOVER

Department of Zoology,
University of Michigan,
Ann Arbor, Michigan

Received April 1, 1971.

[1] Berns, M. W., Cheng, W. K., Floyd, A. D., and Ohnuki, Y., *Science*, **171**, 903 (1971).
[2] Berns, M. W., Ohnuki, Y., Rounds, D. E., and Olson, R. S., *Exp. Cell Res.*, 60 (1970).
[3] Berns, M. W., Rounds, D. E., and Olson, R. S., *Exp. Cell Res.*, **56**, 292 (1969).
[4] Hsu, T. C., Brinkley, B. R., and Arrighi, F. E., *Chromosoma*, **23**, 137 (1967).

Single-gene Heterosis in *Drosophila* revealed by Inbreeding

KIMURA and others[1-4] have suggested that most observed isoallelic variation in natural populations is maintained by mutation pressure and drift alone, and that selection is not involved. This viewpoint has been criticized[5,6], and several models[7-11] have been proposed which permit large amounts of selectively maintained polymorphism without impossibly high genetic loads. The controversy between proponents of selection and those favouring neutrality is far from resolved, and it has stimulated a great deal of work. We report here a case of conditional heterosis in *Drosophila pseudoobscura* which we feel can reasonably be attributed to the polymorphic locus being examined.

One consequence of a model proposed by Milkman[9] and elaborated by Wills, Crenshaw and Vitale[11] is that if there are many polymorphisms maintained by heterotic selection in a population, the selection coefficients associated with each of these polymorphisms will be very small. If most of the genes in the population are monomorphic, however, the selection coefficients associated with the remaining polymorphisms will be much greater. This is because the relative, rather than the absolute, heterozygosity of each organism would determine its relative fitness in the population. We reasoned that inbreeding might reveal selection coefficients associated with loci which seemed to be neutral in outbred populations.

We used a highly polymorphic population of *Drosophila pseudoobscura*, obtained in 1964 by C. W. in Strawberry Canyon, Berkeley, California, and maintained since by Dr S. Prakash. A large number of single pair matings were set up using these outbred flies, and several pure lines were obtained for each of the following electrophoretically distinguishable isoalleles: octanol dehydrogenase (ODH, autosomal, not chromosome III) 1.00 and 1.22, and esterase-5 (E-5, sex-linked) 1.00 and 1.21 (refs. 12 and 14). Both these enzymes are dimeric, and form a hybrid band in heterozygotes. The pure lines of each allelic type were pooled and maintained subsequently in mass culture to form the P_1. Single pair matings between ODH 1.00/1.00 and 1.22/1.22 gave flies all of which had the genotype 1.00/1.22. Brother–sister single pair matings were made from these cultures in turn to give the first inbred generation (I_1). Brother–sister single-pair matings were then made from the progeny of these crosses. When larvae appeared in the vials the parents were removed and their genotypes were determined (E-C vertical acrylamide gel electrophoresis). In one quarter of the vials both parents were heterozygous 1.00/1.22. These vials were retained and the remainder discarded. Flies emerging from them formed the I_2 generation, and these in turn were brother–sister mated. Cultures in which both I_2 parents were heterozygous were retained to give the I_3, and this process was continued.

Table 1 Genotypes and Numbers of Outbred and Inbred *Drosophila pseudoobscura* surviving on Stress Media

Medium	Inbreeding level			Genotype*			χ^2	P
(*a*) Flies segregating for ODH								
				1.00/1.00	1.00/1.22	1.22/1.22		
0.25 M KCl	P_1		♂♂	29 (27)	57 (54)	22 (27)	1.24	$0.75 > P > 0.5$
			♀♀	33 (27.25)	52 (54.5)	24 (27.25)	1.72	$0.5 > P > 0.25$
	I_{12}	Line A	♂♂	19 (18)	40 (36)	13 (18)	1.89	$0.5 > P > 0.25$
			♀♀	22 (24)	55 (48)	19 (24)	2.23	$0.5 > P > 0.25$
		Line B	♂♂	26 (23.5)	43 (47)	25 (23.5)	0.70	$0.75 > P > 0.5$
			♀♀	29 (26)	55 (52)	20 (26)	1.90	$0.5 > P > 0.25$
		Line C	♂♂	22 (22)	46 (44)	20 (22)	0.27	$0.9 > P > 0.75$
			♀♀	25 (22.75)	42 (45.5)	24 (22.75)	0.56	$0.95 > P > 0.9$
	I_{12}	Pooled	♂♂	67 (63.5)	129 (127)	58 (63.5)	0.70	$0.75 > P > 0.5$
	I_{12}	Pooled	♀♀	76 (72.75)	152 (145.5)	63 (72.75)	1.74	$0.5 > P > 0.25$
3×10^{-3} M 1-octanol	P_1		♂♂	30 (33.25)	68 (66.5)	35 (33.25)	0.44	$0.9 > P > 0.75$
			♀♀	39 (32.75)	63 (65.5)	29 (32.75)	1.72	$0.5 > P > 0.25$
	I_{12}	Line A	♂♂	9 (18)	44 (36)	19 (18)	6.33	< 0.05
			♀♀	20 (18)	37 (36)	15 (18)	0.75	$0.75 > P > 0.5$
	I_{12}	Line B	♂♂	16 (22.5)	58 (45)	16 (22.5)	7.51	< 0.025
			♀♀	16 (23.75)	59 (47.5)	20 (23.75)	5.91	0.05
		Line C	♂♂	13 (24)	59 (48)	24 (24)	7.56	< 0.025
			♀♀	16 (18.75)	33 (37.5)	26 (18.75)	3.75	$0.25 > P > 0.1$
	I_{12}	Pooled	♂♂	38 (64.5)	161 (129)	59 (64.5)	19.29	< 0.001
	I_{12}	Pooled	♀♀	52 (60.5)	129 (121)	61 (60.5)	1.73	$0.5 > P > 0.25$
(*b*) Flies segregating for E-5								
				Homozygotes	Heterozygotes			
0.25 M KCl	I_{12}		♀♀	143 (142)	141 (142)		0.01	$0.995 > P > 0.99$
3×10^{-3} M 1-octanol	I_{12}		♀♀	109 (120)	131 (120)		2.02	$0.25 > P > 0.1$

* Expected numbers are given in parentheses.

Inbreeding of the E-5 lines was carried out in a similar way, except that because the males were hemizygous brother–sister matings were retained in which the female was heterozygous 1.00/1.12 and the male was either 1.00 or 1.12. This process of inbreeding, then, gradually made more and more of the genome homozygous except for the chromosomal regions immediately surrounding the ODH or E-5 loci. Several separate inbred lines were carried for both ODH and E-5. While there was considerable lethality and infertility in the first generations of inbreeding, by the I_8 this had disappeared. The fertility of the inbred flies, as measured roughly by the proportion of successful single-pair matings, rose by the I_8 to the outbred level of 80–90%.

Pure lines were obtained from the I_{12} and maintained in mass culture. Matings were carried out between P_1 and also between I_{12} pure lines to give flies of the following genotypes: P_1 ODH 1.00/1.22; I_{12} ODH 1.00/1.22; P_1 E-5 1.00 ♂♂ and 1.00/1.12 ♀♀; P_1 E-5 1.12 ♂♂ and 1.00/1.22 ♀♀; I_{12} E-5 1.00 ♂♂ and 1.00/1.12 ♀♀; I_{12} ♂♂ and 1.00/1.12 ♀♀. Flies of each of these types were allowed to mate *inter se*. The progeny of the ODH crosses would be expected to segregate in a 1 : 2 : 1 ratio. The females of the E-5 crosses should segregate in a ratio of one 1.00/1.12 heterozygote to one homozygote for the gene carried by the male parent.

It has been demonstrated repeatedly that unusual or stress media emphasize genetic differences[15,16]. Consequently, parents of the genotypes listed in the previous paragraph were allowed to lay eggs on corn meal–molasses–agar medium to which either 0.25 M KCl or 3×10^{-3} M 1-octanol had been added. Egg-to-adult survival on both these media was poor.

The numbers of progeny flies of each genotype at the P_1 and I_{12} levels of inbreeding surviving on both KCl and octanol stress media are listed in Table 1. In most cases there was no significant deviation from Mendelian expectation. There was, however, a highly significant deviation in I_{12} ODH males surviving on the octanol medium. An excess of heterozygotes and deficiency of 1.00/1.00 homozygotes was observed in three different inbred lines of I_{12} ODH males, the pooled data yielding a χ^2 of 19.3, significant at the 0.001 level. A barely significant deviation similar to that seen in the males was observed in the females of one of the three lines.

Can this heterosis be attributed to the ODH locus itself? We believe it can. No deviation from Mendelian expectation was observed for the P_1 ODH flies on either stress medium. No deviation was observed for the I_{12} ODH flies on KCl medium, even though the genotype of their parents was identical to that of the parents of the flies raised on the octanol medium—and in most cases the parents themselves were identical, having been transferred from one stress medium to the other to continue egg-laying. The heterotic effect was thus confined specifically to the medium containing octanol, a substrate of ODH. That the octanol medium might be the cause of a generalized heterotic effect is rendered unlikely by the observation that I_{12} E-5 flies on either medium showed no excess of heterozygotes. A classical explanation for heterosis, that it is the consequence of masked subvital genes in the heterozygote, cannot be used here since survival of flies carrying all three ODH genotypes was normal on KCl medium.

The most likely explanation for the appearance of heterosis on inbreeding is that polymorphic modifiers of the ODH locus, perhaps other genes in the pathway of octanol metabolism, have been made homozygous by inbreeding. The effects of the ODH isoalleles themselves then become on inbreeding a much more important factor in the survival of the flies. The heterotic effect is far more pronounced in males than in females. Only one of the three I_{12} ODH lines showed a significant excess of heterozygotes in the females. This suggests that even after twelve generations of inbreeding modifiers sufficient to mask the heterotic effect are still segregating in the *X* chromosomes of two of the lines. Further inbreeding should clarify this point.

The use of specific stress media to detect selectional differences in highly inbred flies will, we hope, prove to be a general method for examining whether other polymorphisms are selectively neutral. Selection coefficients, though not heterosis, associated with the alcohol dehydrogenase locus of *D. melanogaster* have been detected by Gibson using a medium containing ethanol[13]. It is important to emphasize that the observed heterosis is conditional; that is, it is dependent on the particular environment. In Strawberry Canyon the ODH 1.22 allele is at a frequency of 0.023[14]. Though we have yet to determine if long-chain alcohols other than octanol produce effects on flies segregating for the ODH locus similar to those described here, one might speculate that large amounts of long chain alcohols are formed by fermentation in only a small proportion of the food supplies on which the larvae habitually grow. Selection for heterozygotes would thus only occur in a small fraction of the broods.

There are some similarities between the conditional heterosis at the ODH locus and that detected in sickle cell anaemia heterozygotes in man[17]. In the latter case, however, the heterotic effect is large enough to be observed in normally outbred humans, and one homozygote is a semi-lethal. Homozygotes for either ODH allele survive perfectly well when there is no octanol in the medium, so that ODH polymorphism imposes a far smaller genetic load on the population than does sickle cell anaemia—a load which might fall effectively to zero under laboratory conditions. Polymorphisms of the ODH type could, if numerous, permit the concealment of large amounts of genetic variability without large genetic loads. Homozygosity for these genes would not confer any great disadvantage on the organism but heterozygosity for them would, under some conditions, confer a slight advantage. How large this class of polymorphisms is remains to be seen.

We thank Mrs Elizabeth Kidder and Mr James Salzer for technical assistance, Dr S. Prakash for the flies and Dr Peter Carlson for valuable discussions. This work was supported by a grant from the US National Institutes of Health.

CHRISTOPHER WILLS
LOIS NICHOLS

Department of Biology,
Wesleyan University,
Middletown, Connecticut 06457

Received April 13, 1971.

[1] Kimura, M., *Nature*, **217**, 624 (1968).
[2] Kimura, M., and Ohta, T., *Nature*, **229**, 467 (1971).
[3] King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).
[4] Arnheim, N., and Taylor, C. E., *Nature*, **223**, 900 (1969).
[5] Richmond, R. C., *Nature*, **225**, 1025 (1970).
[6] Clarke, B., *Science*, **168**, 1009 (1970).
[7] Sved, J. A., Reed, T. E., and Bodmer, W. F., *Genetics*, **55**, 469 (1967).
[8] King, J. L., *Genetics*, **55**, 483 (1967).
[9] Milkman, R. D., *Genetics*, **55**, 493 (1967).
[10] Smith, J. M., *Nature*, **219**, 1114 (1968).
[11] Wills, C., Crenshaw, J., and Vitale, J., *Genetics*, **64**, 107 (1970).
[12] Hubby, J. L., and Lewontin, R. C., *Genetics*, **54**, 577 (1966).
[13] Gibson, J., *Nature*, **227**, 959 (1970).
[14] Prakash, S., Lewontin, R. C., and Hubby, J. L., *Genetics*, **61**, 841 (1969).
[15] Wills, C., *Proc. US Nat. Acad. Sci.*, **61**, 937 (1968).
[16] Wills, C., and Nichols, L., *Genetics*, **63**, 843 (1969).
[17] Allison, A. C., *Cold Spring Harbor Symp. Quant. Biol.*, **29**, 137 (1964).

Survival of Mouse Embryos after Freezing and Thawing

COMPARED with the successful storage of spermatozoa and other tissue cells at sub-zero temperatures, attempts to preserve frozen mammalian eggs have been disappointing (reviewed in refs. 1 and 2), although a few pregnancies have resulted from the transfer of previously frozen fertilized rabbit eggs[3] and unfertilized mouse eggs[4] to recipient foster mothers. I now wish to describe a technique for obtaining a high proportion of viable embryos after subjecting them to sub-zero temperatures.

Random bred female albino mice (CFLP strain, Carworth Europe) 6–8 weeks old were superovulated with gonadotrophins[5]. Eight-cell embryos were flushed from the oviducts and anterior portion of the uterine horns of mated females 68–72 h later and early blastocysts were flushed from the uterine horns a further 24–28 h later. Embryos were collected in Dulbecco's phosphate buffered salt solution[6] supplemented with 0.33 mM sodium pyruvate, 5.56 mM glucose, 3 mg/ml. bovine serum albumin and 100 IU/ml. penicillin G (potassium salt). The medium had a *p*H of approximately 7.2 and an osmolarity of 287.6 ± 0.9 mosmols; it was similar in composition to that used for storage of mouse embryos at temperatures between 0° and 10° C[7]. Embryos were washed in several changes of medium (2 ml./wash) to remove debris.

Between twelve and twenty embryos were placed in 0.25 ml. of the medium in a 5 ml. test-tube and cooled to 0° C on crushed ice. Then 0.25 ml. of medium plus 15% polyvinylpyrolidine (PVP), previously cooled to 0° C, was added and 3–5 min were allowed for the mixture to equilibrate. The final concentration of PVP (7.5%) was half that used to freeze mammalian blood cells[8]. After equilibration the test-tube was placed in a mixture of acetone and dry ice at $-79°$ C for 5 min. The rate of cooling from 0° to $-79°$ C was approximately 1° C/s. The test-tube was transferred to a vacuum flask of dry ice and left for 30 min before thawing.

To thaw, the test-tube was placed in a water bath at 37° C and 1 ml. of medium, also at 37° C, was added to reduce the concentration of PVP as the medium thawed. The rate of warming to 20° C was approximately 1.4° C/s. The contents of the test-tube were emptied into a solid embryological watch-glass and embryos that looked normal were collected and washed through several changes of medium as quickly as possible because exposure to concentrations of PVP greater than 1% for prolonged periods adversely affects further development (my unpublished observations). Embryos were then transferred to a modified Krebs–Ringer bicarbonate medium[9] and cultured by the method described by Brinster[10]. Table 1 gives the combined results of four experiments which were not significantly different. Of 186 eight-cell ova initially frozen, 139 (74.7%) apparently normal embryos were recovered on thawing and ninety-six (69.1%) of these developed into blastocysts after 48 h in culture.

The combined data for two experiments with early blastocysts are given in Table 1, for again there was no significant difference. Fifty early blastocysts were frozen; thirty-four (68.0%), which were apparently normal, recovered, thirty-one (91.2%) of which expanded fully after culturing for a further 24 h at 37° C.

Although there was no significant difference between the number of normal looking eight-cell ova and early blastocysts recovered after freezing, the subsequent development of the early blastocysts in culture was better than the eight-cell ova ($\chi^2_{(1)}=8.02$; $0.01>P>0.001$).

To test further the viability of the embryos after thawing, nineteen eight-cell ova were transferred after thawing to the ampullar region of the left oviducts (six to seven per oviduct) of three F_1 hybrid females (C57Bl × CBA) on day 1 of pseudopregnancy[11] (day 1 being the day on which the vaginal plug was found). On day 18 of pregnancy two females were pregnant; the left uterine horn of one had three normal looking albino foetuses and two resorptions from the six eight-cell ova transferred, the other female had one albino foetus and three resorptions out of seven eight-cell ova transferred. Thus nine out of nineteen (47.4%) implantations and four out of nineteen (21.1%) foetuses resulted from the transfer of eight-cell ova previously frozen and thawed.

Table 1 Development *in vitro* of Mouse Embryos previously Frozen and Held for 30 min at −79° C before Thawing at 37° C

Developmental stage	No. of experiments	No. of embryos frozen	No. of embryos recovered	No. of blastocysts after culture	Blastocysts (%)
Eight-cell	4	186	139	96*	69.1
Early blastocysts	2	50	34	31†	91.2

* Cultured for 48 h after thawing.
† Cultured for 24 h after thawing.
Experiments combined as they were not significantly different.

Thirteen expanded blastocysts, obtained after freezing and thawing the early blastocysts and culturing them for a further 24 h, were transferred to the left uterine horns of two F_1 hybrid females (C57Bl × CBA) on day 3 of pseudopregnancy (six and seven per horn respectively)[12]. Six and three albino mice respectively were born to the two females. Thus nine out of thirteen (69.2%) blastocysts previously frozen developed into normal young after transfer. Again, viability after freezing and thawing was significantly greater than that of the eight-cell ova ($\chi^2_{(1)} = 8.46$; $0.01 > P > 0.001$).

These results demonstrate that mouse embryos can be frozen and thawed successfully if a suitable protective agent is added to the medium before freezing. Protective agents such as glycerol and dimethyl sulphoxide had previously been found to be toxic to embryos above 0° C[7] and I found that 'Dextran T70' (Pharmacia) and sucrose failed to protect the embryos during freezing. 'Ficoll' (Pharmacia) protected a few embryos but so far PVP has been the most effective protective agent. The blastocysts seemed to be more resistant to freezing than the eight-cell embryos, which is inconsistent with the observation[13] that rabbit blastocysts were less resistant than earlier stages to storage at about 10° C.

With the perfection of this technique, the storage of mutant strains of mice not in current use but of potential interest could be possible. At the appropriate time the stored embryos could be transferred to foster mothers and the resulting offspring used to re-establish the mutant strain. The application of this technique to the large domestic animals could facilitate the dissemination of stock of good genetic background and possibly shorten the generation time for progeny testing. Low temperature storage combined with the sexing of embryos, when this method is developed, would have tremendous scope for the improvement of livestock in the future.

I thank Professor C. R. Austin for advice and for facilities. This work was conducted while I was recipient of a Beit Memorial research fellowship and was supported by a grant from the Medical Research Council.

D. G. WHITTINGHAM

Physiological Laboratory,
University of Cambridge

Received May 17, 1971.

[1] Austin, C. R., *The Mammalian Egg*, 116 (Thomas, Springfield, Illinois, 1961).
[2] Hafez, E. S. E., *Acta Endocrinol.*, **62**, Suppl. 140, 24 (1969).
[3] Ferdows, M., Moore, C. L., and Dracy, A. E., *J. Dairy Sci.*, **41**, 739 Abst. (1958).
[4] Sherman, J. K., and Lin, T. P., *Fertil. Steril.*, **10**, 384 (1959).
[5] Whittingham, D. G., *Nature*, **220**, 592 (1968).
[6] Dulbecco, R., and Vogt, M., *J. Exp. Med.*, **99**, 167 (1954).
[7] Whittingham, D. G., and Wales, R. G., *Austral. J. Biol. Sci.*, **22**, 1065 (1969).
[8] Mazur, P., Leibo, S. P., Farrant, J., Chu, E. H. Y., Hanna, M. G., and Smith, L. H., in *The Frozen Cell*, CIBA Foundation Symp. (edit. by Wolstenhome, G. E. W., and O'Connor, M.), 69 (Churchill, London, 1970).
[9] Whittingham, D. G., *J. Reprod. Fertil.* (in the press).
[10] Brinster, R. L., *Exp. Cell Res.*, **32**, 205 (1963).
[11] Tarkowski, A. K., *Acta Theriologica*, **2**, 251 (1959).
[12] Biggers, J. D., Moore, B. D., and Whittingham, D. G., *Nature*, **206**, 734 (1965).
[13] Chang, M. C., *Science*, **111**, 544 (1950).

Unsuccessful Attempts to Transfer Morphine Tolerance and Passive Avoidance by Brain Extracts

SEVERAL positive and negative reports have been reviewed recently[1,2] on the possibility of transferring acquired behaviour from animal to animal by injecting brain extract from a trained donor into an untrained recipient. We were interested in the possibility of transferring morphine tolerance from rats to mice, as reported by Ungar and Galvan[3]. We failed to replicate this finding, as had other investigators[4,5]. Nevertheless, we felt it worthwhile to search for the appropriate conditions and chose Ungar's simpler system of "fear transfer" as a model[6,7]. Here rats are overtrained (by means of foot shock) to avoid entering a black box; recipient mice are then tested in the same box. To enable us to follow accurately the published procedures one of us (A. G.) spent a few days in Ungar's laboratory. In the next 3 months we carried out eighteen unsuccessful experiments with 125 donor rats and 383 recipient and saline control mice. We then did a blind test in our mice using control and trained donor extracts provided by Dr Ungar. Next, we sent 100 of our mice to Houston, for testing as recipients concurrently with the local strain. Finally, we selected, from all our experiments, those mice (of both sexes) which seemed to avoid the black box more often after receiving the extracts. These animals were bred and the offspring were tested as recipients. We hoped to select for recipient capability that might be under genetic influence. The results of all these experiments were negative or equivocal.

For tolerance the procedures of Ungar and Galvan[3] were followed closely. Sprague–Dawley male rats (200–250 g) were made tolerant by three daily subcutaneous injections of morphine sulphate, starting with 10 mg/kg (in terms of the free base) and increasing to 100 mg/kg after 14 days. Rats were decapitated 12 h later, and brains were removed quickly and stored frozen on dry ice until required.

Recipients were thirty-four Swiss–Webster male mice (25–35 g), screened a week earlier as follows. First, they were tested for response on a 56° C hot plate[8]; all reacted within 30 s, after which morphine sulphate was given (35 mg/kg of free base intraperitoneally). After 30 min the mice were re-tested on the hot plate; two which reacted within 30 s were discarded. Morphine-induced running activity was also measured in special counter cages[9]. From the 4 h cumulative record, the sixteen most active runners were ranked, alternate mice being assigned to two groups. A coin was tossed to decide which group would receive physiological saline and which tolerant rat brain extracts. These solutions were administered intraperitoneally, and 24 h later mice were tested as in the screening procedure.

For passive avoidance procedures were as described by Ungar *et al.*[6,7] except as noted. The apparatus for training and testing consisted of a white 'Lucite' box (30×30×15 cm high) with transparent cover, connected by a small black passageway to a black 'Lucite' box (25×10×15 cm high) with an opaque cover and a grid floor which could be electrified (80 V, 60 Hz). The apparatus was built to Ungar's specifications, except that his second white box was omitted. Sprague–Dawley male rats were placed in the white box and shocked for 5 s when they ran into the dark box, the passageway being closed by a barrier during the shock period. A rat which did not run back into the white box after being shocked was placed there by hand. After the first shock the rat had to be forced into the black box each time. A waiting period of 10 s separated successive trials. Five trials constituted a session, and sessions were repeated daily for 6 days. After the first few trials, most rats displayed obvious signs of apprehension (defaecation, urination, attempts to jump out, crouching in a corner) when placed in the white box. Some learned to position their paws on the grid so as to avoid shock, but the experimenter overcame this by grasping the tail (protruding under the passageway barrier) and jiggling the rat back and forth. In two experiments donors were subjected to only a single training session before decapitation 5 days later.

Recipients were Swiss–Webster mice, usually males (25–30 g). The only modification in the apparatus was the introduction of a barrier containing a mouse hole (2.7 cm diameter semicircle) in the passageway. Two measures were used: latency time to enter the hole initially; and black box time, the total spent in the black box (all four paws inside the mouse hole) in a 180 s test period.

Recipient mice received a familiarization session (day 1), then a screening session (day 2). For the 211 mice subsequently given saline or brain extracts from untrained donors, the mean values and standard errors on day 2 were: latency, 14.6±0.7 s; black box time, 138.9±1.4 s. The corresponding values for 172 mice later given brain extracts from trained donors were: 12.6±0.6 and 139.2±1.5. We rarely found mice with black box times less than 90 s, so few were excluded. Injections (intraperitoneal) were given on day 3, testing sessions were conducted on days 4 and 5. The mice (usually twenty-four) were coded, ranked in order of day 2 latency, and assigned alternately to the treatments. All testing was "blind".

Donors were decapitated 1–2 h after their last training session. Brains were cut sagittally, sandwiched between pieces of dry ice within 30 s, and sometimes kept overnight at −20° C. Subsequently the temperature was kept below 5° C. The brains were homogenized in cold distilled water (6 ml./brain) at 850 r.p.m. using a 'Teflon' pestle. The homogenate was adjusted to *p*H 7.5 with NaOH and HCl, then dialysed against 20 volumes of distilled water for 24 or 48 h. In the morphine tolerance experiment the homogenate was centrifuged at 26,000*g* for 2 h and the supernatant was dialysed for 24 h. The dialysate was lyophilized, dissolved in distilled water (1 ml./brain) and stored at −20° C until use. Each recipient was given 0.5 ml. intraperitoneally. Sometimes, instead of dialysis, the homogenate was stirred on a magnetic mixer at 5° C or at room temperature, then centrifuged (39,000*g*, 30 min), and the supernatant was used directly or after lyophilizing.

The attempt to transfer tolerance to mice using tolerant rat brain extracts is summarized in Table 1. The day after receiving physiological saline or tolerant rat brain extract, all the mice reacted on the hot plate. After the test injection of morphine all displayed good analgesia, with one exception. An occasional morphine-treated mouse will react on the hot plate*, so no significance can be attached to this solitary result. There was a tendency in both groups towards enhanced morphine-induced running; the mean increase was 12% in the control group and 20% in the extract-treated group. Our testing procedure (hot-plate analgesia and running activity) differed from that used in Ungar's laboratory[3,10] (tail clip analgesia), and our standard test dose of morphine (35mg/kg) was much larger than theirs (4.5 mg/kg). We have shown[9], however, that after mice are made tolerant by repeated injections, this dose fails to produce analgesia (hot plate), and opiate-induced running is substantially reduced or absent. Thus, in this experiment, no tolerance was observed in the recipient mice.

Table 1 Testing of Recipient Mice for Tolerance to Morphine

	Reaction time on hot plate (s)		Running activity (counts in 4 h)		
	Before morphine	30 min after morphine	7 days before at screening	After morphine	Change (%)
Control					
No. 15	6	NR	1,902	890	−53
24	20	NR	1,650	1,930	+14
11	14	NR	1,268	971	−23
4	15	NR	1,175	1,689	+30
19	7	NR	3,300	3,694	+11
12	10	NR	1,969	3,629	+46
21	18	NR	2,678	4,525	+41
23	7	NR	2,170	3,244	+33
					Mean +12
Extract					
3	7	NR	1,795	1,972	+9
8	9	18	1,148	1,670	+31
6	12	NR	1,179	1,990	+41
10	9	NR	1,512	1,920	+21
17	12	NR	1,992	2,250	+12
7	10	NR	2,762	2,831	+2
13	10	NR	2,526	4,000	+37
20	7	NR	1,906	2,043	+7
					Mean +20

Control mice received physiological saline, extract mice received brain extract from tolerant rats 24 h earlier, one half brain equivalent per mouse. Morphine sulphate (35 mg/kg free base) was given intraperitoneally. NR: no reaction in 30 s.

Two experiments were carried out with mice as both donors and recipients. Donors were made tolerant to increasing doses of the morphine congener, levorphanol tartrate (20–100 mg/kg). Controls received sodium tartrate on the same schedule. In the first experiment, many of the recipients died within a few hours of intraperitoneal administration of either type of extract equivalent to one to two mouse brains. In the second experiment, in which recipients received material equivalent to half a mouse brain, the animals survived. Three hours later, all fifteen controls and all thirteen which had received tolerant extracts reacted within 30 s on the hot plate. They were then given levorphanol tartrate (10 mg/kg of free base) intraperitoneally and were tested after 30 min. Two mice in each group reacted within 30 s; the rest showed typical opiate analgesia. Repeated testing after 1 and 2 h revealed no difference between the groups in the rate of disappearance of analgesia. In agreement with Ungar and Galvan[3], therefore, we could not transfer opiate tolerance from mouse to mouse.

Preliminary to our attempts to transfer passive avoidance, we first examined the effect of foot shock on the subsequent behaviour of twenty-six untreated male mice. On day 1, half of these were individually familiarized with the apparatus for 180 s each, while the other half received shock (up to 5 s) as soon as they ran into the black box. Nearly all ran out again immediately after receiving only 1–2 s of shock. On day 2 all mice were tested again with no shock. On day 3 the unshocked animals were given saline injections and tested again on day 4. The behaviour of the unshocked mice was typical of that observed in all experiments (Table 2). On their first encounter with the apparatus there was much individual variability;

---

* In the screening procedure for this experiment, as noted earlier, two mice out of the thirty-four failed to display analgesia after a morphine injection.

mean latency was 61 s (s.d. 52; range 8–180, with one mouse not entering the black box at all). On day 2 the mean latency had decreased to 16 s and the responses were more consistent (s.d. 16, range 4–64). The same trend continued on day 4. Black box times showed similar effects, with means increasing from 88 to 146 to 157 and s.d. decreasing from 41 to 23 and 24. Thus, after the familiarization day, both latency and black-box times were predictable and reliable, with no remarkable effect from the inert injection on day 3. In contrast, the shocked mice showed a mean latency increase from 33 s to 139 s, and nine of them avoided the black box altogether. The differences between the unshocked and shocked groups were highly significant (t test, two-sample rank test, binomial sign test).

In the transfer experiments there were no evident differences among the several procedures used, so data are pooled for presentation. To minimize variability, each mouse was used as its own control. The results are expressed as a frequency distribution of differences in latencies (Fig. 1) or black-box times (Fig. 2) between day 2 and day 4 and between day 2 and day 5. The null hypothesis predicts a mean difference of zero, but in the controls there is a consistent decrease in latency and increase in black box time, which presumably reflects greater familiarity with the box. For the 211 control mice and the 172 mice receiving extracts from trained donors the frequency distributions are virtually identical.

In spite of negative results for most of the animals, in some mice one can find possible indications of a weak effect in the relevant direction. Latency results on day 4 show only two of the controls having an increase greater than 100 s, whereas seven of the extract recipients fall into this category. On day 5 the corresponding figures are eight and ten. For black box time on day 4, only two control mice but six extract-treated animals show reductions of more than 100 s. On day 5 the corresponding figures were six and twelve.

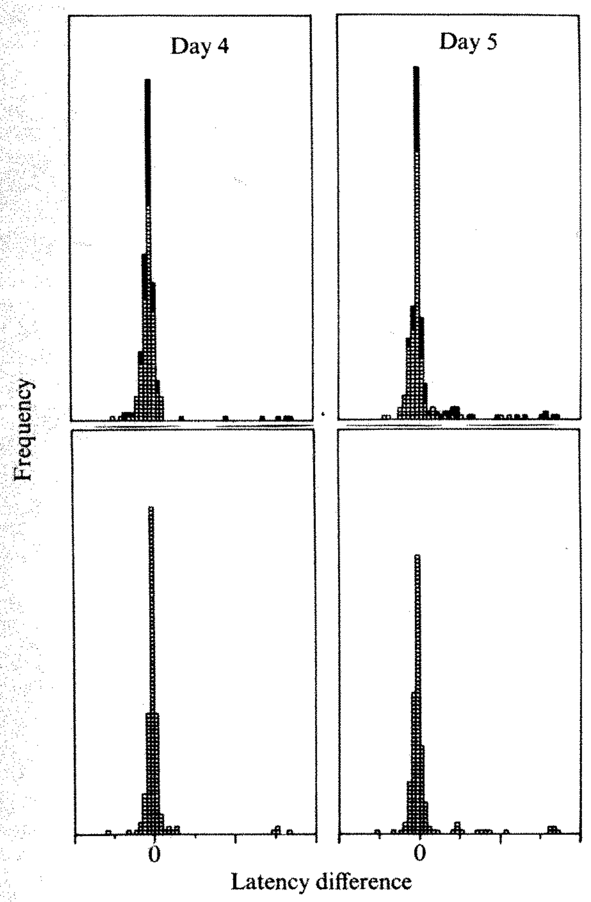


Fig. 1 Frequency distribution of latency differences in eighteen experiments. Ninety-three trained donor rats; seventy-three untrained donor rats; 146 saline injected recipient mice; sixty-five recipient mice receiving brain extract from untrained donor rats; 172 recipient mice receiving extract from trained donor rats. Each box represents one mouse. In upper graphs open boxes are saline controls, black boxes are untrained brain extract controls. In lower graphs all mice received extract from trained donors. Horizontal axis shows change in latency for each mouse from day 2 (screening) to day 4 (or day 5). Each large scale mark represents 100 s; thus the range shown is from −100 to +200 s.

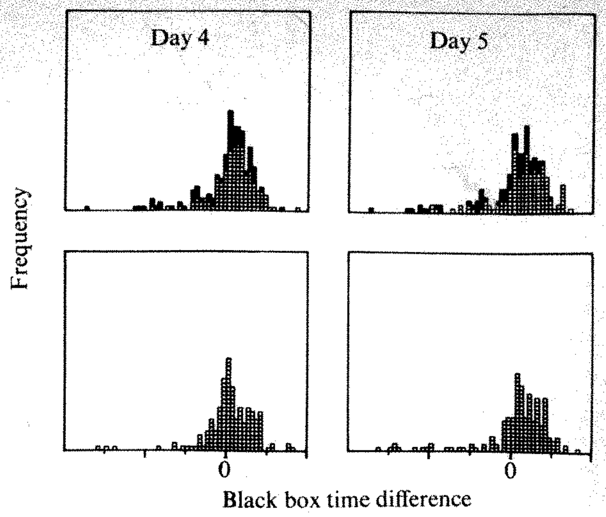


Fig. 2 Frequency distribution of black box time differences in eighteen experiments. Symbols and scales as in Fig. 1.

Table 2 Effects of Shock and No Shock on Latency and Black Box Time

	Latency (s)			Black box time (s)		
	Median	Mean	s.d.	Median	Mean	s.d.
Unshocked group (N=13)						
Day 1	53	61	52	98	88	41
Day 2	11	16	16	150	146	23
Day 3		Injection			Injection	
Day 4	8	12	11	166	157	24
Shocked group (N=13)						
Day 1 (shocked)	30	33	23	–*	–*	–*
Day 2	180	139	70	0	34	61

* No data because mice ran out immediately on being shocked.

Mice were treated as described in the text. Foot shock (80 V a.c.) was administered on day 1 as soon as a mouse entered the black box. Unshocked mice were given a saline injection intraperitoneally on day 3 and retested on day 4.

To test the hypothesis that a few mice were sensitive to the effect of the extract, we examined the extremes of the frequency distributions. In each experiment we noted which three mice had the longest latencies, and which three had the shortest black box times. The results were: latency day 4, twenty-six extract and twenty-four control; latency day 5, twenty-eight and twenty-three; black box time day 4, twenty-one and twenty-eight; black box time day 5, twenty-seven and twenty-two†. There is a very small non-significant trend in the predicted direction.

Dr Ungar kindly supplied us with some of his extract from trained and untrained donors to test in our laboratory. For this experiment fifty-two mice were familiarized, screened, ranked and assigned to treatments as described earlier. One

† The totals sometimes exceed 3×16=48 because if the third-ranking mouse was one of a tied pair, both members of the pair were included.

of us injected the animals, another did all the testing "blind". At the screening session, the mean latencies were: group A = 12 s, group B = 12 s; mean black box times: group A = 145 s, group B = 142 s. Injection dose was 1 g rat brain equivalent/mouse, on Dr Ungar's recommendation (Table 3). In both groups the medians and means indicate a skewing of the frequency distributions towards longer latencies and shorter black box times. The only suggestion of a difference between the groups was at 24 h, where there was a slight (but not significant) preponderance of longer latencies and shorter black box times in group B.

Table 3 Effect of Extract from Untrained and Trained Donors

Test time after injection (h)		Latency change (s)			Black box time change (s)		
		Median	Mean	s.d.	Median	Mean	s.d.
6	*A*	−4	−1	7	16	5	42
	B	0	5	19	4	1	27
24	*A*	0	13	39	3	−1	41
	B	2	30	55	10	−10	57
48	*A*	4	27	59	0	−13	62
	B	3	34	67	12	−11	70
			Extreme latency change (> 50 s)			Extreme change in black box time (> 50s)	
6	*A*		0			2	
	B		2			1	
24	*A*		1			1	
	B		5			4	
48	*A*		3			3	
	B		4			4	

Extracts were supplied by Dr Ungar and tested as described in text. Data in the upper part of the table are changes in latency and black box time (result at time of testing less that at screening session), computed for each individual mouse. Medians, means, and standard deviations are for the set of individual mouse differences. Group A received extracts from untrained donors, group B from trained donors. There were twenty in each group. Data in lower part of table are numbers of mice displaying extreme behaviour in the predicted direction (increased latency, decreased black box time).

When we did not obtain positive results with Ungar's extracts in our laboratory, we concluded that our mouse strain rather than our technique of preparing extracts might be at fault. With Dr Ungar's cooperation, we shipped 100 males of our strain (Bioscience Labs, Inc.) to Houston, where they were tested at various doses of extract along with his Texas inbred strain. Because we were unable to agree with Dr Ungar on the interpretation of the results they are not included here but will presumably be published independently by him.

Finally, although most mice were not good recipients, what few weak effects had been observed might be attributable to a small fraction of the mice having recipient capacity. A transfer factor might be destroyed enzymatically in most recipients, but not in genetic variants. Accordingly, we saved those males and females which showed the greatest prolongations of latency or reductions in black box time in all sixteen experiments. These were bred, and eventually the F1 generation was tested, by randomly assigning littermates to the comparison groups. At screening, results were: mean latencies 12 (s.d. 5) and 11 (s.d. 5), black box times 134 (s.d. 24) and 132 (s.d. 27), for the groups that were to receive extract from untrained and trained donors, respectively. There were no significant differences between the two extracts (Table 4).

We should not dismiss the possibility that acquired behaviour, such as conditioned dark avoidance, can be transferred by brain extracts merely because the proposed mechanisms[11,12] seem fanciful, especially since confirmatory reports have been published from several laboratories[13-15]. On the other hand, our rather exhaustive but unsuccessful attempts to replicate the findings suggest, at the least, that the precise conditions for successful transfer will have to be worked out and defined much more carefully than heretofore if this phenomenon is to meet the test of reproducibility. As matters stand now, it would be more useful for the proponents to establish rigorously and in great detail the conditions for reliably transferring one kind of behaviour in a single species, than to publish marginally successful experiments using different paradigms in a variety of animals.

Table 4 Testing of F_1 Offspring of Best Recipients

Test time after injection (h)		Latency change (s)			Black box time change (s)		
		Median	Mean	s.d.	Median	Mean	s.d.
24	Untrained extract	0	0	7	−2	−10	43
	Trained extract	−2	−1	6	16	11	27
48	Untrained extract	−2	−1	8	21	18	18
	Trained extract	−2	−2	6	10	5	37

Offspring of best recipients from sixteen experiments. Five litters were screened, then littermates assigned randomly to the two groups. $N = 14$ for each group. Extracts from untrained and trained donors were prepared as described, and injected.

We thank Dr George Ungar for hospitality and cooperation, and Barbara A. Judson, Valerie Cunningham, Dennis Uyemura and Ellen Fitzsimmons for technical assistance. This investigation was supported by a grant from the US National Institute of Mental Health.

AVRAM GOLDSTEIN
PATRICIA SHEEHAN
JOSHUA GOLDSTEIN

Department of Pharmacology,
Stanford University School of Medicine,
Stanford, California 94305

Received September 7, 1970; revised March 19, 1971.

[1] Ungar, G., in *Handbook of Neurochemistry*, 3 (edit. by Lajtha, A.) (Plenum Press, New York, 1970).
[2] Ungar, G., in *Methods in Pharmacology* (edit. by Schwartz, A.) (Appleton-Century-Croft, New York, 1970).
[3] Ungar, G., and Galvan, L., *Proc. Soc. Exp. Biol. Med.*, **130**, 287 (1969).
[4] Tirri, R., *Experientia*, **23**, 278 (1967).
[5] Smits, S. E., and Takemori, A. E., *Proc. Soc. Exp. Biol. Med*,. **127**, 1167 (1968).
[6] Ungar, G., Galvan, L., and Clark, R. H., *Nature*, **217**, 1259 (1968).
[7] Ungar, G., *Fed. Proc.*, **28**, 647 (1969).
[8] Eddy, N. B., and Leimbach, D., *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953).
[9] Goldstein, A., and Sheehan, P., *J. Pharmacol. Exp. Ther.*, **169**, 175 (1969).
[10] Ungar, G., and Cohen, M., *Intern. J. Neuropharmacol.*, **5**, 183 (1966).
[11] Ungar, G., *Perspect. Biol. Med.*, **11**, 217 (1968).
[12] Ungar, G., Chapter 5 in *Molecular Mechanisms in Memory and Learning* (Plenum Press, New York, 1970).
[13] Gay, R., and Raphelson, A., *Psychon. Sci.*, **8**, 369 (1967).
[14] Wolthuis, O. L., *Arch. Intern. Pharmacodyn.*, **182**, 439 (1969).
[15] Goeub, A. M., Epstein, L., and McConnell, J. V., *J. Biol. Psychol.*, **11**, 44 (1969).

Formation of Cerebrospinal Fluid in Spinal Subarachnoid Space

IT has been generally accepted that the fluid bathing the central nervous system is produced within the cerebral ventricular system, although some people have favoured extraventricular formation[1-3] as well. Some considered that another site of formation was the spinal subarachnoid space[4,5] (Fig. 1), but others disagreed[6,7].

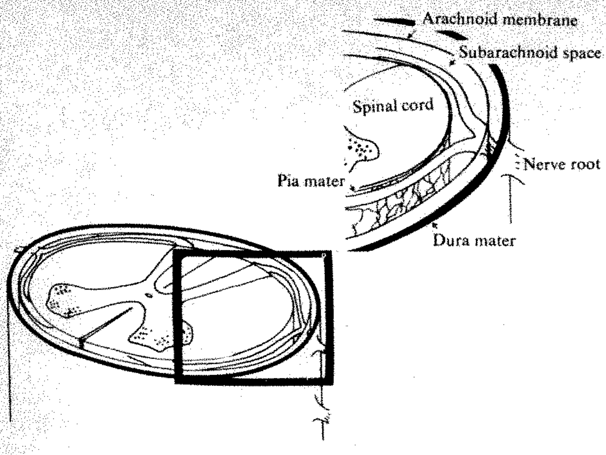


Fig. 1 Section through spinal cord showing position of subarachnoid space.

We have now investigated this problem, performing the inulin perfusion through the spinal subarachnoid space of mongrel dogs (12–15 kg). For anaesthesia 30 mg of pentobarbital/kg body weight was given intraperitoneally and each dog was endotracheally intubated and connected to a respirator after receiving 20 mg of succinylcholine intramuscularly to keep it still.

The dog was fixed in a prone position with the head slightly elevated. Laminectomies were performed over the cauda equina and upper cervical region. The lumbosacral subarachnoid space was punctured with an Argyle 'Medicut' cannula. which was connected to an inflow tubing through which passed an artificial CSF containing 25 mg% of inulin at a rate of about 0.3 ml./min. An Argyle cannula was also inserted into the upper cervical subarachnoid space and connected with outflow tubing which could be raised or lowered to change the CSF pressure during the experiment. No CSF was allowed to leak in and around the puncture sites.

Outflow samples were obtained every 10 min, after a steady state had been reached, at different pressures. These were analysed by the resorcinol recovery method for inulin concentration determination.

Every experiment was followed by autopsy to see whether the perfusion fluid was limited in the spinal subarachnoid space and whether it was bathing the entire system. This was confirmed by perfusing methylene blue dye in the same condition immediately after the final sample had been collected. We found no mixing of subarachnoid fluid between intracranial and spinal subarachnoid spaces.

CSF formation rate was calculated by the method of Heisey *et al.*[1], combining the effects of hydrostatic pressure on outflow–inflow differences and on clearance of inulin, which measured only bulk absorption. The notation to be used in the following description is that of Heisey *et al.* The use of clearance of inulin as a measure of absorption was based on the assumption that the diffusive absorption of inulin was negligible. Therefore the relation between inulin clearance and bulk absorption can be written

$$\dot{V}_a = C_{in} \qquad (1)$$

where $\dot{V}_a$ is the rate of bulk absorption in ml./min; C_{in} is the steady state clearance in ml./min of inulin out of the perfusion system per unit concentration; $C_{in} = \dot{V}_i C_i - \dot{V}_o C_o / \bar{C}$; $\bar{C}$ is mean concentration in the system $= C_o + 0.37\ (C_i - C_o)$; $\dot{V}_i$ and $\dot{V}_o$ are the influx and efflux flow rates in ml./min, and C_i and C_o are the concentrations of inulin in the influx and efflux respectively.

When the outflow volume was equal to the inflow, the rate of absorption was equal to the CSF production rate ($\dot{V}_f$). Therefore, the relation between outflow–inflow difference, formation and absorption of CSF can be expressed as

$$\dot{V}_o - \dot{V}_i = \dot{V}_f - \dot{V}_a \qquad (2)$$

Substituting $\dot{V}_o - \dot{V}_i$ for $O - I$ and $\dot{V}_a$ for C_{in}, from equation (1), the formation rate can be calculated by the following equation.

$$\dot{V}_f = (O - I) + \dot{V}_a \qquad (3)$$

The outflow rates are therefore less than inflow rates, and more fluid is absorbed than is produced. The outflow rate increased as the outlet was lowered and decreased when it was raised. In spinal subarachnoid perfusion, expressions for $O - I$, $\dot{V}_a$ and $\dot{V}_f$ are:

$$O - I = 0.0206\ (\pm 0.00165) - 0.348 \times 10^{-3}\ \text{CSFP ml./min}$$

$$\dot{V}_a \ = 0.0005\ (\pm 0.00005) + 0.320 \times 10^{-3}\ \text{CSFP ml./min}$$

and

$$\dot{V}_f \ = 0.0201\ (\pm 0.00244) - 0.288 \times 10^{-4}\ \text{CSFP ml./min} \quad (n = 41)$$

where CSFP stands for cerebrospinal fluid pressure when zero pressure was taken at the level of the spinal cord during the perfusion. From these equations the production rate is 0.018 (±0.002) ml./min when outflow is equal to inflow rates at the pressure of 59 mm of water.

Although the results give no data as to the actual site or mechanism of the formation of fluid in the space, these experiments have conclusively demonstrated that CSF is being formed in the spinal subarachnoid space and that it is important for this extraventricular production of fluid to be recognized, particularly when one considers the problem of the CSF circulation.

Osamu Sato
Takahiko Asai
Yoshiyuki Amano
Makoto Hara
Ryuichi Tsugane
Michiyuki Yagi

Department of Neurosurgery,
Nagoya University,
School of Medicine,
65, Tsurumai-cho, Showa, Nagoya

Received January 11; revised April 7, 1971.

[1] Heisey, S. R., Held, D., and Pappenheimer, J. R., *Amer. J. Physiol.*, **203**, 775 (1962).
[2] Rall, D. P., Oppelt, W. W., and Patlack, C. S., *Life Sci.*, **2**, 43 (1962).
[3] Sato, O., and Bering, E. A., *Brain and Nerve*, **19**, 31 (1967).
[4] Boldrey, E. B., Low-Beer, B. V. A., Stern, W. E., and Adams, J., *Bull. Los Angeles Neurol. Soc.*, **16**, 225 (1951).
[5] Eichler, O., Linder, F., and Schmeister, K., *Nachgewiesen mit Radionatrium Klimsche Wochenschrift*, **29**, 9 (1951).
[6] Coben, L. A., and Smith, K. R., *Exp. Neurol.*, **23**, 76 (1969).
[7] Hammerstad, J. P., Lorenzo, A. V., and Cutler, P. W. P., *Amer. J. Physiol.*, **216**, 353 (1969).

Reflecting Spheres in the Eyes of Weakfishes (Sciaenidae)

A REFLECTING layer or tapetum lucidum occurs in the eyes of many fishes; in teleosts, it lies in the pigment epithelium[1]. The reflecting material is usually considered to be guanine, but in weakfishes or seatrouts (Sciaenidae) it is a lipid[2,3]. We have been able to characterize the lipid material.

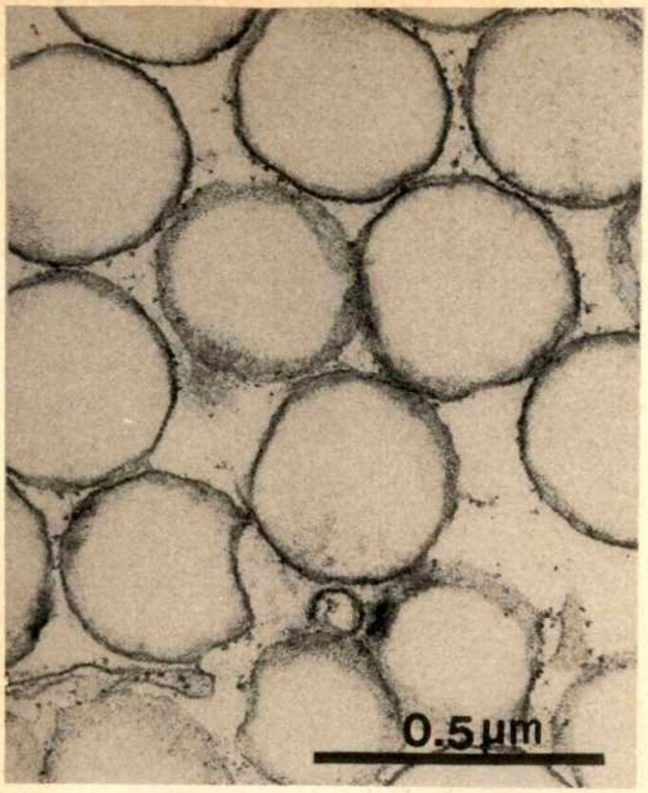


Fig. 1 Lipid spheres in a pigment epithelial cell of the sandtrout (*C. arenarius*) fixed in 3% glutaraldehyde in a 0.1% phosphate buffer, postfixed in 2% osmium tetroxide in the same buffer. This fixation technique does not preserve all the contained lipid, but does define the size and shape of the spheres. The boundary layer is associated with some of the contained lipid and appears to be thicker than an ordinary cytomembrane. Other fixation techniques retain the lipids[2].

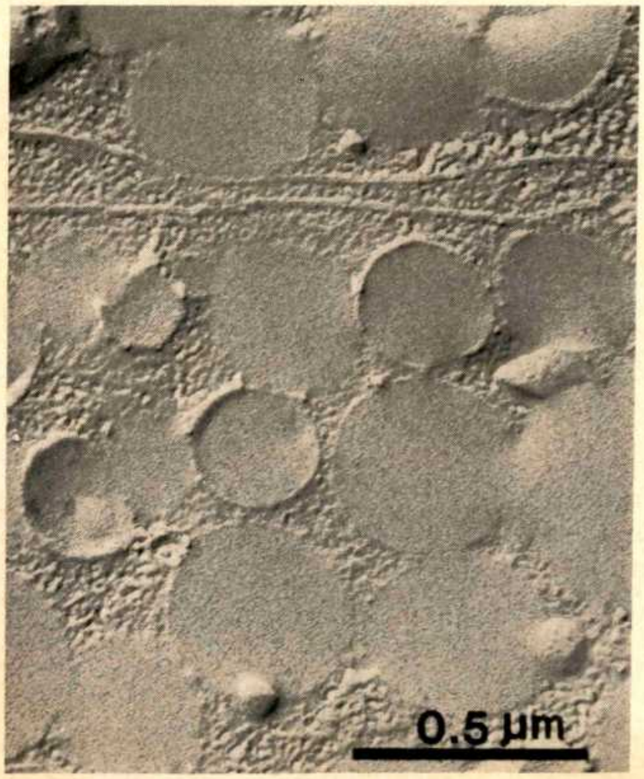


Fig. 2 Lipid spheres from the pigment epithelial cells of the sandtrout (*C. arenarius*) as they appear in freeze-etch preparations. Note the plasma membranes of two pigment epithelial cells and compare these cytomembranes with the surface of the lipid spheres.

The pigment epithelial cells of seatrouts (*Cynoscion arenarius* and *C. nebulosus*) are densely packed with small spheres, about 0.4 µm in diameter (Fig. 1), which are present throughout the length of the cell, but most abundant distal (that is, vitread) to the nucleus. There are about fifteen spheres per µm^3. The cytoplasm in the terminal process of the pigment epithelial cells contains no other visible structures, apart from small vesicles and occasional membranes, when the eye is dark-adapted and the retinal pigment withdrawn. Each sphere is surrounded by a thin membrane, visible as a fine dark line in electron micrographs when part or all of the lipid content of the sphere has been dissolved away. When the material was examined by the freeze-etch technique, the membrane could often be seen as a separate structure, cleaving differently from the internal component. The freeze-etch electron micrographs (Fig. 2) also provided confirmatory evidence of the shape, dimensions, and distribution of the tapetal spheres. The membrane is not a "unit membrane", but is similar to those associated with some plant lipid bodies[4-6], and in lymph chylomicrons[7]. The membranes surrounding the lipid spheres (bodies) in the corn root were termed "boundary membranes" by Trelease[6] who was able to observe them both *in situ* and after isolation. The tapetum has a fairly high refractive index, judged to be close to n_D^{23} 1.50, as determined by the immersion method using Thoulet's solution.

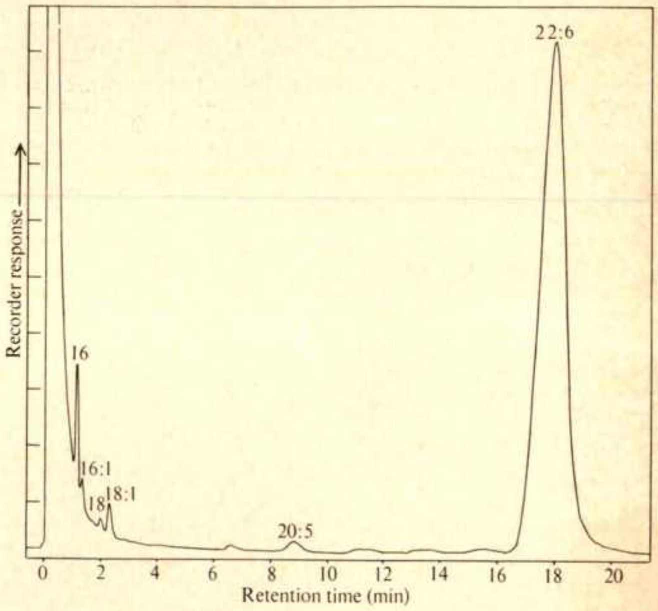


Fig. 3 Gas-liquid chromatograph of a transesterified sample of lipid from the tapetum lucidum of the sandtrout. Operating conditions were: Perkin-Elmer gas chromatograph, model 880; the analysis was carried out on a column of copper tubing, 6 feet × 1/8 inch, packed with 'DEGS' 10% on 'Gas-Chrom Z' (mesh size 60/80); carrier gas was helium, flow rate was 45 ml./min; the column was operated isothermally at 180° C.

To characterize the reflecting material, the pigment epithelium was removed from the eyes of dark-adapted fish; the material contained the entire pigment epithelial cell, including the white reflecting material, melanin, and other cellular organelles. It was cytolysed in distilled water and lightly centrifuged, and the supernatant contained the white reflecting material which was then extracted in chloroform and examined by thin-layer chromatography. It consisted almost exclusively of one major component running with triglycerides. Extracts of the entire pigment epithelium and whole retina contained the same principal component running with the triglycerides. Isolated retina did not contain a large triglyceride component. To secure larger amounts of material, therefore, whole retinas

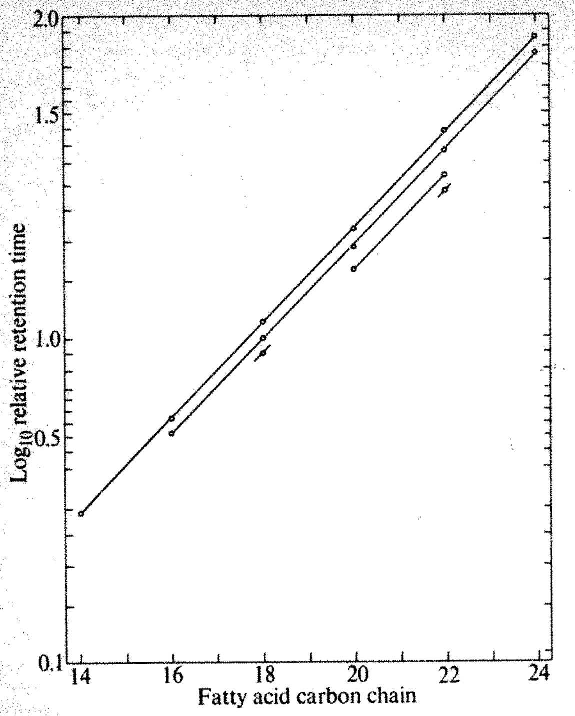


Fig. 4 Plot of $\log_{10}$ retention times against carbon number of fatty acid methyl esters and of the main component from the tapetum lucidum of the sandtrout. Retention times relative to methyl octadecaenoate. Lines from above downwards, saturates, monoenoic, dienoic (n-C18 : 2) only, pentaenoic, hexaenoic (authentic n-C22 : 6 and sample of tapetum). Data from a gas-liquid chromatograph record of a standard mix co-injected with a sample of unknown. Operating conditions: column of copper tubing, 5 feet × 1/8 inch packed with 10% 'Apiezon-L' on 'Gas-Chrom Z', carrier gas helium, flow rate 45 ml./min, isothermal run at 225° C.

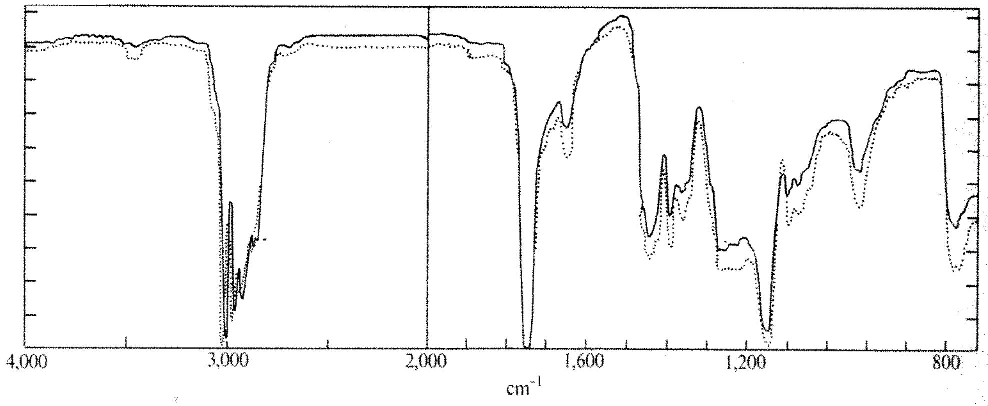


Fig. 5 IR spectra of lipid from the tapetum lucidum of the sandtrout (continuous line) and of authentic tridocosahexaenoin (Hormel Institute, estimated purity >90%, interrupted line). Spectra obtained on a Perkin-Elmer grating infrared spectrophotometer, model No. 237B. Solvent, carbon tetrachloride in sodium chloride cells. Fast scan.

including the pigment epithelium were collected (fifty eyes). They were extracted in chloroform–methanol by the method of Folch *et al.*[8], and the major component was isolated by column and preparative TL chromatography[9]. The eluant, examined by thin-layer chromatography in three different solvent systems, resembled triglycerides in chromatographic behaviour, and was well demarcated from phospholipids, sphingomyelin, cerebrosides, mono- and dipalmitins, cholesterol, cholesteryl esters, waxes, and diacyl glyceryl ethers. It was hydrolysed, esterified or transesterified and the presumptive methyl esters examined by gas-liquid chromatography on four different columns, ranging from non-polar to very polar (FFAP on 'Gas-Chrom Q', 'Apiezon-L' on 'Gas-Chrom Z', 'DEGS' on 'Gas-Chrom Z', and 'EGSS-X' on 'Gas-Chrom P'). The sample showed some minor peaks corresponding to various methyl esters of fatty acids from C14 to C22 : 5, and one major peak corresponding to n-C22 : 6 (Fig. 3). The latter comprised 95% or more of the fatty acids present. Confirmation was offered by a plot of log retention times against carbon number (Fig. 4). Further evidence for the chain length was provided by hydrogenation after which more than 95% of the fatty acid was converted to C22 : 0.

The evidence indicated that the reflecting material was a triglyceride containing docosahexaenoic acid. The original purified extract ran at the same rate on thin-layer chromatography as tridocosahexaenoin, and infrared spectra of sample and of authentic tridocosahexaenoin were closely similar (Fig. 5). It was concluded that the reflecting material consisted largely or almost exclusively of tridocosahexaenoin.

The occurrence of a simple unmixed triglyceride in a tissue is unusual; in depot fats of invertebrates and fishes, triglycerides are usually asymmetric, and n-C22 : 6 occurs preferentially in position 2 (proportionality constant $y_2 = 2.06\chi$, where χ = mol% 22 : 6 in the total triglycerides[10]).

The refractive index measurement of tridocosahexaenoin is not available, but that of fatty acid n-C22 : 6 is 1.49, and that of tridocosahexaenoin may be close to it.

The extract is transparent and very refractile; the diameter of the lipid spheres is about one wavelength (of visible light) and they act as Mie scatterers. Measurements and calculations (to be presented elsewhere) show that the tapetum is an efficient diffuse reflector. The system allows the fish to use light more efficiently in dim illumination, when rod vision or twilight vision is operative. Seatrouts along the coast of the Gulf of Mexico live in shallow waters containing a great deal of suspended material, and often encounter low levels of illumination during daylight hours. Light which passes through the photoreceptor layer of the retina without being absorbed is reflected back into the photoreceptor zone by the lipid spheres in the pigment epithelial cells. This second passage through the photoreceptors allows more light to be absorbed and seems to be effective in promoting vision in dim light conditions.

This work was supported by a US Public Health Service grant and a National Institutes of Health and Biomedical Services support grant. We thank Miss N. J. Maciolek, Dr Julia R. Sever and Mr J. K. Winters for assistance, Dr Henry Aldrich (University of Florida) for the use of his freeze-etch equipment, and Dr Guy Thompson for suggestions about the manuscript.

H. J. ARNOTT

Department of Botany and
Cell Research Institute,
University of Texas at Austin

J. A. C. NICOL

University of Texas Marine Science Institute,
Port Aransas,
Texas

C. W. QUERFELD

High Altitude Laboratory of the National Center
for Atmospheric Research,
Boulder, Colorado

Received February 3; revised February 16, 1971.

[1] Walls, G. L., *The Vertebrate Eye and its Adaptive Radiation* (Hafner, New York and London, 1967).
[2] Arnott, H. J., Maciolek, N. J., and Nicol, J. A. C., *Science*, **169**, 478 (1970).
[3] Arnott, H. J., Maciolek, N. J., and Nicol, J. A. C., *J. Cell Biol.*, **47**, 9a (1970).
[4] Jacks, T. J., Yatsu, L. Y., and Altschul, A. M., *Plant Physiol.*, **42**, 585 (1967).
[5] Ory, R. L., Yatsu, L. Y., and Kircker, H. W., *Arch. Biochem. Biophys.*, **123**, 255 (1968).
[6] Trelease, R. N., thesis, Univ. Texas (1969).
[7] Zilversmit, D. B., in *Structural and Functional Aspects of Lipoproteins in Living Systems* (edit. by Tria, E., and Scanu, A. M.) (Academic Press, New York and London, 1969).
[8] Folch, J., Ascoli, J., Lees, M., Meath, J. A., and LeBaron, F. N., *J. Biol. Chem.*, **191**, 833 (1951).
[9] Stahl, E., *Thin-layer Chromatography: a Laboratory Handbook* (Academic Press, New York and London, 1965).
[10] Litchfield, C., *Lipids*, **3**, 417 (1968).

Mycorrhiza and Litter Decomposition

FORMATION of the mor type of forest litter, characterized by slow decomposition, has been attributed to a number of factors[1-3], but the effect of mycorrhizal fungi, though postulated[4,5], has not been studied experimentally. We have conducted an experiment in which root activity and thus mycorrhizal activity were reduced in small areas of an unthinned *Pinus radiata* stand. The following treatments (three replicates in a randomized block design) were applied to 1 × 1 m plots sited between the trees.

(*A*) The plot boundary was cut to a depth of 30 cm at commencement and recut every 2 weeks. All litter was removed to a sheet of hessian with minimum disturbance. As many roots as possible were removed from the soil to a depth of 30 cm and the litter was carefully replaced.

(*B*) The plot boundary was cut as above and the litter was removed. The soil was dug over to a depth of 30 cm but no roots were removed. The litter was replaced.

(*C*) The plot boundary was cut as above. The plot was not dug, nor were any roots removed. The litter was removed and replaced.

(*D*) The control plot was neither dug nor cut, but the litter was removed and replaced.

Table 1 Dry Weight of Litter from Sample Plots after 12 Months

Treatments			
A	*B*	*C*	*D*
474.8 ± 211.5	351.6 ± 133.7	659.0 ± 328.4	1,681.7 ± 213.9

The figures represent the means (in grams) of three replicates ± the standard deviation.

After 12 months the weight of litter per plot which had received treatment *D* was significantly ($P<0.001$) greater than in those given treatments *A*, *B* and *C*. Experimental design ruled out the effect of soil disturbance or decomposition of severed roots. It seems clear that the presence of living roots suppressed litter decomposition in some way. *Pinus* is a highly mycotrophic genus[6], and for practical purposes *P. radiata* roots in the field may be considered as mycorrhizas. The observed effect could be a result of some physiological activity of the tree roots functioning as a separate entity. On the other hand, it was observed that basidiomycetous mycelial wefts, normally very conspicuous in the litter, were completely absent from plots which had received treatments *A*, *B* and *C* although they were obvious in plots given treatment *D*. We suggest that these wefts were the external mycelium of the mycorrhizas. An alternative explanation for the litter weight results is that fungi of the mycorrhizal partnership, having a biological advantage because they are not dependent on litter for an energy source, become dominant in the litter and suppress the activity of saprophytic organisms. Litter decomposition slows down and conditions favouring mor formation result.

RUTH L. GADGIL
P. D. GADGIL

Forest Research Institute,
Private Bag, Rotorua,
New Zealand

Received March 29; revised May 17, 1971.

[1] Handley, W. R., *Forestry Commission Bull.*, No. 23 (1954).
[2] Kononova, M. M., *Soil Organic Matter* (Pergamon, Oxford, 1966).
[3] Kühnelt, W., *Soil Biology* (Faber and Faber, London, 1961).
[4] Meyer, F. H., in *Soil Micromorphology* (edit. by Jongerius, A.), 23 (Elsevier, Amsterdam, 1964).
[5] Mikola, P., in *Mykorrhiza: Internationales Mykorrhizasymposium Weimar 1960*, 277 (G. Fischer, Jena, 1963).
[6] Lobanow, N. W., *Mykotrophie der Holzpflanzen* (VEB Deutscher Verlag der Wissenschaften, Berlin, 1960).

Immunological Suppression of the Occurrence of Spontaneous Mammary Tumours in C3H/He Mice

MICE immunized with syngeneic tumours acquire resistance to subsequent transplantation of mammary tumours[1-3], but there have been no reports of the immunological suppression of the spontaneous occurrence of the tumours by the use of appropriate specific antigens. Mouse mammary tumours appear in favourable conditions in a high percentage of both virgin and bleeding female C3H mice[4]. Mice of the C3H/He strain are genetically pure (syngeneic) and acquire the mammary tumour inducing virus (MTV) by vertical transmission.

We have assumed that the occurrence of spontaneous mammary tumours in C3H/He mice would be suppressed by immunization with the specific antigens of the mammary tumour.

The tumour specific transplantation antigen (TSTA) of mouse mammary tumours can be extracted from MM102 cells—the ascitic line derived from a spontaneous mammary tumour in a C3H/He mouse[5]. The TSTA extracted from MM-

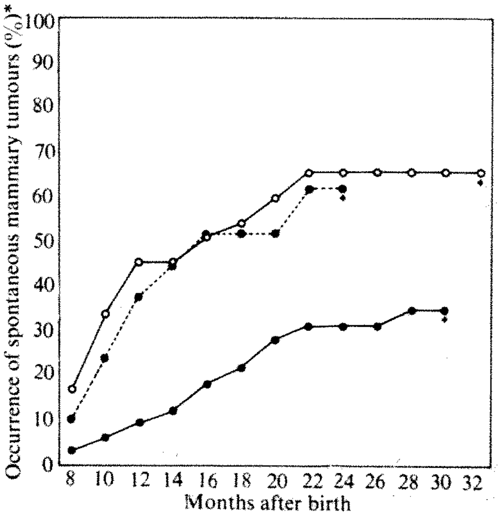


Fig. 1 Occurrence of spontaneous mammary tumours in C3H/He mice. Each plot is figured out the total occurrence within the months. —●—, Immunized with MM-antigen; - - - ● - - -, treated with C3Hf organ extract; —○—, untreated. +, All mice were dead by the marked month.

102 cells is termed MM-antigen. C3H/He mice immunized with MM-antigen acquire resistance to the transplantation of MM102 cells[5] and antibodies which specifically elicit immunological cytotoxicity on MM102 cells can be demonstrated in both the sera and the lymphatic cells of the resistant mice[6]. Further, two experiments suggest the existence of an antigen common to spontaneous mammary tumour cells and MM-antigen. Electron dense precipitates on the cell surface of the microvilli of spontaneous mammary tumour cells treated with RMS (syngeneic antiserum against MM-antigen) can be seen under the electron microscope, using the peroxidase labelled antibody method[7]; and immune cytotoxic activity of RMS can be demonstrated in primary tissue culture cells of mammary tumours[8]. It therefore seemed possible that spontaneous mammary tumours in C3H/He mice might be suppressed by immunization of these mice with MM-antigen.

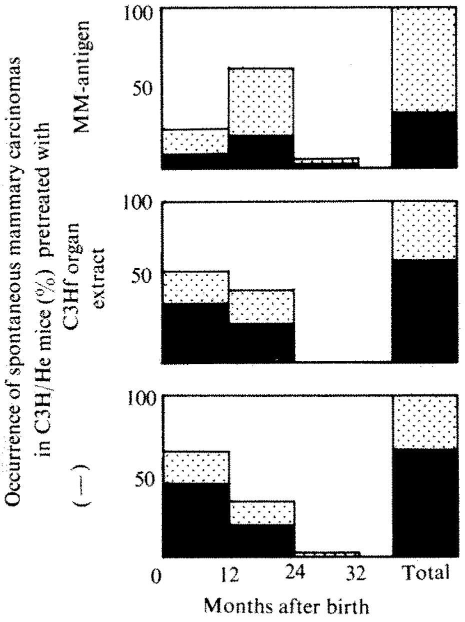


Fig. 2 Occurrence of spontaneous mammary tumours in C3H/He mice. The results are summarized at the end of each year. Shaded bar indicates the percentage of mice with spontaneous mammary tumours. Stippled bar indicates the percentage of dead mice not bearing the tumour.

We divided 105 C3H/He mice about 8 weeks old into three groups of thirty-five. Mice of the first group were immunized with MM-antigen. The visceral organs apart from the lymphatic organs of C3Hf mice were treated beforehand with the same method as extracting MM-antigen from MM102 cells (C3Hf mice are syngeneic with C3H/He mice but do not possess MTV). The second group of mice was immunized with this extract. The third group of mice was untreated. Immunization was carried out as follows. On the first, third and fifth days the mice of the first group were injected intraperitoneally with 0.5 ml. of 1 : 100 dilution of the MM-antigen (1 ml. of the undiluted antigen used here was extracted from 2×10^7 MM102 cells). On the seventh, ninth and eleventh days the mice were injected with 0.5 ml. of 1 : 50 dilution of the antigen. On the thirteenth, fifteenth and seventeenth days the mice were injected with 0.5 ml. of 1 : 25 dilution of the antigen, and on the nineteenth day the mice were injected with 0.5 ml. of 1 : 10 dilution of the same antigen. The mice were then boosted with 0.5 ml. of the undiluted MM-antigen once a month for 3 months. Immunization of the mice ended within 6 months after birth. The injection of extract from C3Hf organs into mice of the second group was carried out by the same method as that of MM-antigen into the mice of the first group.

In the sera of the mice in the first group the antibody reacted specifically with MM102 cells; this could be demonstrated by the immune adherence method until the thirteenth month after the end of immunization, although the antibody titre gradually decreased.

All mice were given dry feed and water *ad libitum* in the same conditions. Three mice from the first group and six mice from the second died during the immunization process, and were omitted from all calculations. The mice were examined for spontaneous mammary tumours once a week after the end of the immunization schedule.

The occurrence of the tumours in the first group was considerably suppressed compared with the second and the third groups (Fig. 1). No significant differences appeared between the second and the third groups. All the results (Fig. 2) were summarized at the end of each year for a period of 3 years. The suppression within the first year was quite drastic in the first group. There was no significant difference in deaths from other causes in the first year. The total occurrence of the tumours over 3 years in the first group was approximately one half of those in the second and third groups.

We thank Drs K. Nishioka and S. Takeuchi for helpful discussions and Miss K. Ueda for technical assistance.

KENJI IRIE
REIKO F. IRIE

Department of Surgery,
Tokyo Metropolitain Okubo Hospital,
Nishiokubo 1-Chome,
Shinjukuku, and
Virology Division,
National Cancer Center Research Institute,
Tsukiji 5-Chome,
Chuoku,
Tokyo

Received January 18, 1971.

[1] Morton, D. L., *Proc. Amer. Assoc. Res.*, **3**, 346 (1962).
[2] Weiss, D. W., Faulkin, jun., L. J., and DeOme, K. B., *Cancer Res.*, **24**, 732 (1964).
[3] Riggins, R. S., and Pilch, Y. H., *Cancer Res.*, **24**, 1994 (1964).
[4] Heton, W. E., Deringer, M. K., Dunn, T. B., and Levillain, W. D., *J. Nat. Cancer Inst.*, **10**, 1139 (1950).
[5] Irie, R. F., Tachibana, T., Nishioka, K., and Takeuchi, S., *Intern. J. Cancer*, **4**, 150 (1969).
[6] Takeuchi, S., *Immunopathological Studies on Tumor-host Relationship in Obstetrical and Gynecological Fields*, 67 (University of Tokyo Press, 1968) (in Japanese).
[7] Suzuki, I., Takahashi, M., Irie, R. F., and Nishioka, K., *J. Electron Microsc.*, **19**, 299 (1970).
[8] Takeuchi, S., Irie, R. F., Inoue, M., Irie, K., Izumi, R., and Nishioka, K. *Intern. J. Cancer*, **4**, 130 (1969).

Chromosome Polymorphism in American Negro and White Populations

THE biological and clinical implications of variations of human chromosome lengths are poorly understood.

In the study we now report, minor variations in chromosome length were correlated with clinical and sociological data obtained from each patient for 4,482 consecutively born infants. By far the strongest correlations were between certain minor chromosome variants and race. Most variants showing a racial difference of frequency were twice as common in Negroes as in Caucasians.

The human variants can be considered as polymorphisms as in *Drosophila* and other species[1-3] because (1) they occur at relatively high frequencies; (2) they are usually associated with normal phenotypes and (3) they are frequently inherited from generation to generation[4,5]. Racial variation of the length of the *Y* chromosome[6], and the question of racial variation in the size and morphology of the short arms of the chromosomes in groups *D* and *G*[7] have already been discussed.

All infants of more than 20 weeks gestation born at Yale–New Haven Hospital from October 1967 to October 1968 were included in the study and good preparations were obtained from the cord blood of 4,366 of 4,482 newborns[8,9]. Cells were treated with 0.075 M KCl and stained with orcein. Two karyotypes were examined from each infant and variation in length was scored using callipers. Variants were detected by comparing the lengths of individual chromosomes against internal standards within each cell (Table 1) and were recorded only when present in both cells. A long *Y* chromosome, for example, was recorded if the *Y* chromosome in both cells was greater than or equal to *F*19 in length, and an unusually long *Y* recorded if it was greater than the total length of *E*18 (Fig. 1). All the results are based on samples of two cells per individual. Studies with larger samples show that, in general, two-cell ascertainments are correct except for the commoner variants. The frequencies of $Y \geq 19$, short arm of *D* or *G* = *E*18 short arm, and both *E*16 variants were, however, overestimated, and the frequencies reported in Table 1 should not be used to estimate the actual frequencies of these variants.

Table 1 Frequencies of Polymorphisms in Caucasian and Negro Infants

Type of variation	Children of 3,476 Caucasian mothers *N*	%	Children of 807 Negro mothers *N*	%	*P*
Increased secondary constriction region *A*1	8	0.23	3	0.37	ns
Unusually metacentric *C* chromosome	2	0.06	10	1.24	<0.01
Short arm of a group *D* chromosome:					
Increased length (= *E*18 short arm)	480	13.80	171	21.18	<0.01
Increased length (> *E*18 short arm)	0	0.25	7	0.87	<0.01
Large satellites (> short arm same chromosome)	78	2.24	34	4.21	<0.01
Tandem satellite	3	0.09	2	0.25	nt
Streaked satellite	1	0.03	1	0.12	nt
Short arm absent	2	0.06	0	0	nt
Variations in long arm of *E*16:					
Increased length (> short arm *C*6)	71	2.04	32	3.96	<0.01
Decreased length (*E*16—*E*18)	19	0.55	3	0.37	ns
Short arm of a group *G* chromosome:					
Increased length (= *E*18 short arm)	81	2.33	45	5.58	<0.01
Increased length (> *E*18 short arm)	2	0.06	0	0	nt
Large satellites (> short arm same chromosome)	92	2.65	36	4.46	<0.01
Tandem satellite	3	0.09	0	0	nt
Streaked satellite	1	0.03	0	0	nt
Variations in length of the *Y* chromosome in 1,741 Caucasian and 411 Negro males:					
Y long (> F19)	253	14.53	60	14.59	ns
Y long (> E18)	6	0.34	1	0.24	ns
Y short (< G22)	7	0.40	2	0.48	ns
Y metacentric	2	0.11	0	0	nt
No polymorphism	2,131	61.31	389	48.20	<0.01

nt, Not tested because of small *N*. ns, χ^2 test for difference in frequency between Caucasian and Negro infants not significant (>0.05).

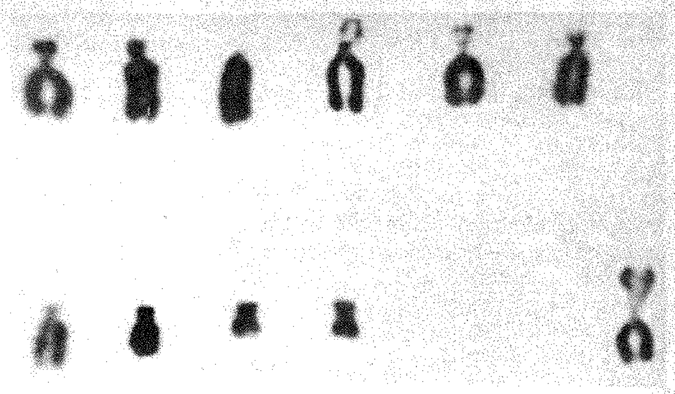


Fig. 1 Minor chromosome variants. In the top row, six polymorphisms in group *D* are shown (left to right): very long short arm ≥E18 short arm, long short arm = short arm *E*18, deleted short arm, long streaked satellites, large satellites (> short arm), and tandem satellites. *Y* chromosome polymorphisms in the bottom row are: *Y*> *E*18, *Y*> *F*19, *Y*< *G*22, *Y* metacentric. An example of a metacentric *C* chromosome with a secondary constriction adjacent to the centromere is shown at the far right, bottom row. Conventional staining with orcein.

The most marked difference between races occurred in the frequency of metacentric *C* chromosomes (Fig. 1). This chromosome was observed in only two of 3,176 Caucasian children but was a relatively common variant in Negro children occurring in ten of 807 children (1.2%, a twenty-fold difference). In every case the chromosome was characterized not only by an unusual degree of metacentricity but a secondary constriction adjacent to the centromere (Fig. 1). All the children were phenotypically normal except for one of the two Caucasian children who had a birth weight below 2,500 g. Precise identification of this chromosome is difficult with conventional staining, but in two cases restaining with quinacrine mustard[9] showed it to be chromosome *C*9. Of sixteen newborns with a short arm of a *D* chromosome greater than the short arm of *E*18, seven were Negro and nine Caucasian, a four-fold difference in frequency. The most common autosomal minor variants occurred twice as often in Negro children as in Caucasian children (Table 1). The common denominator in each of these seven variants was the presence of a secondary constriction in each of the involved chromosomes. Moreover, each of the acrocentric short arm variants is believed to share a common function of nucleolar organization and association, unlike the metacentric *C* and *E*16 variants, although secondary constrictions are present in both.

The relative differences in frequencies in the *D* and *G* group between Caucasian and Negroes were consistent with those found in the smaller sample of twenty Caucasian and twenty Negro children reported by Starkman and Shaw[7]. Similarly, the earlier work by Cohen and Shaw[6] did not show a difference between twenty Caucasian and twenty Negro adults, although a long *Y* chromosome occurred more commonly in twenty Japanese males. Data are presented in this study only in Caucasian and Negro infants because the number of other infants included in the study was small, but of nine unusually long *Y* chromosomes (*Y*> 18) one occurred in a Chinese baby and one in a child of Turkish parents. Only one Turkish child and nine male Chinese infants were included in the study. The clinical significance of these minor variants is also under study and in at least one case a statistically significant correlation has been established: the presence of a *Gp*(sat)+ variant in Caucasian infants was associated with a two-fold increase in the frequency of low birth weight[8]. Certain of the rare variants may also carry an increased risk in Caucasian infants[9]. Racial polymorphisms, however, seem to be the most common correlate of chromosomal variation.

The relative frequencies of the variants may represent an equilibrium between their rates of generation in and elimination from the two populations. The lower frequency of chromosome polymorphism in the Caucasian population may be related to with certain of the polymorphisms in Caucasians. The rate of elimination of the polymorphism from the Caucasian population would be more rapid, hence the equilibrium value would also be lower. Such an argument implies a similarity in the genetic effects for the numerous chromosomal variants observed, and it is difficult to imagine such a unifying factor. It is interesting, however, that most variants occur in heterochromatic regions of the chromosomes, either adjacent to centromere regions or in the distal part of the *Y*. These regions have been shown to contain redundant DNA which probably does not code for mRNA. Changes in the amount and/or distribution of heterochromatin may affect the expression of genes. The regulation of nucleolar organization regions, which occur in the short arm of the *D* and *G* chromosomes, may be particularly affected. It is also possible that the heterochromatin is basically different in the two populations or that differences in the genetic backgrounds respond to similar modifications of heterochromatin in different ways. More definite statements may be possible after examination of the variant chromosomes using newly developed techniques for the visualization of heterochromatin[11-14].

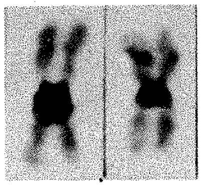


Fig. 2 Increased centromeric heterochromatin in chromosome *A*1. *A*1 homologous chromosomes from an individual heterozygous for increased long arm length. Ascertainment was first made by length measurement. Increased length in one *A*1 chromosome is correlated with increased heterochromatin. Polymorphism is heritable. Heterochromatin is visualized by first denaturing the chromosomal DNA *in situ*, then staining with Giemsa. Details of this method are presented elsewhere[12-14].

A family possessing an unusually long arm of chromosome *A*1 has been recently studied using these methods[12-14]. The results show that the extra length of the chromosome *A*1 long arm is caused by an increase (approximately × 2) of centromeric associated heterochromatin (Fig. 2) which provides preliminary support for the arguments presented here and suggests that other minor variants may be causally related to polymorphisms restricted to heterochromatic areas. Another finding in support of this viewpoint is the high frequency of length variation associated with the *A*1, *C*9, *E*16 and *Y* chromosomes. These chromosomes all contain very large blocks of heterochromatin[12-14]. We are re-examining our families possessing these polymorphisms. If variations in length generally prove to be due to variation in the length of heterochromatin areas, this would explain the benign effect of most such variants on phenotype and hence their high frequency. Lastly, because the polymorphic homologue can now be readily identified by a simple staining technique, it should be a simple matter to determine its pattern of inheritance in kindreds. This should provide extremely useful material for the purpose of establishing gene linkage to specific chromosomes.

This work was supported by the Division of Biologics Standards, US National Institutes of Health.

H. A. LUBS

University of Colorado Medical Center,
Denver,
Colorado 80220

F. H. RUDDLE

Department of Biology,
Kline Biology Tower,
Yale University,
New Haven, Connecticut 06520

Received November 5, 1970; revised March 22, 1971.

[1] Patterson, J. T., and Stone, W. S., *Evolution in the Genus Drosophila* (Macmillan, New York, 1952).

[2] White, M. J. D., *Animal Cytology and Evolution*, second ed. (Cambridge University Press, 1954).

[3] Ford, C. E., in *Human Population Cytogenetics* (edit. by Jacobs, P., Price, W. H., and Law, P.) (University of Edinburgh Press, Edinburgh, 1970).

[4] German, J., *Texas Reports of Biology and Medicine*, suppl., **24**, 347 (1966).

[5] Court-Brown, W. M., Buckton, K. E., Jacobs, P. A., Tough, I. M., Kuenssberg, E. V., and Knox, J. D. E., *Chromosome Studies in Adults* (Cambridge University Press, London, 1966).

[6] Cohen, M. M., and Shaw, M. W., *Ann. Human Genet.*, **31**, 129 (1967).

[7] Starkman, M. N., and Shaw, M. W., *Amer. J. Human Genet.*, **19**, 162 (1967).

[8] Lubs, H. A., and Ruddle, F. H., in *Human Population Cytogenetics* (edit. by Jacobs, P., Price, W. H., and Law, P.) (University of Edinburgh Press, Edinburgh, 1970).

[9] Lubs, H. A., and Ruddle, F. H., *Science*, **169**, 495 (1970).

[10] Caspersson, T., Zech, L., and Johansson, C., *Expt. Cell Res.*, **62**, 490 (1970).

[11] Pardue, M. L., and Gall, J. G., *Science*, **168**, 1356 (1970).

[12] Arrighi, F. E., and Hsu, T. C., *Cytogenetics* (in the press).

[13] Chen, T. R., and Ruddle, F. H., *Chromosoma* (in the press).

[14] Ruddle, F. H., and Chen, T. R., *Colorado Springs Conference on Cytogenetics* (in the press).

Role for Ferredoxins in the Origin of Life and Biological Evolution

THE ferredoxins are members of a class of metalloproteins known as iron–sulphur proteins which act as electron carriers in such diverse biochemical processes as carbon metabolism, nitrogen fixation, photosynthesis and steroid hydroxylation[1,2]. They are relatively small proteins with molecular weights of 6,000 to 12,000; they contain 2–8 atoms of iron and equivalent amounts of inorganic sulphur per molecule, transferring electrons at low redox potentials (-300 to -500 mV at pH 7) and having a characteristic electron paramagnetic resonance signal at $g=1.94$ in the reduced state. Ferredoxins have been found in a wide range of organisms, from the primitive anaerobic bacteria to all higher plants and animals. A knowledge of the changes in their amino-acid sequence[3] is therefore of great value to the study of the evolution of these organisms.

We propose that a ferredoxin may have been among the earliest proteins formed and that ferredoxins may be very suitable for use in the study of the evolution from anaerobic to aerobic organisms. The ferredoxins from anaerobic bacteria have a very simple composition which seems to have developed into the more complex ferredoxins of the photosynthetic bacteria. The final stage of development was reached with the appearance of the primitive blue–green algae whose ferredoxin seems to be similar to those of all other algae and higher plants.

When life started, the principal mode of metabolism is thought to have been anaerobic fermentation of organic compounds which had formed abiogenically in the primaeval environment[4,5]. The ferredoxins probably played a significant role in the development of the fermentative bacteria. In present day organisms of this type such as the clostridia, the ferredoxins are simple, small proteins of fifty-five amino-acids and molecular weight 6,000 with a primitive polypeptide structure[6]. The amino-acid sequences of three of them are known[7-9]. These contain fourteen different types of amino-acids but only nine are common to all three ferredoxins. Significantly, the six amino-acids detected[11,12] in the Murchison meteorite (which fell in Australia in 1969)[10]—glycine, alanine, valine, proline, glutamic acid and aspartic acid—constitute 64% of the total amino-acid content of *C. butyricum*[8], while the nine "common" amino-acids (the six plus serine, cysteine and isoleucine) comprise 91% of the ferredoxin molecule of *C. butyricum*. All nine of these amino-acids have been synthesized abiogenically in conditions which simulated the primitive Earth environment in the laboratory[4]. The amino-acids methionine and histidine, which are difficult to synthesize in these laboratory conditions, are absent from these ferredoxins.

Unlike other electron-transferring proteins, such as cytochromes and flavoproteins, which have complex organic molecules in their active site, the ferredoxins have only iron and inorganic sulphur. Once the polypeptide chain of ferredoxin was formed, these could readily be added as an iron salt and sulphide, both of which were abundant in the primitive Earth. This process is known to occur readily in anaerobic conditions[13].

	1																			20
(A)	Ala–	Thr–	Tyr–	Lys–	Val–	Thr–	Leu–	Lys–	Thr–	Pro–	Ser–	Gly–	Asp–	Gln–	Thr–	Ile–	Glu–	Cys–	Pro–	Asp
(B)	Ala–	Ala–	Tyr–	Lys–	Val–	Thr–	Leu–	Val–	Thr–	Pro–	Thr–	Gly–	Asn–	Val–	Glu–	Phe–	Gln–	Cys–	Pro–	Asp
(C)		Ala–	Phe–	Lys–	Val–	Lys–	Leu–	Leu–	Thr–	Pro–	Asp–	Gly–	Pro–	Lys–	Glu–	Phe–	Glu–	Cys–	Pro–	Asp
(D)	Ala–	Thr–	Tyr–	Lys–	Val–	Lys–	Leu–	Val–	Thr–	Pro–	Ser–	Gly–	Gln–	Gln–	Glu–	Phe–	Gln–	Cys–	Pro–	Asp
	21																			40
(A)	Asp–	Thr–	Tyr–	Ile–	Leu–	Asp–	Ala–	Ala–	Glu–	Glu–	Ala–	Gly–	Leu–	Asp–	Leu–	Pro–	Tyr–	Ser–	Cys–	Arg
(B)	Asp–	Val–	Tyr–	Ile–	Leu–	Asp–	Ala–	Ala–	Glu–	Glu–	Glu–	Gly–	Ile–	Asp–	Leu–	Pro–	Tyr–	Ser–	Cys–	Arg
(C)	Asp–	Val–	Tyr–	Ile–	Leu–	Asp–	Gln–	Ala–	Glu–	Glu–	Leu–	Gly–	Ile–	Asp–	Leu–	Pro–	Tyr–	Ser–	Cys–	Arg
(D)	Asp–	Val–	Tyr–	Ile–	Leu–	Asp–	Gln–	Ala–	Glu–	Glu–	Val–	Gly–	Ile–	Asp–	Leu–	Pro–	Tyr–	Ser–	Cys–	Arg
	41																			60
(A)	Ala–	Gly–	Ala–	Cys–	Ser–	Ser–	Cys–	Ala–	Gly–	Lys–	Val–	Glu–	Ala–	Gly–	Thr–	Val–	Asp–	Gln–	Ser–	Asp
(B)	Ala–	Gly–	Ser–	Cys–	Ser–	Ser–	Cys–	Ala–	Gly–	Lys–	Leu–	Lys–	Thr–	Gly–	Ser–	Leu–	Asn–	Gln–	Asp–	Asp
(C)	Ala–	Gly–	Ser–	Cys–	Ser–	Ser–	Cys–	Ala–	Gly–	Lys–	Leu–	Val–	Glu–	Gly–	Asp–	Leu–	Asp–	Gln–	Ser–	Asp
(D)	Ala–	Gly–	Ser–	Cys–	Ser–	Ser–	Cys–	Ala–	Gly–	Lys–	Val–	Lys–	Val–	Gly–	Asp–	Val–	Asp–	Gln–	Ser–	Asp
	61																			80
(A)	Gln–	Ser–	Phe–	Leu–	Asp–	Asp–	Ser–	Gln–	Met–	Asp–	Gly–	Gly–	Phe–	Val–	Leu–	Thr–	Cys–	Val–	Ala–	Tyr
(B)	Gln–	Ser–	Phe–	Leu–	Asp–	Asp–	Asp–	Gln–	Ile–	Asp–	Glu–	Gly–	Trp–	Val–	Leu–	Thr–	Cys–	Ala–	Ala–	Tyr
(C)	Gln–	Ser–	Phe–	Leu–	Asp–	Asp–	Glu–	Gln–	Ile–	Glu–	Glu–	Gly–	Trp–	Val–	Leu–	Thr–	Cys–	Ala–	Ala–	Tyr
(D)	Gly–	Ser–	Phe–	Leu–	Asp–	Asp–	Glu–	Gln–	Ile–	Gly–	Glu–	Gly–	Trp–	Val–	Leu–	Thr–	Cys–	Val–	Ala–	Tyr
																96	97			
(A)	Pro–	Thr–	Ser–	Asp–	Cys–	Thr–	Ile–	Ala–	Thr–	His–	Lys–	Glu–	Glu–	Asp–	Leu–	Phe–				
(B)	Pro–	Val–	Ser–	Asp–	Val–	Thr–	Ile–	Glu–	Thr–	His–	Lys–	Glu–	Glu–	Glu–	Leu–	Thr–	Ala			
(C)	Pro–	Arg–	Ser–	Asp–	Val–	Val–	Ile–	Glu–	Thr–	His–	Lys–	Glu–	Glu–	Glu–	Leu–	Thr–	Ala			
(D)	Pro–	Val–	Ser–	Asp–	Gly–	Thr–	Ile–	Glu–	Thr–	His–	Lys–	Glu–	Glu–	Glu–	Leu–	Thr–	Ala			

Fig. 1 Sequences of type 4 (plant) ferredoxins. The underlined amino-acids, of which there are sixty, are invariant. A, *Scenedesmus*; B, spinach; C, *Leucaena*; D, taro.

There seem to be four types of ferredoxins and they may be linked in the evolutionary pattern: (1) anaerobic fermentative bacteria ⟶ (2) green photosynthetic bacteria ⟶ (3) red photosynthetic bacteria ⟶ (4) algae and higher plants. The final stage in the development of ferredoxins seems to have occurred in the change from anaerobic photosynthetic bacteria to the aerobic photosynthetic organisms. The process of photosynthesis requires a donor of electrons which, with energy from the Sun, is used to reduce carbon dioxide to organic material. In the earlier photosynthetic bacteria the electron donor was probably H_2S or some other inorganic molecule; in algae and plants the donor was water and oxygen was evolved. This process is thought to have brought about the conversion of the primaeval reducing atmosphere into an aerobic one[14] so that animal life could develop. The first organisms to carry out this type of photosynthesis were probably the blue–green algae. These are prokaryotic, having no nuclear membrane or chloroplast outer membrane, but they differ from bacteria in that their photosynthetic apparatus is organized in a system of intracellular lamellae and they have type 4 ferredoxins. There is a big difference between these plant-type ferredoxins and the photosynthetic bacterial ferredoxins, and this probably reflects the large evolutionary change in photosynthesis that this step involves. The length of the polypeptide chain was increased, the additional protein moiety probably being necessary for the ferredoxin to interact effectively with the specialized photosynthetic lamellar systems. Thus so far no ferredoxin intermediate between types 3 and 4 has been found.

The type 4 plant ferredoxins have molecular weights of about 11,500; they contain two atoms of iron and sulphur, and transfer only one electron[15]. All the plant ferredoxins are very similar in optical absorption, optical rotatory dispersion and electron paramagnetic resonance (our unpublished observations). Thus it seems that with the appearance of the blue–green algae, the development of the ferredoxin molecule was complete.

In the evolution of green algae and higher plants from the blue–green algae, the oxygen-evolving photosynthetic system and the type 4 ferredoxins seem to have remained essentially unchanged. This observation supports the suggestion that plant and algal chloroplasts are the descendants of blue–green algae which lived symbiotically inside the early plant cells[16]. Thus all chloroplast ferredoxins would be derived from a common ancestor, namely blue–green algae.

The type 4 ferredoxins listed in Table 1 span the range from the blue–green algae to higher plants. Although these ferredoxins are very similar in overall structure, there are differences in the detailed amino-acid sequences which offer potential means of following evolutionary relationships within this very wide range of organisms. At present, however, too few sequences are known to permit any attempt to construct an evolutionary sequence. The amino-acid sequences of four type 4 ferredoxins are shown in Fig. 1. Although these sequences are very different from the bacterial ferredoxins, evolutionary relationships between types 1 and 4 have been demonstrated by Matsubara *et al.*[17]. Four of the cysteines are invariant (there is no cysteine at position 18 in *Equisetum* ferredoxin[18]) and they are thought to be structurally involved in the active site.

A proposed model[19] for the active site of type 4 ferredoxins is shown in Fig. 2. Whether a similar type of unit is applicable to the other types of ferredoxin is unknown but it may be possible to extend the model to the 2-electron transfer ferredoxins of types 1, 2 and 3 as has been suggested by Gibson *et al.*[20].

The occurrence of iron–sulphur proteins in animals has not been dealt with here because little is known about them at present. But because of the recent recognition of their ubiquitous occurrence and role in important reactions such as oxidative phosphorylation and hormone synthesis, they will undoubtedly be investigated intensively in the next few years. As an example, the hydroxylase iron–sulphur protein adrenodoxin from adrenal glands contains two iron atoms and two sulphur atoms and transfers one electron; its chromophore

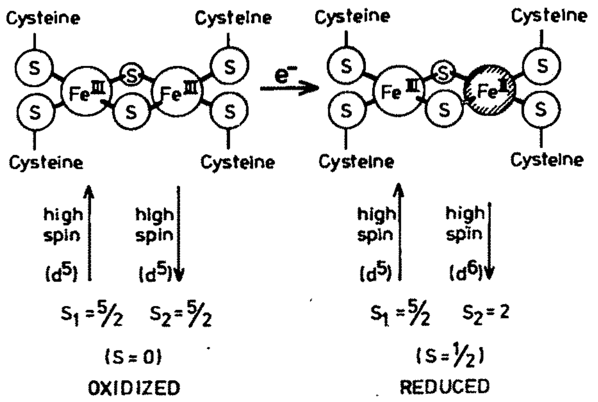


Fig. 2 Model of the iron–sulphur group in plant ferredoxins.

group seems very similar to the plant-type ferredoxins[21] but its amino-acid sequence[22] is quite dissimilar. Iron–sulphur proteins have also been detected in aerobic bacteria, fungi and yeasts[1].

Table 1 Amino-acid Composition of Type 4 (Plant) Ferredoxins

Amino-acid	1	2	3	4	5	6	7	8
Lysine	3	5	4	4	4	5	5	4
Histidine	2	1	1	1	2	1	1	1
Arginine	2	1	1	1	1	1	2	1
Tryptophan	1	0	0	0	0	1	1	1
Aspartic acid	11	14	12	9	14	10	10	13
Threonine	6	6	10	7	6	6	4	8
Serine	7	7	8	8	8	8	7	7
Glutamic acid	11	15	10	16	9	16	16	13
Proline	4	3	4	4	5	4	4	4
Glycine	6	6	7	9	9	8	6	6
Alanine	8	11	10	6	7	6	7	9
Cysteine	5	4	6	4	5	5	5	5
Valine	5	6	5	6	5	10	6	7
Methionine	1	1	1	1	2	0	0	0
Isoleucine	6	5	3	5	6	4	4	4
Leucine	9	9	7	8	7	6	9	8
Tyrosine	4	4	4	2	3	4	3	4
Phenylamine	2	3	3	4	4	2	3	2
Total amino-acids	93	101	96	95	97	97	96	97
References	*	23	24	18	25	26	27	28

1, *Microcystis* (blue–green alga); 2, *Bumilleriopsis* (yellow–green alga); 3, *Scenedesmus* (green alga); 4, *Equisetum* (horsetail; primitive plant); 5, fern (*Polystichum munitum*); 6, taro (*Colocasia*) (monocotyledonous higher plant); 7, *Leucaena glauca* (leguminous tree); 8, spinach (*Spinacea oleracea*) (dicotyledonous higher plant).

* K. T. Yasunobu, R. V. Smith, M. C. W. Evans and K. K. Rao, unpublished observations.

In conclusion, ferredoxins may have been among the simplest proteins, being first formed as a short polypeptide chain of twenty-six amino-acids comprising only nine different amino-acids which are easily formed in simulated primaeval conditions. It might have been possible for the primitive organism to utilize the amino-acids from its environment to synthesize this simple protein. Inorganic iron and sulphur were available in abundance and could readily combine with the apoprotein in a non-enzymatic reaction to form the chromophore group of a ferredoxin which could transfer electrons at reducing potentials of −420 mV similar to those of H_2 gas. From studies of amino-acid sequence the ferredoxins seem to have evolved from the anaerobic fermentative bacteria to the anaerobic photosynthetic bacteria and thence to oxygen evolving, photosynthetic organisms. At some stage, proteins similar to the ferredoxins were incorporated into the metabolic pathways of animals, yeasts and fungi, also catalysing electron transfer reactions. Thus the ferredoxins are a class of proteins in which patterns of evolution can be followed.

We thank Professor F. R. Whatley, Drs M. C. W. Evans and R. V. Smith for valuable discussions.

D. O. Hall
R. Cammack
K. K. Rao

Department of Botany,
King's College,
68 Half Moon Lane,
London SE24 9JF

Received April 5; revised June 24, 1971.

[1] Hall, D. O., and Evans, M. C. W., *Nature*, **223**, 1432 (1969).
[2] Buchanan, B. B., and Arnon, D. I., *Adv. Enzymol.*, **33**, 119 (1970).
[3] Nolan, C., and Margoliash, E., *Ann. Rev. Biochem.*, **37**, 727 (1968).
[4] Kenyon, D. H., and Steinman, G., *Biochemical Predestination* (McGraw-Hill, New York, 1969).
[5] Lemmon, R. M., *Chem. Rev.*, **70**, 95 (1970).
[6] Lipmann, F., in *Chemical Evolution and the Origin of Life* (edit. by Buvet, R., and Ponnamperuma, C.) (North Holland, Amsterdam, in the press).
[7] Tanaka, M., Nakashima, T., Benson, A., Mower, H. F., and Yasunobu, K. T., *Biochemistry*, **6**, 1666 (1966).
[8] Benson, A. M., Mower, H. F., and Yasunobu, K. T., *Proc. US Nat. Acad. Sci.*, **55**, 1532 (1966).
[9] Rall, S. C., Bolinger, R. E., and Cole, R. D., *Biochemistry*, **8**, 2386 (1969).
[10] Lovering, J. F., Le Maitre, R. W., and Chappel, B. W., *Nature*, **230**, 18 (1971).
[11] Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperuma, C., Kaplan, I. R., and Moore, C., *Nature*, **228**, 923 (1970).
[12] Kvenvolden, K. A., Lawless, J. C., and Ponnamperuma, C., *Proc. US Nat. Acad. Sci.*, **68**, 486 (1971).
[13] Malkin, R., and Rabinowitz, J. C., *Biochem. Biophys. Res. Commun.*, **23**, 822 (1966).
[14] Bernal, J. D., *The Origin of Life* (Weidenfeld and Nicolson, London, 1967).
[15] Evans, M. C. W., Hall, D. O., Bothe, H., and Whatley, F. R., *Biochem. J.*, **110**, 485 (1968).
[16] Echlin, P., *Symp. Soc. Gen. Microbiol.*, **20**, 221 (1970).
[17] Matsubara, H., Jukes, T. H., and Cantor, C. R., *Brookhaven Symp. Biol.*, **21**, 201 (1968).
[18] Aggarwal, S. J., Rao, K. K., and Matsubara, H., *J. Biochem.* (Tokyo), **69**, 601 (1971).
[19] Rao, K. K., Cammack, R., Hall, D. O., and Johnson, C. E., *Biochem. J.*, **122**, 257 (1971).
[20] Gibson, J. F., Hall, D. O., Thornley, J. H. M., and Whatley, F. R., *Proc. US Nat. Acad. Sci.*, **56**, 987 (1966).
[21] Johnson, C. E., Cammack, R., Rao, K. K., and Hall, D. O., *Biochem. Biophys. Res. Commun.*, **43**, 564 (1971).
[22] Tanaka, M., Haniu, M., and Yasunobu, K. T., *Biochem. Biophys. Res. Commun.*, **39**, 1182 (1970).
[23] Böger, P., *Planta*, **92**, 105 (1970).
[24] Sugeno, K., and Matsubara, H., *J. Biol. Chem.*, **244**, 2979 (1969).
[25] Schürmann, P., Buchanan, B. B., and Matsubara, H., *Biochim. Biophys. Acta*, **223**, 450 (1970).
[26] Rao, K. K., and Matsubara, H., *Biochem. Biophys. Res. Commun.*, **38**, 500 (1970).
[27] Benson, A. M., and Yasunobu, K. T., *J. Biol. Chem.*, **244**, 955 (1969).
[28] Matsubara, H., Sasaki, R. M., and Chain, R. K., *J. Biol. Chem.*, **243**, 1723 (1968).

Structure of α-Keratin

Low angle X-ray diffraction is a powerful means of investigating long range axial order in fibrous protein structures, and α-keratin was one of the earliest materials to be studied by this method[1]. The observed meridional and near-meridional reflexions have been indexed[2–5] as orders of an axial period of 198 Å, but there are indications[6,7] that this might not be correct. This possibility has been investigated using a high resolution focusing X-ray camera[8] to obtain diffraction patterns from several native keratins and from certain heavy-atom derivatives of porcupine quill.

It is difficult to calibrate fibre patterns with sufficient precision to combine results from different specimens and for the purpose of comparison it was assumed that the well resolved meridional reflexion about 24.8 Å corresponded to the eighth order of a 198 Å axial period. Thus the reciprocal space coordinate ζ of this reflexion was taken as 0.0404 Å^{-1} in each case and the indices of the remaining reflexions calculated on this basis. The results (Table 1) show that the measurements on keratins from different sources and on different derivatives agree in general to within the estimated experimental error of $\pm 0.2 \times 10^{-3}$ Å^{-1}. The ζ-values calculated on the basis of a 198 Å axial period are also listed and it will be seen that there are discrepancies considerably in excess of experimental error between the observed and calculated values. It seems therefore that the axial period of α-keratin is not 198 Å, as is generally supposed.

From a consideration of the results given in Table 1 an alternative axial period of 470 Å was derived and it will be seen (Table 1) that this enables all the observed meridional reflexions to be indexed in a satisfactory manner. In no case does the difference between the observed ζ-value and that calculated on the basis of a 470 Å axial period exceed the estimated experimental error. Further measurements on non-meridional low angle reflexions and on near-meridional high angle reflexions indicate that an axial period of 470 Å is likely to provide a satisfactory basis for indexing the complete diffraction pattern. For comparison, the diffraction patterns of the materials listed in Table 1 were placed on a common scale but the absolute value of the axial period is known to increase with hydration[2]. Measurements on dry porcupine quill dusted with a powdered calibrating substance give an absolute value of 470 ± 3 Å for the axial period in this material.

At low angles the prominent meridional reflexions fall on layer lines with indices (based on a 470 Å period) which satisfy the selection rule $l = 19\,m$, $19\,m \pm 5$ and $19\,m \pm 7$, where $m = 0$, 1 and 2. This type of distribution is characteristic of a periodically distorted structure[9,10]. A likely cause of such a distortion is that the fibrous elements (microfibrils) have an initial screw axis with 19 units in t turns of pitch $470/t$ Å which is disturbed when close packing of the microfibrils takes place during biogenesis. Strong near-meridional diffraction occurs on $l = 17$ and the lateral coordinate of the maximum is such that it could only be due to a first order Bessel function. From the Bessel function selection rule for a helical structure with u units in t turns ($l = um + tn$; where m and n are integers)[11] it follows that with $l = 17$ and $u = 19$, $t = 2$ for $n = -1$. This helix is illustrated in Fig. 1.

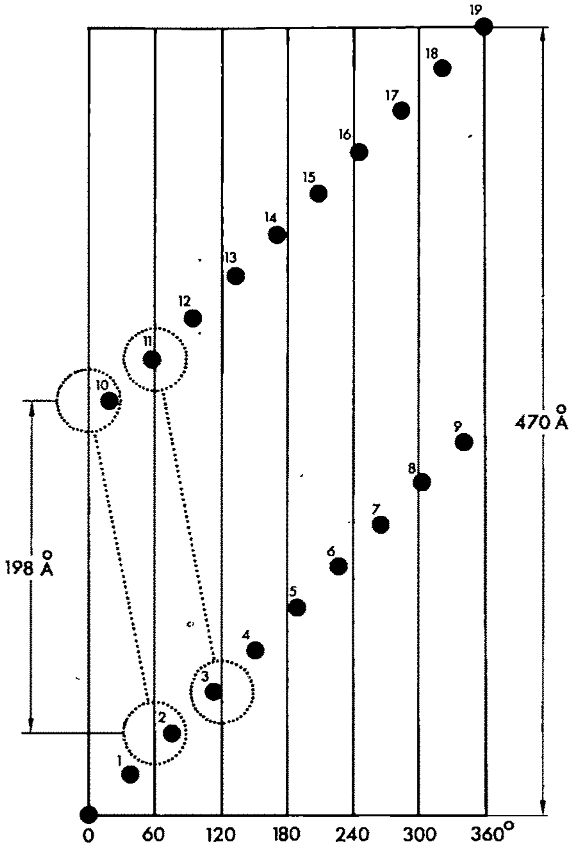


Fig. 1 Radial projection of the axial repeat of structure in the α-keratin microfibril. An isolated microfibril contains 19 units in 2 turns of a helix of pitch 235 Å giving an axial repeat of 470 Å. The vertical lines represent lines of contact with neighbouring microfibrils in a hexagonally close packed structure. Units separated by an axial translation of 198 Å (dotted lines) have almost identical environments and presumably similar distortions. The screw-sense of the helix cannot be determined directly from the X-ray data, but considerations of the way in which left handed coiled coil ropes could be packed side by side with a displacement of 24.8 Å suggest that better packing would be obtained with a right handed screw axis for the microfibril.

The origin of the 198 Å pseudo-period in the axial projection of the structure is immediately apparent from Fig. 1 where the contacts between a microfibril and its six nearest neighbours are indicated by vertical lines. Units separated by an axial translation of 198 Å have almost identical environments and

Table 1 ζ-Values of Meridional Reflexions in the X-ray Diffraction Pattern of α-Keratin (10^{-3} Å^{-1})

African porcupine quill										
Dry		14.8		25.4			40.4	55.3	80.9	95.7
Hydrated	10.8	14.9		25.5			40.4	55.1	80.5	95.2
Silver derivative	10.7	15.0	21.3		29.9		40.4			
Osmium derivative	10.8	14.7		25.4	29.8	34.1	40.4		80.7	
Chromium derivative		14.9		25.6	29.7		40.4	55.1	80.8	95.7
S. American porcupine quill	10.7	14.9		25.6	29.9		40.4	55.3	80.7	95.4
Lincoln wool fibres		15.0		25.6	29.8		40.4			
Mean ζ-value	10.8	14.9	21.3	25.5	29.8	34.1	40.4	55.2	80.7	95.5
Index with 198 Å period	2	3	4	5	6	7	8	11	16	19
Calculated ζ-value	10.1	15.2	20.2	25.3	30.3	35.4	40.4	55.6	80.8	96.0
Error	0.7	−0.3	1.1	0.2	−0.5	−1.3	0	−0.4	−0.1	−0.5
Index with 470 Å period	5	7	10	12	14	16	19	26	38	45
Calculated ζ-value	10.6	14.9	21.3	25.5	29.8	34.0	40.4	55.3	80.8	95.7
Error	0.2	0	0	0	0	0.1	0	−0.1	−0.1	−0.2

presumably therefore undergo similar distortions. The pseudo-period of 198 Å is thus a consequence of the pattern of distortion rather than an indication of molecular dimensions. The redistribution of intensity which occurs at low angles when α-keratin is hydrated[7] therefore reflects a change in the nature of the distortion pattern at the surface of the microfibril.

Our conclusions have several implications for further studies of the structure of α-keratin. (*a*) The microfibril has a nineteen-fold rotation axis in projection and a radius ~36 Å[12], and so it is unlikely that the individual structural units could be resolved when viewed in axial projection in the electron microscope[13,14]. (*b*) The interactions along the lines of contact between microfibrils are quite precise, suggesting that bridges exist between them or that the intervening matrix has, at least in part, a regular structure. (*c*) The microfibril in α-keratin is believed to be composed of molecules of molecular weight ~45,000 (ref. 15). If the number of such molecules associated with each unit in Fig. 1 were 1, 2 or 3 the corresponding microfibril radii would be, respectively, ~27, 38 or 47 Å assuming a density of 1.3 g cm^{-3}. The value derived for two molecules per unit gives the best agreement with the estimate of 36 Å for the radius of the microfibril in African porcupine quill derived from the low angle equatorial X-ray pattern[12]. A consideration of the distribution of intensity in the meridional pattern in various heavy atom derivatives, which will be discussed in detail elsewhere, suggests that the two molecules may be related by a horizontal dyad. (*d*) The prominent 5.16 Å arc in the high angle diffraction pattern does not index satisfactorily on a 198 Å axial period[16,17] but seems to fall on the ninety-first layer line of the 470 Å period. Weaker arcs at 5.06 and 4.96 Å fall on the ninety-third and ninety-fifth layer lines respectively. The diffraction in this area originates from the coiled coil ropes in the interior of the microfibril[12] rather than from the surface and the screw axis is less likely to be disturbed by inter-microfibrillar interactions. The appearance of low order Bessel functions on $l=91$, 93 and 95 is to be expected from the selection rule $l=19\ m+2\ n$ for a helix with 19 units in 2 turns.

R. D. B. FRASER
T. P. MACRAE

Division of Protein Chemistry, CSIRO,
Parkville (Melbourne),
Victoria 3052

Received May 24, 1971.

[1] Corey, R. B., and Wyckoff, R. W. G., *J. Biol. Chem.*, **114**, 407 (1936).
[2] MacArthur, I., *Nature*, **152**, 38 (1943).
[3] Bear, R. S., *J. Amer. Chem. Soc.*, **66**, 2043 (1944).
[4] Bear, R. S., and Rugo, H. J., *Ann. NY Acad. Sci.*, **53**, 627 (1951).
[5] Lang, A. R., *Acta Cryst.*, **9**, 446 (1956).
[6] Onions, W. J., Woods, H. J., and Woods, P. B., *Nature*, **185**, 157 (1960).
[7] Dobb, M. G., Fraser, R. D. B., and MacRae, T. P., *Proc. Third Intern. Wool Text. Res. Conf. Paris, 1965*, **1**, 96.
[8] Franks, A., *Brit. J. Appl. Phys.*, **9**, 349 (1958).
[9] James, R. W., *The Optical Principles of the Diffraction of X-rays*, 563 (Bell and Sons, London, 1948).
[10] Caspar, D. L. D., and Holmes, K. C., *J. Mol. Biol.*, **46**, 99 (1969).
[11] Klug, A., Crick, F. H. C., and Wyckoff, H. W., *Acta Cryst.*, **11**, 199 (1958).
[12] Fraser, R. D. B., MacRae, T. P., Millward, G. R., Parry, D. A. D., Suzuki, E., and Tulloch, P. A., *J. Appl. Polymer Sci.* (in the press).
[13] Filshie, B., and Rogers, G. E., *J. Mol. Biol.*, **3**, 784 (1961).
[14] Millward, G. R., *J. Ultrastruct. Res.*, **31**, 349 (1970).
[15] Thompson, E. O. P., and O'Donnell, I. J., *Austral. J. Biol. Sci.*, **18**, 1207 (1965).
[16] Astbury, W. T., *Proc. First Intern. Wool Text. Res. Conf., Australia 1956*, B, 202.
[17] Fraser, R. D. B., MacRae, T. P., and Miller, A., *J. Mol. Biol.*, **10**, 147 (1964).

Geometry of the Myosin Dimer

MONOMERIC myosin in aqueous solutions of high ionic strength is in rapid reversible equilibrium with a dimer species[1-3]. Because this dimer is likely to be the initiating and possibly the building unit in the formation of the thick filament of muscle, its three dimensional geometry is of considerable importance.

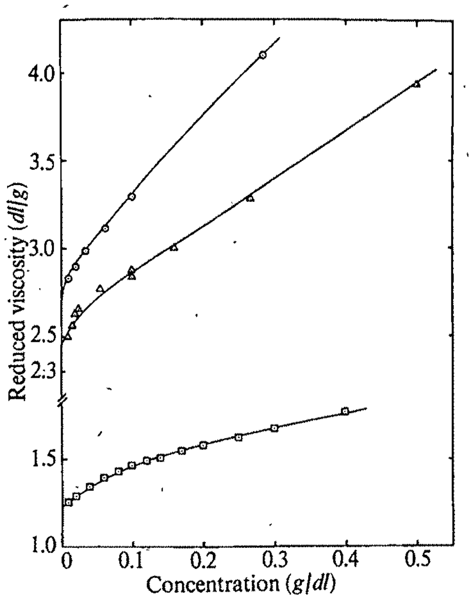


Fig. 1 The reduced viscosity as a function of concentration for myosin (△), LMM (□) and rod (○) at 5.0° C. A 10 min tryptic digestion of myosin was used to isolate LMM, while insoluble cellulose-papain was used for limited digestion of myosin to yield rod[13,14]. Both fragments were purified from low molecular weight components by fractionation on a column of 'Sephadex G-200'. All protein solutions were made up gravimetrically using solvents which had been passed through 'Millipore' filters. An Ostwald-type viscometer was used with an average shear gradient 300 s^{-1}. The outflow time for the solvent at 5.0° C was 146 s and the kinetic energy correction was found to be negligible. The solid curves represent the best computed fit to the experimental data (see text) with the corresponding parameters given in Table 1.

The solubility properties of light meromyosin (LMM) and "headless" myosin (rod), as well as X-ray[4] and electron microscopic evidence[5-8], suggest that the interaction sites responsible for the association of myosin reside primarily along the surface of its rod segment. Since the tendency for self-association of the myosin molecule is not completely repressed in high ionic strength media[2], both LMM and rod may also exist under these conditions in a rapid, reversible monomer⇌dimer equilibrium. This equilibrium may have been overlooked since both fragments, like myosin, exhibit single peaks in sedimentation velocity experiments, thus satisfying the criteria for either an apparently monodisperse non-interacting system or a rapid, reversible monomer⇌dimer equilibrium[9,10]. In the case of myosin, the presence of a rapid, reversible monomer ⇌dimer association[1] was established only after careful sedimentation equilibrium study and computer analysis[2,11,12].

We have found that both LMM and rod self-associate in high ionic strength media by a rapid, reversible equilibrium of the monomer⇌dimer type exhibited by myosin. This suggests that measurements of the asymmetry of these particles in dimerizing conditions could resolve the problem of whether the myosin dimer is formed through parallel or antiparallel association. The myosin molecule can be represented as a rod with a ball at one end. If two such structures are placed side by side in either parallel (head to head) or antiparallel (head to tail) arrangement and one of the two molecules is displaced axially with respect to its neighbour, for each displacement there are, for both LMM and rod, unique dimer structures which characterize the geometry of the parent myosin dimer. We have investigated the concentration dependence of the viscosity of LMM and rod in the ionic medium in which myosin is in a rapidly reversible monomer⇌dimer equilibrium.

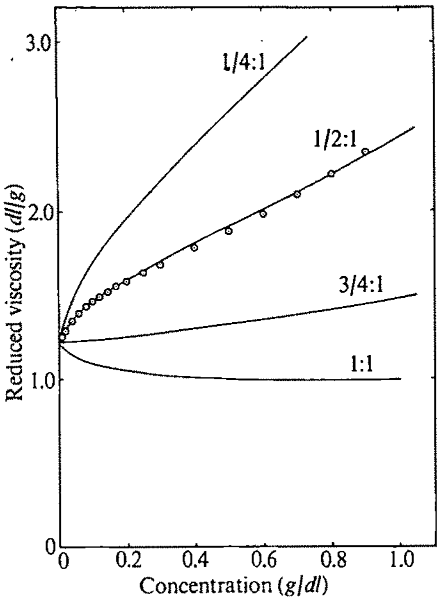


Fig. 2 The viscosity versus concentration dependence of light meromyosin (○) at 5.0° C. The solid curves represent the theoretical reduced viscosity versus concentration plots for LMM in a monomer ⇌ dimer equilibrium with respect to the specified dimer geometry. The parameters used to construct the theoretical plots are given in Tables 1 and 2. A value of 0.45 was assigned to the Huggins constant k'.

Plots of viscosity versus concentration of LMM, rod and myosin in 0.5 M KCl, 0.2 M PO_4 and 0.01 M EDTA, *p*H 7.3 (Fig. 1), all curve downwards at low concentrations in the manner expected for an associating system in which the associated species is more asymmetric than the monomer. In earlier studies[13,14], downward curvature was not detected since measurements were generally made at concentrations greater than 0.5 mg/ml. Moreover, there was no reason to assume that a monomer⇌dimer association reaction was occurring in these early studies. The downward curvatures of Fig. 1 cannot be due to binding of the protein to the capillary wall, since plots of the difference in outflow time between solution and solvent ($t-t_o$) versus protein concentration for these three particles extrapolated through zero at $c=0$[15]. The theoretical reduced viscosity versus concentration plots expected for a monomer⇌dimer equilibrium as a function of dimer geometry were calculated for light meromyosin (Fig. 2) by using the parameters given in Tables 1 and 2. The intrinsic viscosities of the dimer were evaluated from hydrodynamic studies of macromodels as described later. Assuming that a monomer⇌dimer equilibrium does exist and that the specific viscosity (η_{sp}) of the mixture will be an additive function of the specific viscosities of each of the species present, the specific viscosity of the equilibrium system can be expressed by the equation

$$\eta_{sp_{mixture}} = \sum_{i=1}^{2} \eta_{sp_i}$$

where

$$\eta_{sp_i} = \Big[[\eta_i] + k'\,[\eta_i]^2\,c_i\Big]\,c_i$$

c_i is the concentration of the i^{th} species. (*dl*/*g*) and k' is the dimensionless Huggins constant which, for non-associating systems, has values between 0.4 and 0.5.

The experimental data in Fig. 1 have been fitted using various values of K (the equilibrium constant) and $[\eta]_d$ with k' equal to 0.45. The intrinsic viscosity of monomer, $[\eta]_m$, was obtained by extrapolation of the experimentally obtained reduced viscosities to infinite dilution. The derived values of $[\eta]_d$ and K which best fit the experimental points are given in Table 1 and the curves generated from them are shown in Fig. 1. In the case of myosin the value of 10 *dl*/*g* was assigned to the equilibrium constant, this being the value found by high speed equilibrium sedimentation[2].

Table 1 Hydrodynamic Parameters derived from Reduced Viscosity versus Concentration Dependence of Myosin and its Proteolytic Fragments LMM and Rod

Particle	K(*dl*/*g*)	Theoretical (model studies) $[\eta]_m$(*dl*/*g*)	Experimental $[\eta]_m$(*dl*/*g*)	$[\eta]_d$ (*dl*/*g*)
LMM	5.52	0.910	1.23	1.90
Rod	27.5	1.73	2.65	3.42
Myosin	10.0(2)	—	2.45	3.11

To find the geometry of the dimer structure which would be consistent with the derived $[\eta]_d$ values of the three particles, we investigated the hydrodynamic behaviour of macromodels of differing dimer geometry[15-18]. The scaled up models were constructed from precision ground stainless steel rods of 0.317 cm diameter or from brass rods of 0.236 cm diameter. It was assumed that the LMM particle was 860 Å long and 20 Å in diameter and that the rod was 1290 Å long and 20 Å in diameter. Very close agreement was found between the experimental torque and that predicted by the Broersma expression for single rods of varying axial ratio[18].

The axial ratio of an effective ellipsoid having the same volume as the dimer was calculated from measurements of the torque required to rotate the specific dimer model with unit angular velocity in a solution of invert sugar of viscosity 200 poises at 22° C. The intrinsic viscosities expected for the various dimer geometries were derived from the axial ratio utilizing the Perrin[19] and Simha[20] equations and assuming apparent specific volumes of 0.710 and 0.728 for LMM (rod) and myosin, respectively[1]. The intrinsic viscosities expected for the monomeric LMM and rod particles, based on modelling studies, were smaller than those observed experimentally (Table 1). This discrepancy probably arose from the assumption of an incorrect geometry of the monomer used in the construction of our models. We have normalized the viscosities obtained from the model studies to take into account the geometry differences (Table 2).

Table 2 Intrinsic Viscosities derived from Hydrodynamic Studies of Macromodels of Differing Dimer Geometry

LMM			Rod		
Fraction of overlap	$[\eta]_d$(*dl*/*g*)	Dimer length (Å)	Fraction of overlap	$[\eta]_d$(*dl*/*g*)	Dimer length (Å)
1.0	0.613	~860	1.0	1.52	1290
0.75	1.23	1080	0.75	2.65	1610
0.50	1.82	1290	0.66	3.10	1720
0.25	2.74	1500	0.33	5.41	2150

In the case of LMM, the $[\eta]_d$ derived from the experimental curve is best fitted by a dimer geometry corresponding approximately to a 430 Å overlap. Similarly, in the case of the rod, the best dimer geometry corresponds approximately to a 780 Å overlap. Although various geometries for the myosin dimer would be consistent with the experimentally deduced dimer viscosities of LMM and rod taken separately, only one structure is consistent with the combined results from both particles. This structure is a parallel myosin dimer in which the head of one myosin molecule is displaced 430–510 Å with respect to that of its neighbour. The intrinsic viscosities of myosin should be somewhat lower than those of the corresponding rod structures because of the presence of the globular head. The value of $[\eta]_d$ for myosin (Table 1) is therefore consistent with the parallel-type geometry.

These results do not preclude the possibility that other dimeric structures exist, but in the conditions of these experiments the most likely structure is the dimer described. Moreover, Herbert and Carlson have shown, from their light scattering results, that the z-average radius of gyration of myosin has a concentration dependence that strongly supports the view that this parallel dimer is formed on self-association (unpublished results).

The geometry of the myosin dimer deduced here has interesting implications in the assembly of the thick filament of muscle. It is clear from the model of the thick filament proposed by Pepe[6] that the structure can be constructed in all its features entirely from this specific parallel-type dimer in which the head of one molecule is displaced about 430 Å with respect to its neighbour. The bare central region of the filament would be constructed by antiparallel (tail to tail) association of the dimer units. The filament would grow by head to tail addition of the dimers. Moreover, such a dimer has been invoked by one of us as the basic transducing element of skeletal muscle[21]. This proposal requires that the head of one molecule is in juxtaposition with the hinge section (trypsin sensitive region) of its neighbour.

We thank Dr T. J. Herbert and Dr F. D. Carlson for allowing us to quote from their findings before publication. We also thank Dr J. S. Barton and Miss T. Karr for assistance with the computer program and Miss S. Himmelfarb for technical assistance. This research was supported by the National Institute of Arthritis and Metabolic Diseases.

M. BURKE
W. F. HARRINGTON

Biology Department,
The Johns Hopkins University,
Baltimore, Maryland 21218

Received April 22, 1971.

[1] Godfrey, J. E., and Harrington, W. F., *Biochemistry*, **9**, 886 (1970).
[2] Godfrey, J. E., and Harrington, W. F., *Biochemistry*, **9**, 894 (1970).
[3] Herbert, T. J., and Carlson, F. D., *Biophys. Soc. Abst.*, **11**, 198a (1971).
[4] Huxley, H. E., and Brown, W., *J. Mol. Biol.*, **30**, 383 (1967).
[5] Huxley, H. E., *J. Mol. Biol.*, **7**, 281 (1963).
[6] Pepe, F. A., *J. Mol. Biol.*, **27**, 203 (1967).
[7] Cohen, C., Lowey, S., Harrison R. G., Kendrick-Jones, J., and Szent-Györgyi, A. G., *J. Mol. Biol.*, **47**, 605 (1970).
[8] King, M. V., and Young, M., *J. Mol. Biol.*, **50**, 491 (1970).
[9] Gilbert, G. A., *Disc. Faraday Soc.*, **20**, 68 (1955).
[10] Gilbert, G. A., *Proc. Roy. Soc.*, A, **250**, 377 (1959).
[11] Yphantis, D. A., *Biochemistry*, **3**, 297 (1964).
[12] Roark, D. E., and Yphantis, D. A., *Proc. NY Acad. Sci.*, **164**, 245 (1969).
[13] Young, D. M., Himmelfarb, S., and Harrington, W. F., *J. Biol. Chem.*, **239**, 2822 (1964).
[14] Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H., *J. Mol. Biol.*, **42**, 1 (1969).
[15] Reisler, E., and Eisenberg, H., *Biopolymers*, **9**, 877 (1970).
[16] Kuhn, H., and Kuhn, W., *J. Polymer Science*, **9**, 1 (1952).
[17] Haltner, A. J., and Zimm, B. H., *Nature*, **184**, 265 (1959).
[18] Broersma, S., *J. Chem. Phys.*, **32**, 1626 (1960).
[19] Perrin, F., *J. Phys. Radiat.*, **5**, 497 (1934).
[20] Simha, R., *J. Chem. Phys.*, **13**, 188 (1945).
[21] Harrington, W. F., *Proc. US Nat. Acad. Sci.*, **68**, 685 (1971).

Extended Sleep and Performance

MOST studies of the psychological consequences of sleep have been concerned with the way in which sleep deprivation decreases performance[1,2]. Little has been done to discover the relationship between extended sleep and performance. It may therefore be of interest to describe a study in which unnaturally extended sleep has been found to be followed by decreased performance in certain tasks.

Twelve normal male college students, who regularly slept 7–8 h nightly, were requested to abstain from drugs including alcohol for at least 5 days before their first night in the experiment and until after the last. They typically averaged 7–8 h of sleep per night according to their sleep logs. On the days of the experiment latency from the last meal was controlled and subjects were instructed not to nap or to drink caffeinated beverages after 1800 h and to maintain their usual level of physical activity. Subjects were informed that the purpose of the experiment was to investigate the relation between sleep and personality.

An adaptation night preceded 2 control nights of habitual sleep and 2 nights of extended sleep with a 1 day recovery period between the last two conditions. Subjects slept in pairs and were always awakened at 0900 h following both conditions, but were not told this, and were served 6 ounces of fruit juice as a partial nutritional control for blood sugar level. In the extended sleep condition subjects retired at 2200 h and in the control condition they were in bed by 2200 h engaged in quiet activities until 0100 h when they were requested to sleep.

Calculation and vigilance tasks similar to those used by Wilkinson *et al.*[3] and a complex motor performance (pinball, Gottlieb "swing along") task were administered as performance measures at a fixed time to control for circadian effects. Order of presentation for the pinball and vigilance tasks as well as conditions of sleep were counterbalanced across subjects. The 50 min calculation task which involved adding 100 columns of five two-digit numbers was given 30 min after awakening. The number of sums added incorrectly and the time required to complete it was scored. Following the calculation task a 45 min pinball task and a 45 min version of the Wilkinson vigilance task[2] were administered simultaneously and alternately to each of the pair of subjects. For the vigilance task the measure of performance taken was the combined number of signals missed (omissions) and incorrectly detected (false reports). For the pinball task average score per game was scored, efficient performance being represented by a high score. Electroencephalographic, electromyographic and electro-oculographic activity was recorded and the records scored according to standard procedures[4].

Table 1 shows the number of minutes spent in each sleep stage, total sleep and wakefulness time and total time spent in bed during both nights of each sleep condition. Because of technical difficulties, the adaptation night was substituted for one of the control nights in computing the various means in Table 1. On nights of extended sleep, the mean time allowed for sleep was 10.6 h and, of this, subjects slept 9.1 h on average, with a range of 7.5–10.7 h. Most of the time spent in bed awake on these nights was generally attributable to long sleep latencies which averaged 47 min, a smaller portion of this period being due to terminal wakefulness, which averaged 21 min. On control nights the mean time allowed for sleep was 7.6 h and, of this, subjects slept 7.1 h on average with a range of 6.2–7.8 h. Wakefulness on these nights was minimal for most subjects. Comparisons between the two conditions for the amount of time spent in each sleep stage were reported previously[5].

Decrements in performance with extended sleep were significant for two measures: combined number of omissions and false reports on the vigilance task (Table 2; $P<0.005$, one-tailed Wilcoxon test) and average score per game on the pinball task (Table 2; $P<0.025$, one-tailed Wilcoxon test). There were no significant differences in performance between the control and extended sleep condition on the calculation task for either errors or speed of calculation (control condition: mean errors = 5.7, s.d. 3.53, mean speed = 25.4 min, s.d. 6.4; extended sleep condition: mean errors = 6.3, s.d. 2.03, mean speed = 24.2 min, s.d. 3.5).

It seems unlikely that the effect of prolonged sleep was due to variations in nutritional intake or less activity consequent to

Table 1 Sleep Latency, Wakefulness, Minutes Spent in Each Sleep Stage, Total Sleep Time and Total Time in Bed for Control (C) and Extended Sleep (E) Conditions

Subject	Sleep latency (min)		Wakefulness during sleep (min)		Terminal wakefulness (min)		Stage 1		Stage 2		Stage 3–4		Stage REM		Total sleep time (h)	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
1	2	35	17	3	0	3	48	54	201	252	85	86	100	169	7.2	9.4
2	10	11	0	6	80	7	24	26	173	254	79	118	93	192	6.2	9.9
3	3	110	21	8	12	67	19	30	190	262	67	68	112	88	6.5	7.5
4	0	107	18	21	0	0	30	32	197	212	72	103	110	161	6.8	8.5
5	7	45	1	5	0	19	31	25	217	296	86	103	111	140	7.7	9.4
6	0	32	37	8	22	68	38	24	205	271	95	104	76	138	6.8	8.8
7	22	27	18	86	0	13	45	65	254	251	61	104	57	87	7.0	8.4
8	2	14	46	16	0	18	14	34	155	297	145	82	99	166	6.8	9.7
9	2	2	3	10	0	0	22	33	247	288	97	124	103	194	7.8	10.7
10	3	89	9	7	0	0	26	21	216	290	95	83	126	161	7.7	9.3
11	2	54	13	31	8	17	34	53	191	227	122	121	94	135	7.3	8.9
12	2	36	4	74	0	4	24	70	185	293	141	81	107	75	7.6	8.7
Means	6	47	16	22	10	18	30	39	203	266	95	98	99	142	7.1	9.1

a prolonged period in bed, for there were controls for these factors, but it is possible that the effect is related to sleep onset occurring at an earlier phase of the circadian rhythm. Because wakefulness tended to increase in the extended sleep condition, this might account for the decrements in performance. This increase, however, was primarily in sleep latency at the beginning of the night. Increased wakefulness during sleep increased at a rate of only 0.2 min/h. The increase in terminal wakefulness of 8 min does not seem to be a crucial factor, especially because testing on pinball and vigilance tasks did not begin until 75 min after subjects rose from bed. As in sleep deprivation experiments[6] and a previous study on extended sleep[2], accuracy on the subject-paced calculation task was insensitive to changes in amount of sleep, whereas the experimenter-paced pinball and vigilance tasks proved to be sensitive measures of impaired performance following manipulation of sleep variables.

Table 2 Average Performance on the Vigilance and Pinball Tasks after Two Nights of the Control (C) and Extended Sleep (E) Conditions

Subject	Combined errors of commission and omission on the vigilance task		Average score per game on the pinball task	
	C	E	C	E
1	7.5	8	592	643.5
2	9	8.5	557.5	583.5
3	14	15	464	516.5
4	4	1.5	559.5	550
5	11.5	21	571	515.5
6	10.5	15.5	654.5	516
7	3	9	638	378
8	25.5	38	525	469
9	2.5	10	533	481
10	10.5	10.5	531.5	522.5
11	9.5	16	586	505.5
12	20.5	41	548	490.5
S.D.	6.8	12	51.2	63.7
Means	10.6	16	563.5	514

It is well known that sleep deprivation impairs waking behaviour[7]. This investigation indicates that deviating from a habitual regime of sleep by extending sleep is detrimental to subsequent waking behaviour as well. We suggest that any general theory of sleep should attempt to explain both phenomena.

This study was supported in part by the US Public Health Service, Environmental Control Administration. We thank Drs Ralph Berger, Barry McLaughlin and Emanuel Lask for advice and assistance.

JOHN M. TAUB
GORDON G. GLOBUS
ERIC PHOEBUS
ROBERT DRURY

Department of Psychiatry and Human Behavior,
College of Medicine,
University of California,
Irvine, California 92664

Received August 3, 1970; revised March 25, 1971.

[1] Globus, G. G., *Psychosom. Med.*, **31**, 528 (1969); Globus, G. G., in *Intern. Psychiat. Clin.* (edit. by Hartmann, E.), 78 (Little, Brown, Boston, 1970).

[2] Taub, J. M., and Berger, R. J., *Psychon. Sci.*, **16**, 204 (1969).

[3] Wilkinson, R. T., Edwards, R. S., and Haines, E., *Psychon. Sci.*, **5**, 471 (1966).

[4] Rechtschaffen, A., and Kales, A. (eds), *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects* (US Government Printing Office, Washington, 1968).

[5] Phoebus, E., Taub, J. M., Globus, G. G., and Drury, R., *Percept. Motor Skills*, **31**, 526 (1970).

[6] Williams, H. L., Lubin, A., and Goodnow, J. J., *Psychol. Monog.*, **73**, 26 (1959).

[7] Wilkinson, R. T., in *The Physiology of Human Survival* (edit. by Edholm, O. G., and Bacharach, A. L.), 399 (Academic Press, New York, 1965); Wilkinson, R. T., in *Progress in Clinical Psychology* (edit. by Abt, J. A., and Reiss, B. F.), 28 (Grune, New York, 1969); Johnson, L. C., in *Sleep: Physiology and Pathology* (edit. by Kales, A.), 206 (Lippincott, Philadelphia, 1969).

[8] Jones, H. S., and Oswald, I., *Electroencephalogr. Clin. Neurophysiol.*, **24**, 378 (1968).

Single Cell Protein and the Exploitation of Mutant Algae lacking Cell Walls

MICROBIAL cells are potentially attractive sources of protein food, but this usefulness is at present limited, not merely by technical and economic difficulties[1–4], but also because the indigestibility of the cell walls limits the extent to which they

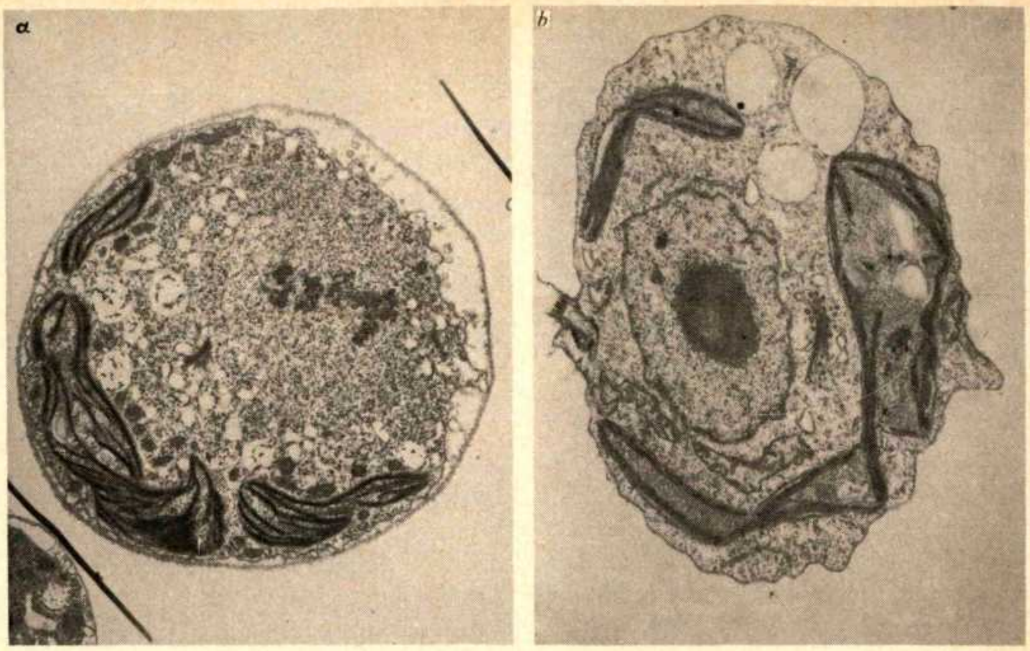


Fig. 1 Longitudinal section of (*a*) a wild type cell, (*b*) a mutant CW15, lacking a cell wall. Note cell is bound only by a plasma membrane. (Electron micrograph ×7,200.)

can be incorporated directly into human food. Indeed it has been suggested that microbial cells[5] and the alga *Chlorella*[6] cannot be used in human food without removal of the cell walls. For algae, there are two ways of overcoming this difficulty—either the material can be fed to ruminants, or mechanical, chemical or enzymatic techniques[7] can be used for the fractionation and removal of the cell walls. Enzymatic methods are as yet inefficient and, whereas industrial scale production in Czechoslovakia involves mechanical disruption of the algal cells with Ballotini beads, Japanese commercial production involves chemical extraction. Although all these methods are easy and efficient on a laboratory scale, on an industrial scale problems of fractionation are as formidable as those of cultivation[2]. Furthermore, the cost involved militates against a more general utilization of this food source. There is now evidence that by genetic manipulation it is possible in some instances to overcome these problems posed by the presence of the cell wall.

Table 1 Amino-acid Composition of Wild Type Cells and of a Mutant Strain CW15 Lacking Cell Walls

Amino-acid	mg in WT	mg in CW15
Lysine	0.78	0.85
Histidine	0.21	0.29
Arginine	0.81	0.86
Aspartic acid	1.11	1.21
Threonine	0.60	0.64
Serine	0.54	0.53
Glutamic acid	1.30	1.27
Proline	0.60	0.57
Glycine	0.68	0.64
Alanine	0.92	0.90
Half cystine	0.09	0.07
Valine	0.76	0.73
Methionine	0.35	0.49
Isoleucine	0.61	0.48
Leucine	1.08	1.08
Tyrosine	0.50	0.55
Phenylalanine	0.67	0.63
Total protein	11.61 mg	11.79 mg

20 mg of acetone–ether washed cells was used in each instance.

Following treatment with the chemical mutagen N-methyl-N′-nitro-N-nitrosoguanidine we have isolated in the unicellular green alga *Chlamydomonas reinhardi* a large number of mutants with various defects in cell wall biogenesis (ref. 8 and unpublished work). These are readily distinguished from wild type cells by their altered colony morphology. A few of these mutants produce no detectable quantities of cell wall (Fig. 1); in spite of this, some can show comparable growth rates to the wild type and in all respects they seem to be perfectly viable and normal when grown in liquid culture. Furthermore the cells retain ample mechanical strength to allow aeration, stirring, and harvesting by centrifugal separation. Since the nature and quantity of protein are of primary relevance in the context of single cell protein production comparative assays of protein content and of amino-acid composition of one of the mutant strains and of the wild type were undertaken. The results are given in Table 1. The percentage of protein in the wall-less mutant is comparable with that in the wild type although the wall itself does contain protein. The total weight of the wall, however, is small in comparison with that of the whole cell. The differences in amino-acid composition between the two strains are of little significance.

The potential value of such mutant forms of green algae lacking cell walls is immediately apparent in that it overcomes one of the most serious problems facing those who have sought to exploit them as a source of single cell protein[9]. While the particular mutants of *C. reinhardi* may have no significance as sources of single cell protein there is clearly every incentive for undertaking programmes to induce and isolate comparable mutant strains, not only of the algae now being grown on a commercial scale[4] but also of other microorganisms.

I thank Mr M. Rees for amino-acid analyses, and Miss K. Plaskitt for electron micrography.

D. Roy Davies

John Innes Institute,
Colney Lane, Norwich NOR 70F

Received May 19; revised June 9, 1971.

[1] Wilkinson, F. F., *Soc. Gen. Microbiol. Symp.*, **21**, 15 (1971).
[2] Vincent, W. A., *Soc. Gen. Microbiol. Symp.*, **21**, 47 (1971).
[3] Vilenchich, R., and Akhtar, W., *Proc. Biochem.*, 41 (February 1971)
[4] Enebo, L., *Chem. Eng. Proc. Ser.*, **65**, 80 (1969).
[5] Shapira, J., and Mandel, A. D., *Nature*, **217**, 1061 (1968).
[6] Mitsuda, H., Yasumoto, K., and Nakamura, H., *Chem. Eng. Proc. Ser.*, **65**, 93 (1969).
[7] Hedenskog, G., Enebo, L., Vendlova, J., and Prokes, B., *Biotechnol. Bioeng.*, **11**, 37 (1969).
[8] Davies, D. R., and Plaskitt, K., *Genet, Res.*, **17**, No. 1, 33 (1971).
[9] Sparks, R. E., *Chem. Eng. Proc. Ser.*, **65**, 120 (1969).

BOOK REVIEWS

Success Stories

Contemporary British Chemists. By W. A. Campbell and N. N. Greenwood. Pp. 286. (Taylor and Francis: London, June 1971.) £5.

In a technological, numerate, post-Dainton society there is great advantage in producing graduates with some understanding of a physical science such as chemistry, without expecting them to rely on it too heavily in their post-university careers. To ignore details of the second law of thermodynamics matters little, provided there is some notion of the thought processes used in deriving it. Chemistry has become a core subject or, according to one eminent chemist in industry, has ceased to exist.

This point of view represents a revolution against the consensus of the past. Until the late fifties of this century, professors of chemistry walked ten feet high. The aspiring young chemist, thankful for intelligence and luck which allowed him to be accepted for and surviving an honours course, emerged at the end bursting with facts, more or less well digested, but certain that society will reward him appropriately for the years spent in dark satanic labs. These beautiful simplicities eroded under the twin pressures of facts and industrial forces. As the number of facts multiplied, it became simply impossible to digest more than a fraction in the three to four years available. A substantial portion of industry began its clamour for more and more of new style scientists, whose education would render them more suitable for managerial positions.

The response of chemical educators has been the notion of chemistry not as a vocational but as an educational discipline. Improvements in teaching methods both at school and at university resulted in enormously improved curricula and methodologies, and the new views have obtained if not universal at least widespread approval. Yet a paradoxical situation remains. To make science as attractive as possible to schoolchildren and undergraduates, their enthusiasm has to be aroused, but not to such a pitch that they should elect, in the vast majority of cases, to carry on with science after graduation. For the individual, education in science represents no longer a vocation for the discipline, but rather one for worldly success. Irrespective of any other consideration, the functional success of such an argument requires that for those who study chemistry, or the sciences generally, the resulting qualification should open all doors from Whitehall to Throgmorton Street and offer a level of comfort the young aspirant would like to be accustomed to.

The main argument of this book is that it is indeed possible to scale professional heights with a chemistry degree, even in fields that have little to do with chemistry itself. Its method is to present scientific and personal biographies of 145 successful people—all but five of them men—based on 180 questionnaires sent out by the authors. While the aim is excellent and the presentation of the book first class, the very complexities of changing society have introduced a number of difficulties. The selection of top people, not even within a profession but based loosely on it, is always a hazardous procedure: since under our system it is extremely unlikely for anybody to become obviously eminent very early in life, the success stories are products of the very educational ideas that have lost ground. Thus the vast majority of the 145 are professors of chemistry: undeniably important figures in the scientific landscape but hardly calculated to convey the message of a wide variety of career choices. The successful chemist appears to be doing what chemists have always been doing: research. *Se monumentum requiris, circumspice.* Yet this year the highest level of graduate unemployment since the war will certainly force all new science graduates to explore careers outside the confines of their discipline.

P. J. FARAGO

Astrochemistry

Geochemical Exploration of the Moon and Planets. By I. Adler and J. I. Trombka. Vol. 3. Pp. x+243. (Springer-Verlag: Berlin and New York, 1970.) 58 DM; $16.

This book represents an attempt to review past and present efforts to determine the geochemical composition of the Moon and planets. It is essentially concerned with the techniques of geochemical exploration and, as such, could have been given a more specific and informative title. That it contains a fairly extensive review in its 239 pages cannot be denied; however, the organization of this review leaves much to be desired.

The book is arranged, or more accurately, is arbitrarily divided, into six chapters. The first chapter is an introduction which briefly summarizes some of the scientific problems to be solved by exploration of the Moon and planets. The next chapter is entitled "Instruments Used for Compositional Exploration", and describes the instrumentation and experimental results of the Surveyor, Ranger and early Luna spacecraft. The third chapter, "Instruments and Techniques under Development", contains much of the meat of the book in terms of the range of techniques discussed. The authors describe experimental arrangements developed, and being developed, to carry out chemical analysis of the planets using X-ray spectroscopy, X-ray diffraction, neutron activation and mass spectrometry in surface experiments, and X-ray, γ-ray, α-particle and infrared spectroscopy in orbital experiments. The fourth chapter, "Apollo Surface Missions and the Lunar Receiving Laboratory Program", is concerned with the analysis of the returned lunar samples. Presumably because of the breadth of the overall programme, the discussion is restricted to describing NASA's Lunar Receiving Laboratory programme. This is done in unnecessary detail and makes very tedious reading. For example (when describing the Gas Analysis Laboratory), "This laboratory is located on a mezzanine above the Vacuum Laboratory on the third floor of the LRL". The whole chapter, including a description of the preliminary results of Apollo 11, seems to have been taken, with minimal editing or reorganization by the authors, from NASA reports. The fifth chapter, "Data Processing and Analysis", discusses in some detail the problems of data acquisition and analysis from remote environments. The final chapter, "Orbital and Surface Exploration Systems after Early Apollo", discusses a number of projected integrated missions incorporating and extending the techniques discussed earlier.

It is inevitable that much of the book will be rapidly outdated by events in the fast changing field of space exploration. Unfortunately, the way the book is organized accentuates this problem, and in reading it I found myself constantly thinking of how the arrangement of topics could have been improved to make something of more lasting value. The authors do not seem to have set out with a well defined purpose for the book, and this is probably its major flaw. As an honest-to-goodness reference manual of current techniques for space exploration, it is too diffuse. The discussions of scientific results are also scattered about the book and of necessity too cursory to be of any value. As a historical record it suffers from the fact that much of the record is of projected experiments.

People seeking an introduction to the techniques of remote geochemical exploration may find this book useful. They will not find that it has the polish of a good textbook. Perhaps that is too much to expect of an up-to-date review.

GRENVILLE TURNER

Search for Spectra

The Spectra and Structure of Simple Free Radicals. By Gerhard Herzberg. Pp. xi+226. (Cornell University: Ithaca and London, June 1971.) £5.25.

THE 1968 Baker lectures at Cornell University by Gerhard Herzberg, now published in this slim volume, fulfil the ambition of the author to write an introduction to molecular spectroscopy. Few would argue that he is the greatest exponent of the subject still in active research, and on the basis of his three comprehensive books one might suppose that anything he does not know about molecular spectroscopy is not worth knowing.

The book has a title and a sub-title: an introduction to spectroscopy. The latter gives a better representation of its character because free radicals are used only to illustrate general features of molecular spectroscopy and little emphasis is placed on any unique features. In fact the definition of radical which has been adopted ("any species with a short life in the gas phase under normal laboratory conditions") would not be generally accepted in other branches of chemistry. For example, the molecule C_2 is included but not NO. The omission of detailed discussion of electron spin resonance makes the title slightly inappropriate.

The introductory chapter covers some of the special problems that arise in the measurement of the spectra of transient species, although the book as a whole has a theoretical rather than an experimental approach to the subject. There is an enjoyable account of the search for the spectrum of CH_2 which was stimulated by the observation in 1941 of some unidentified lines in the spectrum of a comet. These were finally assigned to molecule C_3 but CH_2 was eventually tracked down in 1961 in the flash photolysis of diazomethane.

The main part of the book is divided into three sections: diatomics, linear polyatomics and non-linear polyatomics. As an indication of the level of the book, the diatomic chapter gives an account of rotation–electronic coupling, the symmetry properties of rotational levels, of Hund's coupling cases *a* and *b* (but not the less important cases) and the rotational structure of electronic bands. It can therefore be seen that the book goes much further than is usual in undergraduate spectroscopy texts.

The chapters on polyatomic radicals give very good accounts of vibronic interactions (the Renner and Jahn–Teller effects) and of vibration–rotation interactions (Λ doubling and Coriolis interaction). There is also an extensive discussion of molecular orbital correlation diagrams and their relevance to the understanding of state symmetries and molecular shapes.

Finally the book is completed by a short chapter on radiationless processes: dissociation, predissociation and recombination. A description of the mechanism of these processes is given and a discussion of selection rules, but no reference has been made to the recent work on predissociation line widths which has been undertaken in several different laboratories.

The book has been elegantly produced and is a pleasure to read. It is a pity that it is so highly priced, as it should be widely used as a textbook for graduate courses in spectroscopy.

J. N. MURRELL

Chemical Mutagenesis

Chemical Mutagenesis in Mammals and Man. Edited by F. Vogel and G. Röhrborn. Pp. xiv+519. (Springer-Verlag: Berlin and New York, 1970.) 124 DM; $34.10.

VARIOUS expert committees have recently emphasized the critical need for mutagenicity testing of chemicals before their registration for commercial or other uses. These committees include the Task Force on Research Planning in Environmental Health Science, March, 1970; the Food and Drug Administration Advisory Committee for Protocols on Safety Evaluation, Panel on Reproduction Studies in the Safety Evaluation of Food Additives and Pesticide Residues, December, 1969; and the Advisory Panel on the Mutagenicity of Pesticides, the Secretary's Commission on Pesticides and their Relationship to Environmental Health, December, 1969. Such recommendations not only reflect critical concerns as to the public health implications of chemical mutagenesis, but also the recent development of a variety of test methods, data from some of which have a high degree of presumptive human relevance.

Chemical Mutagenesis in Mammals and Man is the first of several recent books addressed to this problem with particular emphasis on the importance of *in vivo* mammalian systems in testing for chemical mutagens. This book is a compilation of papers presented at a symposium held on October 7, 1969, in Mainz, Germany. The editors, Drs Vogel and Röhrborn, are admirably qualified for this purpose, as they are internationally recognized and respected workers in the respective fields of population genetics and mutagenicity testing in mammals.

The book contains thirty chapters by authors who include many well known authorities in chemical mutagenesis, including Barthelmess, Ehling, Kuhlmann, Legator, Malling, and Chu. Most chapters deal comprehensively with the specifics of mutagenicity testing in mammalian systems. The editors have also contributed chapters on genetic monitoring of human populations and on the dominant lethal assay in rodents, and also a very useful summary. The stylistic heterogeneity of the book, which the editors have wisely preserved, makes for dynamic and stimulating reading.

The editors are to be warmly congratulated for their timeliness in publishing this volume, which will be of great value, not only to research workers in the field, but also to the chemical and pharmaceutical industries, and to government agencies.

SAMUEL S. EPSTEIN

Designing Libraries

New Library Design: Guide Lines to Planning Academic Library Buildings. By Stephen Langmead and Margaret Beckman. Pp. ix+117. (Wiley: Toronto and Chichester, August 1971.) £5.75.

THE ideas for this book came from the authors' collaboration as project architect and systems librarian for the McLaughlin new library building at the University of Guelph, Canada. They have subsequently acted as consultants to several other Canadian libraries and they lecture in library architecture at the Graduate School of Library and Information Science at the University of Western Ontario. Experience taught them that there are certain steps fundamental to the planning process which are ignored at peril and others which, while not absolutely essential, greatly smooth the passage of a plan through its various stages. In consequence they have set down a systematic schedule of steps to be taken from beginning to end of the planning of a large university library. The resulting guidelines enable anyone planning a new academic library to identify the problems which will need to be solved, make it quite clear who will need to be consulted and why, and provide many of the factual details and most up-to-date accepted standards which both architect and librarian will need. As an illustrative example the McLaughlin Library and its planning brief are described, with added diagrams and photographs.

This must surely be a classic case of joint consultation, the very antithesis of the Columbia Law Library which was described as "reflecting, by its symmetry and balance, the triumph of the Architect over the Librarian". Here the librarian takes his proper place as a qualified person who is well able to organize and administer the extremely complex functions of a modern library intent on communication as well as conservation, who understands the needs of faculty and students, and who controls a management system which designs and evaluates his services. The architect is a professionally competent adviser, planner, designer, coordinator, cost estimator, arbitrator and supervisor. The two clearly respect each other's contribution. The library design favoured is a completely flexible modular plan, flexibility being carried to the point of specially designed handling equipment which can move a fully loaded bookstack.

There is nothing essentially new about library design in the book. The novelty exists in putting it all down on paper so concisely and usefully to produce a book which will take an immediate place as a standard text.

W. Ashworth

Processing Pictures

Picture Language Machines. Edited by S. Kaneff. (Proceedings of a Conference held at the Australian National University, Canberra, February 24–28, 1969.) Pp. xiv+425. (Academic: London and New York, December 1970.) £4.50.

Although the title of this book reflects an overall concern for some general understanding of what would characterize an acceptable picture processing system, individual papers span a variety of topics. The collection starts with four reviews, by Narasimhan on picture languages, Bobrow on question–answer systems, Clowes on a layman's guide to Chomsky, and Jacks on design principles for an industrial graphics system. Then jackets are taken off and the fun begins. The general effect of the book is that of a broadside. Away with the restrictive attitudes of classical pattern recognition with its naive hope for classification based on independent feature extraction. In with the view that any picture processing system worthy of its salt must come to grips with the full range of deep problems associated with systems which can bring to bear an open ended corpus of knowledge about depicted domains to the interpretation of what is depicted. It is this last theme, aspects of which are referred to as "an ability to appropriately articulate structure" (paraphrasing Clowes) and an "ability to use models which are isomorphic to those of the human interacting with the system" (paraphrasing Stanton) which is emphasized in various forms in different papers. It is this theme which motivates the inclusion of the particular review papers and also interesting papers on topics such as natural language behaviour, the role of learning in picture processing, and on dynamic data structuring.

Several stimulating discussions have the theme of the desirability of problems posed by picture processing systems which would make use of descriptions to generate appropriate and different algorithmic behaviour in different contexts.

I found the collection heavy going, largely because of the prolix style of many of the authors—why use a phrase when a paragraph will do? By contrast, the unedited discussions at the end of each paper have an Alice in Wonderland flavour, but like Alice they are sometimes remarkably rewarding.

Nevertheless, given that one is not reading to obtain answers but rather to seek for sensible questions, and those not just about picture processing, the book is well worth the effort involved in reading much of it two or three times.

E. W. Elcock

Flying Mammals

Biology of Bats, Vol. 1. Edited by William A. Wimsatt. Pp. xii+406. (Academic: New York and London, December 1970.) £11.65.

Biology of Bats, Vol. 2. Edited by William A. Wimsatt. Pp. xv+477. (Academic: New York and London, January 1971.) £12.15; $26.

Every biologist secretly believes that his "group" is underrated by the scientific community which fails to appreciate the variety of its forms, the beauty of their adaptations and the importance of the problems posed. Those who study bats are of course quite different: they know their subjects get a raw deal. One has only to analyse any general zoology text or university course: bats fly and thus have unique skeletal modifications (differing from those of pterodactyls and birds) and most use echolocation to catch insects (the rest eat fruit). But whoever quotes a desert bat in an essay on water regulation?

The reasons for all this are quite simple. In addition to the irrational popular repugnance or indifference due to lack of confrontation, there is a natural reluctance to tackle the large and diffuse literature. The lack of a general account of bat biology has been remarkable. Allen's excellent monograph of 1938 was republished, unaltered twenty-four years later and still seems to be in demand after a third of a century. Yet its treatment is semi-popular and necessarily superficial; more recent texts, in German and French, are even shorter.

The deficiency is now being made good by a treatise, edited by William Wimsatt, of which two volumes are now published. It would be unfair to try to assess the scope of the work at present—a third volume and possibly a fourth are planned but no information is yet available on their probable contents. One can only hope at this stage that current omissions will be made good when the work is complete. The present two volumes contain seventeen essays by twelve authors, all American and all authoritative. Each volume carries an introduction by the editor, who has not yet contributed to the text, and detailed author and subject indices. They are beautifully produced, with copious illustrations of high quality, except for some old engravings which have not reproduced well, and one diminutive pair of X-ray photographs.

The first volume has ten chapters on a wide variety of topics and they are necessarily very variable in length. The first and longest chapter, on bat origins and evolution by Jepsen, deserves special mention. It contains a more detailed description of *Icaronycteris index* (the oldest and most complete fossil bat) than has hitherto been published and is illustrated by numerous superb stereo photographs. For many workers this material alone will justify the purchase of this volume. In addition the author gives a general discussion of the Chiroptera that forms an excellent introduction to the whole treatise. The general taxonomy of the order has not changed since Miller (1907), but Jepsen adds some stimulating ideas on relationships. He also argues that flying and gliding make different demands on the body so that bats could never have been gliders.

The second chapter is on karyotypic trends by Baker, and then follow three chapters by Vaughan, giving detailed descriptions of the skeletal and muscular systems respectively, and a discussion of flight patterns and aerodynamics. This last is surprisingly brief although new work is known to be in hand. The remaining five chapters are diverse. Orr writes on development, prenatal and postnatal, giving only two and a half pages to prenatal aspects, which will presumably be expanded later under reproduction. Griffin reviews migration and homing, Davis discusses hibernation ecology and physiological ecology and Lyman deals with thermoregulation and metabolism, but not the metabolic and thermal problems of active flight. Finally Rosenbaum gives a detailed comparative account of the urinary system.

The second volume, although longer, contains only seven chapters, all but the last being closely related. Quay, writing on the integument and its derivatives, mentions, but does not describe, the elaborate and varied facial structures. Sensory aspects of the skin lead naturally into the central nervous system, discussed by Henson, the peripheral nervous system (Quay), the ear and audition (Henson), vision, olfaction and taste (Suthers) and finally to a brief account of the pineal by Quay. Other endocrine aspects will presumably follow later. The volume concludes with an enormous consideration (131 pages) of bats in relation to human health. While no one, least of all those who work with living bats, should doubt the importance of this subject, its place in this volume and the mystery about future plans suggest poor coherence in the whole work.

But for the rest we must (and will eagerly) wait and see. I have high hopes and whatever the outcome one thing seems certain: that the current increase in work on bats, which has occurred in spite of the lack of a book of this sort, will gain much impetus now that it has appeared. DAVID PYE

Character and Attitude

Character Structure and Impulsiveness. By David Kipnis. (Personality and Psychopathology: a Series of Monographs, Texts and Treatises.) Pp. xi+133. (Academic: New York and London, March 1971.) $7.95; £3.75.

Attitude and Attitude Change. By Harry C. Triandis. (Wiley Foundations of Social Psychology Series.) Pp. xiii+232. (Wiley: New York and London, April 1971.) £3.50 boards; £1.65 paper.

THE first of these books reports a series of interrelated studies, all based on the author's scale of impulsiveness, a scale which correlates both with Gough's socialization scale and the extraversion scale of the MPI. The scale concentrates on masculine outdoor hobbies, and all studies are therefore wisely limited to male subjects. Kipnis starts with the interesting and important idea that diagnostic instruments in psychology should be used like diagnostic tests in medicine, that is not only to describe the current state of a person but to show how to improve the score of those with a deficit on the test.

After a series of studies designed to establish the validity of his instrument he implements this action-orientation in the following way: high scorers on impulsiveness are underachievers in college examinations; they are also highly motivated by material incentives. Kipnis, accordingly established an ingenious field experiment with proper controls, in which impulsive underachievers were paid money for doing their mathematics course. The results show that the more intelligent impulsive students improved their grade in this condition, which had, however, no effect on the less intelligent. Although the role of intelligence as a moderator of other personality predispositions is interesting, it receives no theoretical treatment in this book, which is altogether deficient on theory. Furthermore, the effect disappeared when payment stopped. The intended "cure" may, for all one knows, only have strengthened the original "illness". Here, as elsewhere, nothing would have been more practical than a good theory to deal with such doubts.

The study of attitudes continues to dominate social psychology and social psychological publications. At least a dozen books on attitude and attitude change have been published during the past year. Triandis's contribution—*Attitude and Attitude Change*—is in the textbook category. As one would expect from this author, it is workmanlike, clearly written and thoroughly up-to-date. The text is refreshingly catholic in approach and goes beyond the currently most fashionable debate about consistency theories. Instructors and students will find the student assignments at the end of each chapter particularly useful; they are imaginative guides to self-instruction and encourage students to think about the underlying assumptions in attitude research. There is a bibliography of well over five hundred items. MARIE JAHODA

Quantum Text

Practical Quantum Mechanics I. By Siegfried Flügge. (Die Grundlehren der Mathematischen Wissenschaften in Einzeldarstellungen, Band 177.) Pp. xiv+341. (Springer-Verlag: Berlin and New York, 1971.) 70 DM; $20.30.

Practical Quantum Mechanics II. By Siegfried Flügge. (Die Grundlehren der Mathematischen Wissenschaften in Einzeldarstellungen, Band 178.) Pp. xii+287.) (Springer-Verlag: Berlin and New York, 1971.) 60 DM; $17.40.

ANYONE who has taught a course of quantum mechanics knows the difficulty of providing practical examples which are within the mathematical competence of the students and can be completed in a reasonable time. In these two volumes, which have been designed as a unified work, will be found 219 problems, together with their solutions, which will greatly extend the repertoire. They are all of moderate length and will be accessible to any student who can recognize the Bessel equation and consult references for properties of its solutions when these are required.

The first volume deals exclusively with one-body problems without spin. Many one dimensional and three dimensional potentials are introduced and the problems of calculating wavefunctions for bound states and for elastic scattering are well represented. Applications are made to some specific situations in atomic and nuclear physics, with numerical values given to all the constants so as to emphasize the order of magnitude of the effects. The problems illustrating scattering resonances and Regge poles are especially welcome. In the second volume the problems cover a wider range and include illustrations of the introduction of spin, the interactions between two and three particles, quantum statistics and the Dirac relativistic equation with shorter sections on non-stationary problems and radiation theory. It will be very useful to have problems on tensor forces, the Tietz approximation in Thomas–Fermi theory and on helicity. Between the two volumes most of the features of elementary quantum mechanics which have to be compared with the corresponding features of classical mechanics are introduced and discussed.

Faced with so many useful problems and elegant solutions I am reluctant to turn to the limitations of this collection. It has to be said, however, that the problems are all of textbook type and deal with the solution of the differential equations for the wavefunctions and the subsequent calculation of properties. The classical techniques of mathematical analysis are emphasized. Matrix techniques, which are often better illustrations of real problems and are extensively used in modern self-consistent field treatments of atoms and molecules, scarcely appear and there is no group theory. The diagram techniques of modern perturbation theories appear briefly in only one solution. As an introduction to the practical problems of quantum theory and to the most used mathematical methods this is a very lopsided and unrepresentative compilation. As a preparation for courses on specialized aspects of quantum theory probably the examples on nuclear theory will be considered most adequate. The student interested in atomic and molecular physics, in field theory or in solid state physics will find few examples that he cannot find elsewhere.

The volumes have been well produced with many well designed graphs. The English style has some reminders of the German of the earlier edition, but the book has been rewritten. The purist will object to the abbreviation of "having no complete set of eigenvectors" to "having no eigenvectors" in some matrix problems.

For the teacher who selects carefully and can supplement from other sources, this will be a valuable and much used addition to the armoury. For the unaided student who learns best by working out examples and attempting open-ended problems the practical introduction to quantum mechanics still has to be written. G. G. HALL

CORRESPONDENCE

Tolerable Pollution

SIR,—I have read with great interest Dr Samuel S. Epstein's article entitled "Control of Chemical Pollutants" (*Nature*, **228**, 816; 1970). Many of the points made by Dr Epstein are very well taken and need emphasis. Unfortunately, however, some of the concepts that he expresses need to be examined more closely and carefully thought through.

For instance, in the determination of "efficacy" the practising physician may have entirely different concepts than the academician who is not dealing with patients on a daily basis. To the academician there is probably no place for a placebo. To the harassed physician trying to bring solace to the whining, complaining patient the placebo may have a most important, though limited, role. I remember clearly a female patient I once had of 70+ years who demanded of me a "tonic". "Tonics," it should be remembered, were practically a part of the culture of people in this age range. Finding no "tonics" in the formulary of the hospital where I was working, I prescribed an innocuous "bladder mixture" made up of potassium iodide and hyoscyamus. Much to my surprise, the patient returned at her next visit lauding the "efficacy" of this "tonic" to the skies and insisted that it be renewed. Obviously this was not a material that I should be permitted to market as "Dr E's famous tonic", but was there any harm in its use in these circumstances? Who, then, is to determine efficacy? Should flavours, colours, antirancidity materials or other such materials be permitted in foods? Do they have "efficacy"? Some might well argue that foods should be eaten only with their natural inherent flavours and colours and that the addition of artificial (or even natural) flavours or colours does not improve the food's efficacy. Rancid food should simply be thrown out. What about the present uproar over the use of tolbutamide in diabetics? Even cyclamates are thought by many physicians to have value in the treatment of obesity and diabetes. Who is to make these important determinations?

Turning to the concepts of carcinogenicity, teratogenicity, and mutagenicity, there are few people who do not believe that we should control them to the best of our ability. However, as I pointed out in my Ramazzini oration (*J. Occ. Med.*, **13**, 161; 1971), the concept of zero is a mathematical one. As our analytic techniques become more and more sensitive, it will ultimately be necessary for us to establish a "practical" zero. In essence this will mean that some finite, measurable quantity will be determined to be "tolerable". Thus a "tolerance" for carcinogens, teratogens, and mutagens will be established. Precedence for this approach has been established in the milk and water sanitation fields. Here a definite number of organisms are allowed, that is, are considered tolerable. It was recognized that to set the levels at zero organisms could well have eliminated these essentials from human consumption. Thus recognizing the impracticality of the so-called Delaney clause and devoting research efforts to a serious attempt to establish tolerances for carcinogens seems a more sensible approach than simply extending this concept to teratogens and mutagens. This approach has been taken by Truhaut and an international committee in attempting to establish a tolerance for aflatoxins in foods.

Lastly, the concept of "disinterested" scientists needs carefully to be examined. What, truly, do we mean by a "disinterested" scientist? Is he "disinterested" because he is an academician? After all, Pasteur made what is probably one of the most important discoveries to mankind when he was working for the French wine industry. Is the scientist directly engaged in or dependent for support from a federal bureaucracy "disinterested" in its perpetuation? Can a scientist's views be modified as he lives longer and becomes more mature and knowledgeable? Is this change in viewpoint due to his having lost his disinterested status?

The concepts I expressed in my editorial on environmental carcinogenesis (*Cancer Res.*, **22**, 395; 1962) seem today to need re-emphasis. We do need to control *significant* exposures of the general population to carcinogens, teratogens, and mutagens. After all, today we do not have any real comprehension of what the teratogenic or mutagenic rates are in the human race as a whole. For all we know, these rates may be on the downswing now because of man's ability to overcome some of the vicissitudes of his former existence. A rereading of the "Report on the Sanitary Condition of the Labouring Population of Great Britain" by Edwin Chadwick, 1842 (edit. by M. W. Flinn, Edinburgh University Press, 1965) should reassure us on the real progress we have made in just a little over 100 years. We still, nonetheless, live in a real world, fraught with real and imminent dangers which we must do our best to minimize in a real and practical way.

Yours faithfully,

R. E. ECKARDT
Director

Esso Research and
Engineering Company,
Medical Research Division,
PO Box 45,
Linden, New Jersey 07036

Obituary

Wendell Meredith Stanley

WENDELL MEREDITH STANLEY, Professor of Molecular Biology and of Biochemistry at the University of California, Berkeley, died suddenly, aged 66, on June 15 in Spain.

His career in biochemistry and molecular biology began at the University of Illinois in 1926 when, as a graduate student of the organic chemist, Roger Adams, he synthesized a series of fatty acids and correlated their structures and physical properties with effectiveness against the leprosy bacillus. After earning his PhD degree in 1929, Stanley went to Germany as a National Research Council Fellow in the laboratory of Professor Heinrich Wieland, where he worked on sterols of yeast. Returning to the United States in 1931, he worked for a year at the Rockefeller Institute for Medical Research in New York with cell physiologist W. J. V. Osterhout, on model chemical systems that simulated cell membranes.

In 1932, Stanley moved to the Rockefeller Institute Laboratories in Princeton, New Jersey. There he began his studies on the chemistry of viruses which culminated in 1935 with the crystallization of tobacco mosaic virus (TMV), the first of these disease agents to be obtained in such a form. Not content with the isolation and crystallization of TMV, he proceeded with a small group of colleagues to develop systematically and imaginatively an entirely new field of science: the biology, chemistry, and physics of viruses. From these efforts, and work elsewhere, the chemical composition and morphology of a variety of viruses were defined in molecular terms. Stanley's initial studies on the chemical modification of viruses later provided the foundation for inactivating viruses for vaccine production and for studies on molecular

genetics. With the onset of the Second World War, his group turned to animal viruses and their fundamental studies on the purification and characterization of influenza virus led to the preparation of a safe and effective vaccine.

Stanley joined the University of California at Berkeley where, in 1948, he founded the Virus Laboratory and built a new Department of Biochemistry which he served as Chairman until 1953. Subsequently he initiated the formation of the Department of Virology in 1958 which he chaired until 1964 when it was expanded to become the present Department of Molecular Biology. In these academic departments, Stanley promoted and fostered the blending of teaching and research in a most fruitful way. His own interests were directed toward the conquest of viral diseases and the potentialities of viruses in the induction of cancer. He encouraged research in this area and exerted considerable influence in programmes on the national level. His contributions to cancer research were recognized by two awards from the American Cancer Society, one in 1959 and another in 1963 and by his selection as President of the Tenth International Cancer Congress, Houston, Texas, in 1970.

Many honours came to Stanley over the years: elections to honorary societies, distinguished lectureships, about a score of honorary degrees, and numerous medals and prizes including the Nobel Prize in Chemistry in 1946. In his lifetime, he had three different and equally productive careers: first as a bold, imaginative scientist whose research revolutionized biological thought; second as an outstanding educational administrator who recruited, supported and inspired many young scientists; and third as a distinguished statesman who played a major role in shaping enlightened national and international policy with regard to scientific research on cancer and the role of viruses.

British Diary

Monday, September 13

Fundamentals of Software for Computer Engineers (vacation school, thirteen days) Institution of Electrical Engineers, in association with the Institution of Electronic and Radio Engineers, at the University of Manchester.

Surface Chemistry of Oxides (three-day Discussion) Faraday Society, in the Chemistry Department, Brunel University, Uxbridge, Middlesex.

Tuesday, September 14

5th Annual Solid State Devices Conference (three days) Institute of Physics and the Physical Society, in collaboration with the Institution of Electrical Engineers, the Institution of Electronic and Radio Engineers, the Institute of Electrical and Electronic Engineers, at the University of Lancaster.

Flexographic Printing Inks (7.30 p.m.) Oil and Colour Chemists' Association, at the Griffin Hotel, Boar Lane, Leeds.

Wednesday, September 15

Centrifugal Methods of Particle Size Analysis (2.30 p.m.) Society for Analytical Chemistry, North East Section and the Particle Size Analysis Group, at Newcastle University, Room L.101, Mertz Court, Queen Victoria Road, Newcastle-upon-Tyne.

Formulation of Two Epoxy Paints (4.30 p.m.) Mr A. McWilliam, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1. (Student Lecture.)

Thursday, September 16

Insect/Plant Relationships (two-day symposium) Royal Entomological Society of London, at the Imperial College of Science Mechanical Engineering Lecture Theatre, Exhibition Road, London SW7.

Modern Analytical Techniques for Bio-Active Substances (two-day meeting) Society for Analytical Chemistry, at the University of Surrey, Guildford, Surrey.

Friday, September 17

The Work of the Government Chemist (7 p.m.) Dr H. Egan, Society for Analytical Chemistry, Western Region, in the Chemistry Department, University of Bristol.

Sunday, September 19

Electrical Measurement Practice (vacation school, thirteen days) Institution of Electrical Engineers, in association with the Institution of Electronic and Radio Engineers, at the University of York.

HOW TO BUY NATURE

Volumes start in January, March, May, July, Septembe and November, but subscriptions may begin at any time.
The direct postal price per subscription is:
12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature + Nature Physical Science	**£24**	**$83**	**$90**
Nature + Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, September 10 1971

NATURE Volume 233
SEPTEMBER 17 1971

Reform by Inches or Half-inches

It is still an open question whether the British Association for the Advancement of Science will manage to survive the years ahead. The steps taken at the Swansea meeting of the association two weeks ago (see *Nature*, **233**, 80; 1971) to modernize the administration of the association are steps in the right direction, but they are manifestly small and even timid steps. There is a chance that the association's management might have moved even more cautiously if it had not been for the unaccustomed toughness of the voluntary committees, sixteen or so of them, which are responsible for the detailed arrangement of the programme of the association's annual meeting. From all appearances, even the heart-searching of the past year, which has at least seen the production of a fearless analysis of the association's financial and intellectual plight (see *Nature*, **231**, 412; 1971), seems not to have created a sufficient sense of urgency. The result is that the Swansea meeting was presented not with a tangible programme of reconstruction but with an invitation to appoint yet another committee to put flesh on the bones of the proposals put forward by the Committee of Review three months ago. The danger, of course, is that the association's claim on public attention, like its investment income, is being drained away with the passage of each week or month.

Why is the association making such heavy weather of its attempt to stay in business? Gone, it seems, is the robust independence of the early nineteenth century, when the British Association came into being (in the year in which the First Reform Bill was being fought through the British Parliament) because its founders recognized the need of an independent and even irreverent forum for interdisciplinary discussions. (Those were bad times for the Royal Society, which might otherwise have done the job.) Only a part of the reason lies in the intricacy of the association's constitution, with its complicated hierarchy of committees intended chiefly to ensure that responsibility lies nowhere identifiable. There is also a serious danger that the association is quite unreasonably imprisoned by conventions which belong to the second half of the nineteenth century, not the first.

The pomp and ceremony which attends each annual meeting, and which must leave a good many of those who went to Swansea hanging their heads in shame, is one obvious sign of this symptom of the association's trouble. Is it really necessary that the first item on each year's programme, increasingly intended to be a means of provoking public discussion of important issues, should consist of a solemn procession in which academics and would-be academics dress up in academic finery and make their way like actors in a bad Greek chorus through a public auditorium to a platform more often used by actors than by participants in a ceremony for awarding honorary degrees? Is it really in keeping with the association's wish to perform a real service in the modern world that there should be yet another procession to the local cathedral to hear some bishop deliver himself of familiar pronouncements on the place of science in society at large? And even if good fellowship (of which there is plenty) must be acknowledged to be an important part of the association's annual rites, is it really necessary that there should be so many invitations for men and women to dress up in evening clothes? Harmless though these conventions may be in ordinary circumstances, an organization seeking to make a mark on public opinion as well as on the opinions of its members must necessarily use its public ceremonies as ways of reinforcing what it has to say. Especially because it is a long time since the association had constructive and sharp things to say about issues of public importance, nobody should be surprised that it has acquired a public reputation much more appropriate to a snobbish and old-fashioned club than to an organization seeking to enhance the public reputation of modern science in a modern and sceptical world.

What is to be done? The first need is that the association should form a clear vision of what it wants to be. If, as seems entirely proper, one important objective is the encouragement of an informed opinion on public issues with scientific antecedents or connexions, the association will have to work out ways of defining what these issues are and what needs to be said about them. This is intellectual work, not simply administration, which means that devices will have to be developed for containing controversy within the framework of the association. If the association is to play a creative part in the encouragement of European collaboration in science, as Professor W. H. McCrea and Sir Brian Flowers (see page 173) have been urging, then it will have to become more professional in organization and more imaginative in innovation. If the association wishes, as it should, to go on providing stimulating intellectual fare for those who turn up at its annual meetings, there is a case for strengthening, not for eroding, the voluntary organization of the association into sections of specialists which has provided the pattern of the past century. It is a great surprise that the Committee of Review, responsible for the past year's self-examination, has done so little to follow up the suggestion that stronger links should be forged between the British Association and the more conventional learned societies.

But is it worth the trouble? This is what a great many British scientists will be asking. That they do so is a measure of how, over the years, the association has lost its hold on the interests and affections of professional scientists in Britain. Luckily, however, there is still enough support to ensure continuity, at least for another year or two. Moreover, it is now more clear than ever that there is a public need for what the association might accomplish. Rarely has the profession of science been as widely misunderstood and wrongly maligned than at the present time; the British Association could help enormously to set the record straight, not so much by means of counter-propaganda as by the objective study of important public issues on which public opinion seems commonly to founder. This is a task which is almost by definition beyond the scope of the orthodox societies. It is also one that cannot be left to an organization such as the British Society for Social Responsibility in Science—that has become such a vigorous pressure group that it cannot be a sounding board for informed opinion at the same time. It is earnestly to be hoped, in the critical months ahead, that professional scientists in Britain will recognize that the fate of this distinguished and potentially valuable institution will depend on their help—or at least on their indulgence.

Opportunities for Collaboration in Europe

In spite of all the enthusiasm for European collaboration that has become common in the sphere of the British Government's influence, very little has been done to show how the institutions responsible for the support of scientific research should be adapted to meet the future needs. Although the imaginative declaration by Sir Brian Flowers at the British Association last week (reprinted in full on page 173 of this issue) is necessarily a first attempt to list the jobs that must at some stage be done, it is encouraging that serious public discussion has at least begun. The question now is what should be done to advance the cause, and to make the prospect of European collaboration a reality. It goes without saying that the first essential is to recognize that in the long run, the creativity of the European scientific community is potentially of inestimable value.

The first thing to be said is that European collaboration in science will not yield its full potential until the university systems of Western Europe are much more closely integrated with each other than at present. There is no point in beating about this bush. At some stage, it will be necessary to ensure that centres of research will be easily accessible to all who work in their fields of excellence. In many cases, this implies that universities should be able to appoint to their faculties people of foreign nationality simply on the strength of their potential contribution to research and teaching. Elsewhere, research institutes will have to be similarly accessible. There are obvious difficulties when systems of higher education are administered as if they were parts of the government, as in France or Italy, but it will probably be much more difficult to arrange that research institutes, most of which are supported by public money, will be similarly accessible. When, for example, will the *Centre Nationale de la Recherche Scientifique* be allowed by the government of France to take on its payroll scientists from elsewhere? In the same spirit, when will the British government agree that the Agricultural Research Council (if it survives the report of the Rothschild Committee) should employ scientists from France or the Netherlands? The Commission of the European Communities has made some progress, in the past few months, towards the definition of rules for regulating the transfer of professional qualifications from one European country to another, but this is a far cry from the arrangements that will eventually be necessary for allowing foreign participation in institutions which are traditionally regarded as national in their essentials.

Beyond that hurdle is the problem of what should be done to rationalize—"to harmonize" as they say in Brussels—the pattern of basic research in Europe. In the past few years, there have been notable successes in collaboration in fields such as high-energy physics, and there is no doubt that CERN is a model of how nations can function in such a way that their collective efforts are more valuable in combination than separately. It is only proper to recall that in the past few years, there have been failures as well as successes. Sir Brian Flowers has done a public service in asking that schemes for collaboration should be designed in a spirit of realism and not with the hope of extraneous benefits. The failure of ELDO shows what is likely to go wrong when institutions are designed to bring political rather than technical benefits, but it is also a great misfortune that, more recently, European nations have made very little headway in collaboration on the construction of large telescopes. But if a sensible coordination of plans is as difficult as this in fields in which there are expensive and conspicuous instruments to be built, what prospect is there for cooperative planning in less spectacular fields of inquiry?

The obstacles are obvious and formidable. The past few years have shown how carefully the Science Research Council in Britain has had to move in its attempts to mould the pattern of research at British universities. It is easy enough to persuade universities and other research organizations to accept that there can be only a few centres of excellence in any field, but the same institutions, however enlightened, are usually unwilling to accept that laudable attempts to concentrate research at selected centres should deprive them of what they consider to be their rights. Some countries on the mainland—France, for example—are better able to work out patterns for the conduct of research because of their centralized systems of support, but elsewhere—as in West Germany—the machinery for the channelling of public money to basic research is an awkward blend of centralized direction and decentralization, with the universities only accidentally involved in the conduct of research. In short, European institutions for the administration of the public money spent on basic research are such an inhomogenous group that structural considerations will impede coordination. To begin with, the best that can be hoped for is that the governments of Europe will establish some kind of talking shop within which research councils, public research foundations and universities can begin to work out what needs to be accomplished. Quite independently of whether the European Community is to be enlarged, this is a task that could be attempted right away.

Further away is the problem of how money should be spent on European research. For the time being, at least, it is to be expected that the member nations of the European Community will regard what they spend on higher education and basic research as a long-term investment in national well-being or even prosperity. Their justification for this expenditure will often rest on the belief that such expenditure will improve their competitive position within the communities With luck, in other words, natural rivalry should help to ensure that European governments spend generously on higher education and basic research in the decade or so ahead. But this is bound to be a passing phase. As soon as there emerges a rational pattern of research, it will also be necessary to work out ways of coordinating expenditures of funds. That will be the point at which governments with large budgets for research and higher education will be looking askance at those who drag their feet. At the same time, it will be necessary for governments (and taxpayers) to accept that national resources should be spent on foreign enterprises. At this stage, it is hard to see how governments can be prevented from tearing each other to pieces without an international budget for research and higher education. The fact that such a goal is bound, at present, to seem distant should not allow those who administer public expenditure on research and development to shy away from the fact that the planning must be done now, and not shelved until some future date.

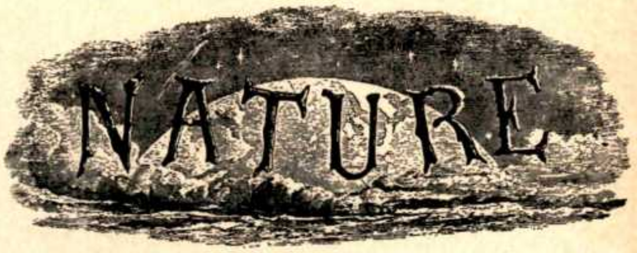
From Prayer-meeting to Trade Fair

THE fourth United Nations Conference on the Peaceful Uses of Atomic Energy, now finishing at Geneva, is a remarkable proof of how rapidly it has been possible to convert a bright idea into a workable and sophisticated technology. It is also a powerful demonstration of the way in which technological innovations should be introduced to those most likely to benefit from them with a greater sense of sobriety than that attending the first United Nations conference in 1955. Will the sponsors learn the lesson now staring everybody in the face?

Fifteen years ago, nuclear power was still largely military in character. The first United Nations conference was remarkable for the way in which it helped the governments of the nuclear powers to put some of their secret cards on an open table. Even such facts of nature as the cross-sections of nuclear reactions involving neutrons were secret, and it seemed at the time to be a great discovery for all concerned that measurements on both sides of what was then called the Iron Curtain tended to be roughly in agreement with each other. To be sure, it was at the time a puzzle to know just why the nuclear powers had decided to make a partially clean breast of the information which they had acquired in the pursuit of military objectives. Perhaps they were responding honestly to the complaint of non-nuclear nations that it would be intolerable if the Great Powers were able to hang on to what seemed to be a monopoly in the exploitation of an important and beneficial technology. It may be that the initiative of President Eisenhower, who launched from the United States an international programme with the slogan "Atoms for Peace", struck a genuine response from the Soviet Union and the United Kingdom. It may even be, as the sceptics were inclined at the time to protest, that all concerned had their eyes on the main chance—the opportunity for making important sales of equipment. In any case, there is in retrospect no doubt that the promise of nuclear energy was spelt out with too much enthusiasm, and that in the process the development of the industry was hindered and not helped. The moral is that in the launching of a new technology, as in the launching of a business enterprise, it is imprudent to publish too cheerful a prospectus.

A second lesson to be learned from the past two weeks at Geneva is that technical progress in nuclear power has been as rapid as anybody could realistically expect. Fifteen years ago, even thermal reactors were entirely novel. Only the foolhardy talked seriously of fast reactors. Although there has since been all kinds of false excitement about fusion reactors, it does now seem as if thermonuclear power is about to be a reality. The importance of this development cannot be exaggerated—it is tantamount to freedom from anxiety about energy supplies in the foreseeable future. That makes up for all kinds of errors.

100 Years Ago



THE preparations made by the Governments of the present age to have every phase of a total eclipse studied and recorded, contrast favourably with the superstition that prevailed a few centuries ago. For instance, the *Scientific American* quotes the following from a German paper :—"The Elector of Darmstadt was informed of the approach of a total eclipse in 1699, and published the following edict in consequence :—'His Highness, having been informed that on Wednesday morning next at ten o'clock a very dangerous eclipse will take place, orders that on the day previous, and a few days afterwards, all cattle be kept housed, and to this end ample fodder be provided ; the doors and windows of the stalls to be carefully secured, the drinking wells to be covered up, the cellars and garrets guarded so that the bad atmosphere may not obtain lodgment, and thus produce infection, because such eclipses frequently occasion whooping cough, epilepsy, paralysis, fever, and other diseases, against which every precaution should be observed.'"

From Nature, **4**, 418, *September* 21, 1871

OLD WORLD

BRAIN DRAIN

The Circus Leaves Town

QUIETLY, without any fuss, the North American Joint Selection Board has ceased to exist due to the withdrawal of the Civil Service Commission (CSC). The board, comprising representatives of the CSC, the United Kingdom Atomic Energy Authority (UK AEA) and the Central Electricity Generating Board (CEGB) toured North America to recruit the best of the young British research scientists and engineers who had emigrated as part of the Brain Drain, and wanted to return.

Established in 1958 (the CEGB joined in 1962) under the chairmanship of Mr Harry Hoff, the board—which became known to applicants as Hoff's Circus—saw its job not as a mission to reverse the Brain Drain but as an attempt to recruit the best people to carry out research work in Britain.

The Civil Service Commission has now withdrawn because it feels that the operation "is no longer cost effective" and because at the moment it is finding "the calibre and quantity of recruits required" in Britain. As the costs do not diminish proportionately because one of the parties has withdrawn, the UK AEA and CEGB have decided not to continue. Costs have risen rapidly despite attempts to cut them to a minimum by introducing a greater degree of preselection of candidates. In the board's 1968 report it was estimated that the cost was "about £500 per man recruited—scarcely any more, if not less, than the cost of recruiting a comparable man in the United Kingdom". The exact figure for last year is not known, but it was in the region of £1,000–£1,200 per man.

The UK AEA and CEGB are not particularly distressed to see the board go, although both would have been happy for it to continue. With postgraduate jobs hard to come by both are getting plenty of applicants. On the other hand, whereas there is no suggestion that all the brightest graduates go abroad, there is no doubt that the standard of the recruits from America, with their extra year or two of experience was very high. Sixty or seventy per cent of those interviewed were recommended for appointment but as can be seen from the table less than 20 per cent were actually placed.

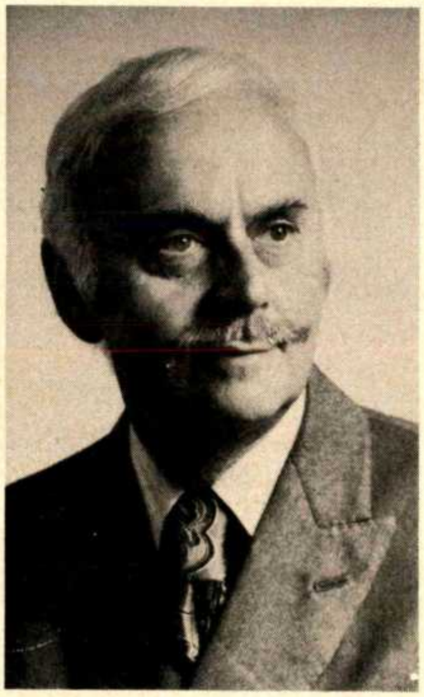
Success of the North American Joint Selection Board 1966-71

Year	Applied	Interviewed	Placed
1966–67	—*	215	39
67–68	—	249	47
68–69	—	257	36
69–70	250	173†	30
70–71	302	186	>25‡

* Figures not known
† Preselection introduced
‡ Final figure not yet known

Until recently it looked as if the board's activities were about to expand. The Post Office was interested in joining and for the past two years information about suitable applicants has been passed on to British Rail and the British Steel Corporation.

But if the board's members no longer need recruits from America, there are still many British subjects in America who would like to return home. The number of applicants has been increasing over the years and Management Selection Ltd (MSL), who provide information and advice to both applicants and employers, say that, if anything, there are more scientists wanting to return. The problem for the exile is that whereas one interview with the board allowed him to be considered for several posts in Britain, he now has to come to this country at his own expense in the hope of finding a job when he gets here. It is ironic that just when the employment situation for highly qualified scientists in this country is very difficult, one of the organizations that made it easier to find a research job has ceased to function.



Photograph by Mark Gerson, FIIP, ARPS

Mr Harry Hoff of the UK AEA, Chairman of the North American Joint Selection Board throughout its thirteen year life.

Companies (and the board members) are perfectly willing to consider the exile for research posts in Britain, but recently the demand for highly qualified people has fallen away, and the companies are finding that by and large they, too, are finding the quantity and quality they want in Britain, and only when they are sure that an applicant from North America is suited to the post are they prepared to pay the fare for the applicant to attend an interview in Britain.

Now that the Brain Drain is no longer a subject for popular concern it is interesting to note that with the employment situation for PhDs becoming increasingly difficult in North America (see *Nature*, **233**, 87; 1971) more Americans are inquiring at MSL about job possibilities in Britain. Seems like the end of a chapter.

MOLECULAR BIOLOGY

EMBO not for Culham

THE decision by the British government not to offer Culham as a site for the proposed European Molecular Biology Organization (EMBO) laboratory last week caused less surprise in scientific circles than it did in the ranks of the Oxfordshire County Planning Committee.

The decision is only one more chapter in the lengthening history of the laboratory. Although conceived in 1963 when EMBO was first formed, no final decision has yet been taken on whether the laboratory will be built but this is expected to be recommended by an international conference next month.

Britain is only one of the countries to consider offering a site for the laboratory and the eventual decision not to do so was taken according to the Department of Education and Science, because "the sites being proposed by West Germany offered better facilities and climate" than the Culham site. In the 1967 proposal for the laboratory it was stated that the site must be in distinguished intellectual surroundings, be easily accessible, have good amenities in terms of climate, housing, international schools, language and countryside, and the revised proposals in 1970 emphasized the need for the laboratory to be near a large research centre in which sophisticated instrumentation and computation are being developed for other purposes. Mr Hugh Farrant, chairman of the Oxfordshire Planning Committee which had welcomed the proposal of Culham as the site last year, claims that apart from the stipulation about climate, the Culham site fills the bill, having adequate communications and being close to both Oxford and Harwell.

The considerations that led to the decision probably included the fact that Germany is prepared to put considerably more money into the project than any of the other governments involved and has two suitable sites to offer, at Munich and Heidelberg, which are both in the centre of Europe. A further consideration is that Dr John Kendrew of the Medical Research Council's Molecular Biology Laboratory at Cambridge is the likeliest candidate to become the first director of the proposed laboratory and a British director of a European laboratory in Britain would not appear very European.

Mr Airey Neave, MP for Abingdon, who described the decision as "rather disappointing" is to ask Mrs Thatcher, Secretary of State for Education and Science, for a full statement, but as the decision was taken after full consultation with EMBO officials it is unlikely to provide ammunition for the dissenters.

COMMUNICATIONS

Dial Around the World

THE largest single contract ever awarded by the British Post Office has been given to Plessey Telecommunications for the first stage of Britain's newest international telephone exchange. The exchange will use crossbar switching and will provide more than a third of the 18,000 international telephone circuits eventually planned to be handled by the Post Office's new Mondial House, now under construction. As part of the contract, worth about £10 million, Plessey will also provide a complex of computers which will perform the complicated accounting procedures involved in international telephony—the cost of each call must be apportioned among the countries of its origin, its destination and its passage. The same equipment can, at the same time, give precise information on how the exchange is routing the calls presented to it.

For the Post Office, the opening of the exchange in Mondial House at the end of 1973 will mean a larger increase in the number of destinations to which British telephone subscribers can dial their own telephone calls. Until this year, the Post Office depended on its exchange at Faraday House, with its 2,000-circuit capacity to handle all international calls. The Wood Street international exchange has now began to operate and will eventually have a capacity of 8,400 circuits. But when Mondial House's 18,000 circuits are available, there will be connexions, by means of cable, microwave and satellite, to 51 countries, including 29 in Europe as well as the United States, India and Australia. As the number of countries which can dial each other increases, thanks largely to satellites, Britain's importance as a transit centre will increase—and so will the Post Office's revenues from external telecommunications.

For Plessey, the award is a reminder that the Post Office has fully repented of its error of the 1950s when it vetoed the crossbar switching system with which the United States, Sweden, Japan and some continental countries were modernizing their telephone networks. Plessey (or the company which it has since absorbed, Automatic Telephone and Electric) developed crossbar privately, without a Post Office subsidy. Only in recent years has Plessey persuaded the Post Office to adopt it as a standard exchange.

Plessey has also developed the newer kind of exchange described as electronic but actually based on reed relays—which the Post Office is also installing. The system is called TXE-2 and the Post Office eagerly publicizes the gradual introduction of electronic exchanges into the telephone network. But Plessey executives last week made it plain that only crossbar, and not the much-publicized TXE-2 switch, could handle a telephone exchange of the size and complexity required by Mondial House.

Considering Plessey's position as British developer of crossbar it was unlikely that the contract would go to the Post Office's other major suppliers, GEC-AEI and Standard Telephones and Cables. Plessey can probably be fairly confident of supplying the rest of the Mondial House exchange. But the Post Office may not always buy British. It is showing a new boldness in accepting foreign suppliers and gave Philips of Eindhoven a much-prized contract for an international telegraph switching exchange just a few weeks ago. Plessey received this big Mondial House contract fairly easily but neither it nor the other two manufacturers can afford to be complacent about the future.

NUCLEAR PHYSICS

Cause for Concern?

CONCERN is growing among nuclear structure physicists in Britain that the proposed national nuclear structure facility will absorb all the finance available for nuclear structure research and that the facilities now available at the universities will be declared obsolete with a consequent withdrawal of Science Research Council support.

Sir Brian Flowers, chairman of the Science Research Council, said last week that when the national facility is built all other accelerators in the country will be outdated and that support would be withdrawn from several of these facilities in order to concentrate on the new machine. In particular, Sir Brian mentioned that the Heavy Ion Linear Accelerator at Manchester University and the Tandem Van de Graaff at Liverpool University would be among the first to have their support cut whereas the most recent accelerators at Oxford University would still be supported. It would all depend, he said, on the nuclear structure budget at the time at which the national centre became operational. Sir Brian emphasized that the matter is now in a state of flux and that nothing definite would emerge until a decision was taken on the national facility; this will probably be in eighteen months' time when the design study team, at present at work at Daresbury, have completed their work (see *Nature*, **232**, 76; 1971).

K. W. Allen, professor of nuclear structure at Oxford University, said this week that it was unreasonable and unthinkable that support for the Oxford accelerators would be withdrawn and that such a decision would be contrary to current policy. The Oxford accelerators are at present supported to the tune of £250,000 a year, but there is no question of this being withdrawn before 1976, the earliest date for completion of the national facility if the green light is given soon.

Scientists at Liverpool are also concerned about the future of their laboratory but they are understandably less worried than the Oxford physicists. Although no final decision has been taken by the SRC on the siting of the national facility, the fact that the design study team is sited at Daresbury tips the scales in favour of a site in the north of England. Daresbury is within easy reach of both Liverpool and Manchester and if funds are withdrawn from both these universities they can still effectively carry on their research. But the position of the Oxford physicists and others farther away from the site is not so cheerful.

Enthusiasm for the nuclear structure centre will undoubtedly wane in some quarters if, on completion of the design study, there is a possibility of closing down all other facilities in favour of the new centre. If this happens, future developments in nuclear structure in Britain will have to turn in some other direction. One possibility is the upgrading of the Oxford accelerators by replacing the 12 MeV tandem Van de Graaff with a more powerful version (see *Nature Physical Science*, **232**, 76; 1971). Such a proposal is under consideration and if the SRC supports this improvement it will possibly signify the beginning of the end for the National Nuclear Structure Facility project.

TECHNOLOGY

Innovation Research

THE smaller member countries of the Organization for Economic Cooperation and Development, such as the Netherlands, Sweden and Switzerland, have been very successful in reaping the benefits of technological innovation. That is one of the conclusions to be drawn from an extensive international review of research on the processes and mechanisms of technological innovation published recently by the OECD (*The Conditions for Success in Technological Innovation*, OECD, Paris; 1971).

The aim of the report is partly to assess how member countries of the OECD have measured up to technological challenges and partly to discover how an increasingly favourable climate for innovation can be created. Some of the conclusions of the report, such as the idea that industrial competition is vital to successful innovation, are, however, still matters of contention which are unlikely to be resolved because of a lack of data. More than half of the 155 accounts of research cited in the report are concerned solely with the United States and the report calls not only for more research in other member countries of the OECD but also for further investigation of particular aspects of the problem, such as the mechanisms of technology transfer between industries and the time scales involved in innovation processes.

The OECD report follows closely on the heels of a British report by the Centre for the Study of Industrial Innovation (see *Nature*, **232**, 432; 1971) which analysed the reasons for the abandonment of technological projects; before that, for example, there was the report of the Charpie committee to the US Department of Commerce which highlighted, possibly to an unrealistic extent, the importance of small companies in the innovative process.

The latest study is intended as a sequel to the series of reports called *Gaps in Technology* and published by the OECD during the past few years. The innovation process is considered from the point of view of industry, government and the universities—the three principal components of the framework in which innovation takes place. Important relationships seem to exist between companies of differing sizes because, for example, small companies tend to specialize in sophisticated technological areas which require smaller production capacity but which often make use of the experience of scientists and engineers who once worked in large industrial (or government) laboratories. The mobility of scientists should be encouraged, says the report, because it accelerates the diffusion of technology and brings fresh minds to bear on particular problems. Industrial management also faces many problems of adjustment to an ever-changing situation but these can be overcome if entrepreneurial attitudes are encouraged, not only in individual managers but also in whole companies and institutions so that information can be exchanged easily across organizational boundaries and changes made quickly and efficiently as the situation demands.

The report also suggests that governments can contribute to the improvement of the innovation process through changes in the tax and patent systems and that government procurement will continue to play a vital role in improving the technological prowess of industry.

Miscellaneous Intelligence

FEARS for the fish stocks in the North Atlantic will be strengthened by the reports of experimental trawling carried out in the sixties by the Fisheries Research Board of Canada (Technical Report 260). Operations on the Scotian Shelf, south-west of Nova Scotia, have been systematic enough in the past few years for accurate estimates of fish stocks to be possible. The Biological Station at St Andrews, New Brunswick, estimates that the standing crop of cod on the Scotia Shelf amounts to 464 million pounds. Argentine, pollock and haddock come next in order, with standing crops of 453 million pounds, 428 million pounds and 391 million pounds respectively. In case this should seem plenty of fish for all the trawlers now operating in the region, however, it is relevant that the laboratory's estimate of the "maximum sustainable yield" of cod—140 million pounds a year—is already less than the sum of the commercial landings of cod each year, 190 million pounds or so. In the circumstances it is forgivable that the report should draw attention to the largely unexploited stock of squid on the Scotia Bank, enough to provide 121 million pounds of edible or nearly edible food a year,

ASLIB is about to take over from the Department of Education and Science the thankless task of trying to maintain an advance list of international scientific conferences. Nobody will begrudge these tireless librarians the £8.00 which they propose to charge subscribers for their quarterly list, but it will be interesting to see precisely how Aslib tries to solve the awkward problem of separating international conferences proper from those to which the organizers have invited a few ritual foreigners, sometimes in the hope that the hospitality will be repaid.

THE first full-scale nuclear power station to be built in Sweden, the 440 MW boiling water reactor plant at Simpevarp on the east coast, supplied electricity to the Swedish grid for the first time last month. Its output is expected to reach 350 MW by the end of this year and full capacity will be achieved during 1972. Most of the construction work was carried out by the Swedish mixed state-private company, ASEA-Atom, which has developed a commercial boiling water reactor capability independently of the United States. A second nuclear power station (580 MW) is expected to commence operation in 1974. During the 1980s, the construction of a further four nuclear power plants is planned and the total nuclear generation capacity should then be about 5,000 MW.

A NEW research vessel for the Natural Environment Research Council, RRS Challenger, was launched last week. The Challenger is the first Council vessel to be purpose-built for research, the other eleven in the NERC fleet have all been converted; she will operate from the council's research vessel base in Barry, Glamorgan. RRS Challenger has a displacement of 1,440 tons but is not the largest of the fleet. The new vessel has accommodation for nine scientists and is equipped for marine, geophysical and other oceanographic research. The Natural Environment Research Council expect the Challenger to be operational by April 1972 and most of her cruises are expected to be in the north Atlantic.

"To conserve is the Museum's first priority; to educate and entertain is a close second. To conserve for future generations whilst ignoring the present generation would be absurd", is one of the opening remarks of *Museums in Education*, Education Survey No 12, HMSO, 50p, published this week. The survey conducted by Her Majesty's Inspectors of Schools, while admitting that much progress has been made in the use of museums in education, calls for a still greater use of them. The desirability of museums and educational institutions to cooperate to a greater extent is emphasized, as is the need for museums to cooperate more. The report says the "happy camaraderie among professionals needs to become a much more flexible and extensive exchange of exhibitions and professional services in many fields"

Clearing the Air from Geneva

from a Correspondent

THE second World Administrative Radio Conference for Space Telecommunications, organized by the International Telecommunication Union, was held in Geneva from June 7 to July 17, 1971, and was attended by some 650 delegates from 100 nations. The decisions taken at the first such conference in 1963 have stood the test of time, but developments in space technology and radio astronomy have made new international agreements necessary to cater for the needs of the next decade.

The demand for radio frequencies shows no signs of abating, and as there is a limit to the extent of the radio spectrum which can be exploited the same frequencies should be used by more than one service whenever this is technically feasible. Much of the discussion, and most of the difficulties in reaching agreements, were concerned with problem sharing. As technology advances some of the increased demands can also be met by using frequencies higher than 40 GHz, the previous upper limit of the allocated bands, and allocations have now been made up to 275 GHz.

There has been a remarkable expansion in satellite communications since 1963, and the next decade is expected to see the implementation of satellite services for aeronautical and maritime navigation and communication, for direct broadcasting to the public (at least on a communal basis), for the study of the Earth's resources, and for monitoring the environment. It is also becoming clear that recent discoveries by radio astronomers are providing new insight into the fundamental physics of matter, and that greater protection from interference in several frequency bands is necessary for this important branch of science to develop.

Most of the work of the conference was organized in three committees. The first was concerned with the administrative procedures by which international negotiations may take place as services are introduced or expanded. It has been necessary to revise the procedures laid down in 1963, particularly as the greater emphasis on frequency-sharing calls for clear and more comprehensive procedures by which different countries may coordinate their activities. An interesting departure from custom is that there is now provision for the adoption of new technical criteria on frequency sharing when these are recommended by the International Radio Consultative Committee (CCIR, an advisory committee of the ITU) without waiting for another administrative conference. Countries will have the option of retaining the existing criteria or adopting the revised criteria when these become available.

The second committee was concerned with establishing the technical criteria such as limits of radiated power and power flux densities which are considered necessary to enable services to share frequencies without mutual interference. This committee also provided the technical data needed first for calculating the coordinating distances within which consultation with other countries need take place, and then for calculating the likelihood of interference, and the necessary separation distances, taking into account the actual radio links in operation or planned. Agreement on comprehensive technical standards, in the available time, was made possible only by the thorough preparatory work carried out in February 1971 by a special joint meeting of relevant Study Groups of the CCIR in Geneva, and before that by a CCIR working party in Nice in December 1970, which assembled the latest data on radio propagation necessary to enable the more general studies to proceed.

Since 1963 it has become clear that the future development of space communications is vitally dependent on the exploitation of geostationary satellites, which remain fixed in relation to the surface of the Earth. The technical committee therefore gave considerable attention to the need for the efficient use of the geostationary satellite orbit, and the criteria which must be adopted to avoid interference between links using different satellites in this orbit, and between these links and terrestrial services.

Although the preparation of administrative procedures and technical criteria is fundamental to the development of communications, it is the frequency allocations themselves which will excite the widest interest. The revision of the frequency allocation tables was the task of the third committee, and some difficult problems were encountered. Although space services can be operated over a wide range of frequencies, certain parts of the spectrum have marked advantages and are in heavy demand. One such range, for example, is in the region of 2–3 GHz; wide bandwidths are not available at much lower frequencies, and propagation at much higher frequencies can be seriously affected by adverse meteorological conditions. This desirable part of the spectrum, which is already used extensively for terrestrial services, is also in demand for Earth exploration satellites, for broadcasting satellites, and for increased bandwidths in space research. An acceptable compromise was finally achieved, by which it should be possible for these services to share certain bands, provided that there are only a few Earth stations for space research and Earth exploration, that the power flux density from broadcasting satellites is kept below an agreed limit, and that full consultation takes place before any new system is established. Similar problems in other parts of the spectrum were treated in similar ways.

The final agreements on frequency allocations are inevitably the result of compromise, and operators of most services will be disappointed that not all their hopes were realized. It has not been possible to make new primary allocations for space research, except in the newly-allocated part of the spectrum above 40 GHz, but several special provisions have been made which will, it is hoped, enable the necessary arrangements for specific operations to be made by negotiation. The basis of this philosophy is that successful operation of a few Earth stations in the space research service, even though a high degree of protection is required, need not inhibit the use of the same frequencies for other services in other parts of the world.

Radio astronomy has received improved status in several bands, and has obtained a new exclusive high frequency band near 22 MHz and new bands above 40 GHz. There is increased recognition of the importance of bands needed for observing natural line emissions. On the other hand, the expected increase in transmissions from satellites, for all purposes, constitutes a threat to radio astronomy which cannot be avoided by locating observatories in remote areas. As an example, there is now an allocation for broadcasting satellites in a band adjacent to the exclusive radio astronomy band at 2,690–2,700 MHz. Careful planning and much goodwill is needed if radio astronomers are to be able to use this band without danger of serious interference.

The new regulations will come into force, formally, on January 1, 1973. But as they reflect the established policies of most countries, many of the provisions will already have been adopted for practical purposes, and others will no doubt be followed well in advance of the formal date of implementation.

NRC Committee Knocks Anti-knock

by our Washington Correspondent

A STUDY of the health hazards associated with airborne lead, conducted by the National Research Council*, achieves the remarkable feat of providing sound biological reasons why lead should be banned from use in petroleum as soon as possible, while at the same time cutting the ground from under the feet of the most vociferous critics of the automobile industry. The chief conclusion of the study is that while airborne lead does not provide a serious threat to the health of the average city dweller—even in Los Angeles—young children and some groups of workers may get enough lead into their bodies from car exhausts to produce biochemical damage and, in severe cases, even clinical signs of lead poisoning. The study, which was conducted under contract to the Environmental Protection Agency by the Panel on Lead of the Committee on the Biologic Effects of Atmospheric Pollutants (a special committee of the National Research Council), will be scrutinized carefully by the EPA, for it is planning to introduce regulations controlling lead in petroleum in December this year.

Based on an extensive survey of the literature on lead pollution, the study comes up with the finding that the concentration of lead in the largest cities in the United States is twenty times greater than over sparsely populated rural areas, and more than 2,000 times greater than in the air over the centre of the Pacific Ocean. There is "little doubt as to whether man has substantially contaminated some parts of his environment with lead", the panel concludes, and "it is also clear that one major source of general environmental contamination is the combustion and dispersal of lead alkyl compounds used as automotive fuel additives".

Before this conclusion can be used as a stick with which to beat the car manufacturers, however, the panel points out that the average lead content in the air over major American cities has not changed greatly for the past fifteen years, and even if cars carry on discharging lead into the atmosphere, there will be little change in the magnitude of the effect on biological systems "for some years to come". The panel also concludes that much the most important source of lead in the blood comes from food and drink. In any case, the average city dweller in the United States receives insufficient doses of lead from both the air he breathes and the food he eats to pose a threat to his health.

Although the panel offers no suggestion on the level of lead in the blood that can be considered safe, running through the report is the assumption that blood lead levels below 40 μg per 100 g are not biologically harmful because it is only above that level that synthesis of the haem part of the haemoglobin molecule is affected, and the first clinical symptoms of lead poisoning (which are usually mild anaemia, headaches and generalized muscle aches) are not noted until blood lead levels exceed about 80 μg per 100 g of whole blood. In contrast, the panel report cites studies that show that the average person living and working in the city of Los Angeles—where the concentration in the atmosphere is twice as high as most other cities—will have a blood lead content of less than 25 μg per 100 g of whole blood, and inhabitants of other cities are likely to have about 15 μg of lead in every 100 g of blood.

On the other hand, however, the panel puts children who live in large cities and some groups of workers, such as policemen on traffic duty and parking lot attendants, at a high risk from airborne lead. The latter group is more or less continually exposed to high concentrations of airborne lead, which may result in blood lead concentrations greater than 40 μg per 100 g of blood, but the panel also points out that "even within this small population, this level of blood lead concentration is probably attained by only a relatively small proportion of those exposed. To reach the very high level of blood concentration compatible with clinical lead poisoning would probably require in these special groups a five-fold increase beyond current levels in lead assimilation for a long period".

But the exposure of city children to airborne lead and also to lead paint and other sources is a much more intractable problem. For one thing, recent studies have shown that many city children have blood lead concentrations of 40–60 μg per 100 g. Leaving aside the ingestion of lead paint, which is a major problem in the poorer areas of large American cities, the panel suggests that children living in large cities are exposed to a high risk of lead poisoning through dust and grit in the streets. Such dust often contains more than 2,000 μg per g of lead, and if a child manages to eat only about 40 mg of dust each day, his blood lead content could be taken above the 40 μg limit. Or, more drastically, if he ate 0·4 g a day, he would show symptoms of clinical lead poisoning, possible even resulting in damage to the central nervous system. This, says the panel, "is an undesirable level of exposure". Unfortunately, but not surprisingly, nobody is clear about how much dust and grit is swallowed or inhaled by children in large cities because no such studies have yet been reported. That is a social study which the panel suggests could well be carried out.

Asked last week whether these findings of blood lead concentrations call for a ban on lead in petroleum, Dr Paul B. Hammond, the panel's chairman, said "on strict biological grounds, I would say that a case could be made out for banning lead from gasoline, but I could also make out a case, on strict biological grounds, for banning the automobile as a lethal weapon". Dr Hammond pointed out that the panel can make a biological judgment, but it is now up to somebody else to make an economic judgment on whether or

Feasibility Study

THE National Academy of Sciences has accepted a contract from the Environmental Protection Agency to determine whether the automobile industry is capable of manufacturing an engine that will meet the standards set by the Clean Air Act. Manufacturers must, by 1975, produce automobiles which emit 90 per cent less carbon monoxide than the engines produced in 1970, and they have until 1976 to reduce emissions of nitrogen oxides by 90 per cent. A committee, under the chairmanship of E. L. Ginzton, chairman of Varian Associates, California, will determine whether it is technologically feasible for the industry to design, mass produce and service such an engine. Reports will be sent to the EPA and to Congress in January and July of each year.

**Airborne Lead in Perspective,* to be published by the National Academy of Sciences, Printing and Publishing Office, 2101 Constitution Avenue, Washington DC 20418.

not lead alkyls should be banned from petroleum. Ironically, it may be the manufacturers themselves who are forced to conclude that they can no longer afford to have their cars run on leaded petroleum, whatever recommendations the EPA comes up with in December. The automobile manufacturers must by 1975 meet strict regulations on the amount of unburnt gases and nitrogen oxides emitted from automobile exhausts, and one method which at present looks promising is to attach a catalyst to the exhaust system to reduce nitric oxide and to oxidize carbon monoxide. Lead, however, poisons the catalysts.

Apart from its findings on the levels of lead in the blood of city dwellers, the panel also pinpointed areas where more research is needed. For one thing, the panel points out that monitoring of both city air and food is totally inadequate to determine either the source of lead which eventually finds its way into human blood, or to provide warnings of potential risk. But the chief areas where more extensive research is called for lie in the biological sciences themselves. For example, the panel says that studies are required to determine whether the body burden of lead increases with age, and the assumption that the non-diffusable fraction is tightly bound in bone, and therefore biologically inert, also requires further detailed examination.

A more important recommendation, and one which throws into relief the panel's tacit assumption that blood lead levels below 40 g are safe, is the suggestion that studies on the biological effects of low levels of exposure to lead are urgently required. "The subtle effects on behaviour of low lead exposure of long duration without prior exposure may be manifest in two types of disorders: the dulling of mentation and chronic hyperkinesis", the panel points out. "No information is available regarding the possibility of cause and effect in what may be an extremely important problem."

TECHNOLOGY ASSESSMENT

Daddario's Kite Flies Yet

by our Washington Correspondent

THE Office of Technology Assessment, like an old soldier, refuses to die. A proposal to set up within the legislative branch of the Federal Government an agency designed to assess the likely consequences of technological projects has been kicking around in the House of Representatives Subcommittee on Research and Development for the past four years, and its ancestry can be traced back a good deal further than that. But, just as the idea seemed finally to have been laid to rest, it has appeared again with enough vigour and support to take it through the rest of the congressional mill and probably even into the statue books. A bill to set up the office was introduced into the House by Mr John Davis, chairman of the Subcommittee on Research and Development and the rest of his committee members a few days before members of Congress dispersed for their Summer vacations. It was referred to Mr Davis' committee, and has since been sent back to Congress without amendment and with a report explaining why it would be "shortsighted in the extreme, if not disastrous" for Congress to turn the idea down.

The bill, which is in most respects identical with a bill introduced by Emilio Q. Daddario, Mr Davis's predecessor as chairman of the research and development subcommittee, would establish an Office of Technology Assessment charged with appraising the impacts of the applications of technology and with helping Congress to determine the relative priorities of the projects before it.

The chief reason for the bill, and without doubt its strongest asset on Capitol Hill, is that the Administration at present has all the resources at its disposal for making decisions on technological matters and Congress is ill-equipped to challenge them. To help redress the balance, the proposed Office of Technology Assessment (OTA) will provide information to congressional committees and serve as an early warning system for likely undesirable social effects of legislation involving technology. All these functions would be performed at a cost of $5 million for the office's first year of operation.

Jobs specifically earmarked for the OTA in the bill are to identify existing or probable impacts of technology, where possible to establish cause and effect relationships between technological advances and social or financial consequences, to determine alternative programmes to achieve specific goals, to identify areas where more research is needed to support the assessment, and to furnish the results of these labours to the appropriate legislative body. The OTA will have the Library of Congress to help carry out its tasks, and the National Science Foundation to cooperate on the development of techniques of technology assessment, and to award grants and contracts for research needed by the OTA.

A panel consisting of eleven members would be responsible for the policy of the OTA and an operational unit, headed by a director, would be charged with the task of carrying it out. Two Senators, two members of the House of Representatives, the Comptroller General, the director of the Congressional Research Service of the Library of Congress, the director of the OTA itself, and "four especially qualified members from the general public" appointed by the President with the Senate's approval would together provide the personnel for the Technology Assessment Board. The director would be appointed for a six-year term and the public members of the board would serve for four years each, with the possibility of being reappointed once. Assessments could be instigated by the board or the director, but the chief users of the office's expertise are expected to be congressional committees whose chairmen could ask for specific studies to be carried out. Like the FBI, the OTA would be expected to furnish a report without coming to any conclusions.

Although the way in which the OTA will in theory carry out its business, and the rationale that underpins the attempt to set it up, is clear, thirteen days of hearings and four years of rumination by the Subcommittee on Research and Development have failed to provide a convincing description of how it will work in practice. For one thing, if the OTA is to be used to check on a particular action of the Executive, it will have to move with sufficient speed to produce a report that is not a post mortem, and for another, independent members of the public who are both equipped and willing to serve on such a body are rare indeed. The new bill has at least alleviated the problem of finding staff for the Technology Assessment Board to the extent that it has cut the number of public members down from seven in the original proposal to six and finally to four in Mr Davis's new bill.

If the bill survives intact the congressional mill—as indeed it stands every chance of doing, for Congress is not noted for turning down measures that are designed to strengthen its hand against the Executive—the Office of Technology Assessment would be essentially Mr Daddario's baby, for it was he who introduced the legislation leading up to the new bill, and it was under his chairmanship that the subcommittee conducted its hearings. Although the need for Congress to be kept informed of the likely consequences of new technology was agreed by all the witnesses before the committee, few aspects of the proposal remained uncriticized. Dr William D. McElroy, Director of the National Science Foundation, for example, told the subcommittee that the office would lack credibility and that it may better serve to coordinate the work of agencies that are already engaged in technology assessment. Those are criticisms that the new legislation has clearly failed to answer.

MARINE POLLUTION

No More Dumping

by our Washington Correspondent

GONE are the days when politicians would complain that there are no votes in sewage, and that there is no political capital to be gained from supporting legislation to clean up the rivers and oceans. With the present public concern about the deterioration of the natural environment, sewage has become a live political issue, and one on which most politicians have something to say. Such was the case last week when the House of Representatives passed, by a vote of 304 to 3, a bill designed to regulate the dumping of waste material in the tidal waters around the United States, and to prohibit entirely the dumping of some particularly toxic material.

In many respects, the bill embodies in legislation the conclusions and recommendations of the Council on Environmental Quality, whose report *Ocean Dumping: A National Priority,* was sent by President Nixon to Congress in October last year. But the bill has already met with opposition from the Bureau of the Budget for extending the council's recommendations to include the designation of marine sanctuaries around the United States and for requesting a further $2 million for research into the long-term effects of marine pollution.

Chief among the provisions of the bill are a total ban on the dumping of chemical and biological weapons and highly radioactive waste materials into the oceans, and a ban on the dumping of other material unless a permit has been issued by the Environmental Protection Agency or the Secretary of the Army. Disposal from outfalls is, however, not covered by the bill because it is already regulated by the Federal Water Pollution Control Act. Permits will be granted by the EPA, or the Secretary of the Army in the case of dredged or fill material, for transportation from the United States of the material to be dumped.

An applicant for a permit must convince the EPA that the dumping will not degrade the environment or present a danger to public health and he may, if necessary, be required to defend his application before a public hearing. If he decides not to bother with the bureaucracy involved in getting a dumping permit, he will be liable to a fine of $50,000 for illegal dumping, and to help ensure that he is discovered in his crime, the bill proposes that members of the public be rewarded by up to $2,500 for providing information which brings the offender to book.

As for research and development on the ecological consequences of marine dumping, the bill provides $2 million to the National Oceanographic and Atmospheric Administration (NOAA) to engage in both long-term and short-term studies. The Committee on Merchant Marine and Fisheries added this clause to the Administration's original proposals because it believes that an early warning system is needed to identify, for example, pesticide residues whose presence is likely to constitute an ecological hazard. Too often, such damage is noticed only when it has become irreversible. The bill also sensibly calls for wide-scale monitoring of the purity of the oceans. The proposal to give more government money to marine research has, however, already fallen foul of the Office of Management and Budget, for Donald Rice, an assistant director of OMB, wrote to Mr Thomas M. Pelly, chairman of the Committee on Merchant Marine and Fisheries, to complain that this section of the act is "unnecessary and undesirable". With curious logic, Mr Rice explained that since the EPA and NOAA are already spending more than $2 million between them on oceanographic research, giving them an extra $2 million may restrict their activities.

An even more curious aspect of the legislation dealing with research is the function envisaged for the National Science Foundation. In the original bill, the NSF was earmarked to receive an extra $1 million along with NOAA, but during the debate, Mr Lennon, from North Carolina, deleted all reference to the NSF and instead substituted the Secretary of State for Commerce. Mr Lennon's amendments were taken immediately after a roll call, and in the confusion, or perhaps in the absence of interest from his colleagues, they were accepted without discussion or division. Mr Lennon later explained, however, that he had decided to cut the NSF out of the bill because NOAA is better equipped to manage the research programme, and by vesting the responsibility in the hands of a single agency, duplication of effort can be cut to a minimum.

The chief fight with the Administration over the bill is likely, however, to involve the section dealing with the designation of marine sanctuaries in which all sorts of activities would be prohibited in an effort to preserve the natural beauty or the wildlife of the sanctuary. NOAA has been charged with the task of deciding which areas of coastal water should be so designated, and the Secretary of State for Commerce will be given the authority to enforce whatever regulations are laid down to protect the sanctuary. Sanctuaries must be designated within two years after the bill is signed by the President, and their siting will be subject to public hearings.

The Administration's chief grumble is that this section of the bill is likely to cost up to $10 million a year for the first three years, mostly for the acquisition of land. But the Administration is also chary of infringing the rights of states whose shoreline borders on a proposed marine sanctuary. A move to delete this section of the bill in the House of Representatives was, however, defeated by a vote of 20 to 33, lack of a quorum notwithstanding, and a motion seeking to prohibit oil prospecting in areas being considered for designation as sanctuaries was turned down with an even smaller vote—10 to 42. But the days of indiscriminate dumping seem over.

Space Collaboration

THE successful meeting of scientists from the USSR Academy of Sciences and from the National Aeronautics and Space Administration (NASA) at Houston in June is to be followed by another meeting between the parties in Moscow in November. The aim of the series of meetings is to discuss the technical requirements for a possible link-up in space between an Apollo-type spacecraft and a Salyut-type orbital scientific laboratory. Even further in the future a link-up between a spacecraft of the Soyuz-type and a Skylab orbiting station might be considered.

The problem facing the scientists is to agree on compatible systems of rendezvous and docking, radio and optical signals, communications, life support and crew transfer. After the Houston meeting it was agreed that the technical feasibility of accomplishing such a docking exists in principle and that further studies would be advantageous.

The current plan is to initiate the Skylab project at the end of the Apollo programme and to launch the first such Earth orbiting workshop in May 1973. Three different groups of astronauts will stay on board for up to twenty-eight days on the first mission and for up to fifty-six days on the second and third missions planned for the end of 1973.

A Salyut spacecraft is already in Earth orbit, and the first stage of the proposed collaboration will ensure that the best developed facets of the United States and USSR space programmes will be combined.

NEWS AND VIEWS

Haemoglobin Synthesized in *Xenopus* Eggs

THE rumours, which have been circulating for the past several months, to the effect that Gurdon and his colleagues at Oxford have observed the translation of mammalian messenger RNAs which were injected into frog eggs and oocytes, were well founded. For on page 177 of this issue of *Nature*, Gurdon, Lane, Woodland and Marbaix describe in detail how to use *Xenopus* eggs and oocytes as an extremely sensitive assay system for amounts of messenger RNA as small as 1 ng. That is not all; with their oocyte system it is now feasible to study the control of the translation of messenger RNAs in living eukaryotic cells. This development, coinciding as it does with the progress several groups have made towards the development of efficient cell-free systems which support the translation of eukaryotic messengers, could scarcely have been better timed. With these two approaches at their disposal, and with the wealth of information about bacterial protein synthesis at hand, biochemists should, with luck, now be able to elucidate the minutiae of protein synthesis in eukaryotes.

9S RNA from reticulocytes is, of course, the most readily available mammalian messenger and the results of initial experiments by Gurdon and his colleagues, which are to be published shortly in the *Journal of Molecular Biology*, proved that oocytes, taken from *Xenopus laevis* induced to ovulate by hormone treatment, synthesize haemoglobin when they are injected with this RNA and haemin. Greatly encouraged, Gurdon's group then set about characterizing more precisely this system and, what is perhaps even more important, proving that the haemoglobin messenger is not a special case and that other foreign messengers can be translated in *Xenopus* oocytes.

Their first problem was discovering the best way to introduce labelled amino-acids into eggs and oocytes. It turned out that injected ^{3}H-histidine leaks out of oocytes far more rapidly than out of eggs; conversely, oocytes take up this label from the medium more rapidly than do eggs. Deciding to label eggs by injection and oocytes by incubation in labelled medium, they followed the rate of synthesis of haemoglobin after the injection of rabbit 9S RNA and compared it with the rate of translation of endogenous messengers. After a lag phase of some 10 to 15 minutes, presumably the time taken for the injected messenger to become associated with the cells' translation apparatus, the synthesis of haemoglobin and the translation of endogenous messenger continues at a constant relative rate for more than 20 hours. Furthermore, experiments with puromycin indicate that both endogenous protein synthesis and haemoglobin synthesis are equally susceptible to inhibition. Plainly there is nothing atypical about the way in which the haemoglobin messenger is translated and the haemoglobin messengers are no less stable in oocytes and eggs than their endogenous counterparts.

The next question to answer was how efficiently do oocytes and eggs translate the rabbit haemoglobin messengers? By measuring the size and specific activity of the pool of histidine in these cells and the amount of this amino-acid incorporated into haemoglobin during given times after the injection of known amounts of messenger preparations, Gurdon and his associates have made an estimate of the number of globin chains made per hour per molecule of messenger. Such estimates are inevitably only approximate for they depend on a variety of unproven assumptions. For example, the size of the pool of histidine in the cells which is drawn upon for protein synthesis might well be significantly less than the total amount of extractable free histidine, which was what Gurdon *et al.* measured. And factors such as the leakage of messenger from injected cells and the failure of some RNA molecules to act as messengers add further elements of uncertainty. Nevertheless the estimates provide a fairly reliable indication of the comparative efficiency of translation of haemoglobin messengers in different systems. It emerges that oocytes are almost as efficient at translating this messenger as are reticulocytes; at 20° C each molecule is translated about 80 times an hour in reticulocytes, 24 times an hour in the best oocyte preparations and a mere 0.1 times an hour in a cell free system, to judge from the published data of Lingrel.

But is the translation of haemoglobin messenger in eggs and oocytes a special case? Obviously if it is the system has only limited potential but, on the other hand, if *Xenopus* oocytes and eggs will support the translation of all manner of foreign messenger RNAs as efficiently as they support haemoglobin synthesis there are countless ways in which this system might be exploited. Unfortunately with so limited a selection of natural messenger RNAs currently available in anything like a pure form, Gurdon's group can only provide a partial answer to this crucial question but their answer is most encouraging. Of the three messengers which they tested a 9–11S RNA from mouse myeloma cells and the synthetic messenger AUGpolyU seem to be translated but phage f2 RNA is not. Oocytes receiving the myeloma messenger incorporate ^{3}H-serine and ^{3}H-threonine into protein which is precipitated by antisera against the myeloma protein and which co-chromatographs on 'Sephadex' with cold carrier myeloma protein. Synthetic AUGpolyU promotes the polymerization of phenylalanine and concomitantly endogenous protein synthesis is severely restricted. In short, more than one natural messenger and a synthetic messenger is translated.

As Gurdon *et al.* point out, this means that oocytes can be used as an assay system for the putative messenger activity of RNA preparations from eukaryotic sources. If injected RNAs either stimulate amino-acid incorporation by these cells or compete with the translation of haemoglobin messenger RNA they will probably be found to contain messengers. And as an example of the sensitivity of this system they show that as little as 2.5 ng of RNA from mouse 3T6 cell polysomes almost halves the amount of haemoglobin made by oocytes injected with saturating amounts of haemoglobin messenger. By contrast 30 ng of ribosomal RNA has no effect on the amount of haemoglobin made. Indeed by increasing the amount and number of radioactive amino-acids in the cells, Gurdon's group calculate that after incubations of about 20 hours it may be possible to detect as little as 1 pg of haemoglobin messenger.

GERONTOLOGY

Living Longer

from a Correspondent

AT the international forum on the control of human ageing, held from September 2–4 at the Gottlieb Duttweiler Institute (The Green Meadow Foundation) in Switzerland, two different points of view became apparent—there were those participants who stated that research in life prolongation must continue and those who were appalled by the social and economic aftermath of such added years.

According to Professor F. Verzar (Institutes für Experimentelle Gerontologie, Basel), this century will be the century of the aged and progress will be made in social gerontology, medical geriatrics and experimental gerontology. A move to the right of the human taximeter clock giving a 10–20 per cent increase in life span may come and batteries of tests to measure ageing rate in the short term must be used. Calorie restriction remains the simplest method of prolonging life and if any active agent such as an antioxidant is used it should be capable of being introduced as late in life as possible to prolong active vigorous life without increasing dependent old age.

The search is now on for a substance which will turn off the process of ageing. Professor B. L. Strehler (University of California) suggested that if the human thermostat could be reprogrammed to 2° C or less life would be increased by 25 per cent.

According to Dr L. Robert (CNRS, Laboratoire de Biochimie du Tissu Conjonctif) immunological manifestations of age related disease are probably themselves part of a general gene-related, differentiation-linked ageing mechanism. Orgel's (*Proc. US Nat. Acad. Sci.*, **49**, 517 ; 1963) theory of the inaccuracy of the protein synthesizing machinery of cells was supported by work with a strain of *Neurospora* (Dr C. M. Lewis, West London Hospital).

Professor I. Sobel (Florida State University) spoke on the employment problem of the over forty-fives. Older workers are already on average unemployed longer. Adding fifteen years to the life span would lead to a period of decline in earnings lasting for twenty-five to thirty years with serious consequential psychological upsets. Each 5-year increase in life span would raise the labour force by about 5 per cent.

Professor W. F. Anderson (University of Glasgow) considered that prolongation of active human life without massive retraining programmes for the older worker and without encouragement to take up part-time employment associated with adequate medical and social resources for the existing elderly would be disastrous.

The pharmacological control of ageing was reviewed by Dr C. G. Kormendy (Bristol Laboratories, Syracuse). He suggested that the most promising substances should be subjected to laboratory studies. Most agents still seem to be too toxic.

Dr M. Goldsmith (Science Policy Foundation, London) stressed that vast socioeconomic implications must be considered before institutions of gerontology are established. Fortunately, gerontologists are unlikely to contribute to demographic pressure before the year 2000 and antioxidants, perhaps the least expensive agents, would have no significant effect until the early decades of the twenty-first century (Dr R. W. Prehoda, Toluca Lake Station, California). Dr R. D. Clarke (Prudential Insurance Company, London) thought that, by 1990, therapies based on two or more ageing theories may extend the life span by 35 per cent. Any modest increase in contraception, abortion or sterilization would have greater impact on demographic pressures in the next thirty to forty years than the probable advances in gerontology.

In discussion, pleas were made for controlled studies now of the effect of certain endocrine substances, for example oestrogens in women, regarding their influence on the life pattern, and Dr J. Huet (International Center of Social Gerontology, Paris) made a forceful argument for an international institute of gerontology under Professor Verzar's administration.

The participants, although differing in the benefits of and means of prolongation of life, were unanimous in the need to plan now for larger numbers of old people. Multidisciplinary action should be taken by scientists, sociologists and physicians.

Drug Resistance in Cancer Chemotherapy

THE problem of the development of resistance to chemotherapy is one of the major difficulties in the path of successful treatment of disseminated cancer. A study of the mechanisms of resistance in man is extremely difficult for most cancers because remissions are uncommon and multiple biopsies of cancer material are not usually possible. A study of resistance to drugs in acute leukaemia, however, is feasible because multiple complete remissions are common and the blood and bone marrow can provide malignant cells at several stages during the development of the disease.

Drug resistance may develop because the agent is not taken up by the malignant cell or because enzymes are produced which catalyse its destruction either in the malignant cell itself or by normal tissues such as the liver. New metabolic pathways may be used to circumvent the metabolic block of the agent used. These types of resistance are likely to be specific for a single group of chemotherapeutic agents with similar modes of action. Another possible mechanism of resistance is less specific and depends on an alteration in the proliferation characteristics of the cancer cell. Such changes could result in an alteration in the effect of all drugs acting on cancer cells at the time of DNA synthesis which includes most anti-cancer chemotherapeutic agents. Resistance to a large number of drugs could therefore develop non-specifically. There is growing evidence that all these mechanisms may be operative in man.

One example of resistance to a drug being modified by metabolic alterations within the cancer cell is provided by the treatment of acute lymphoblastic leukaemia using L-asparaginase. This agent is thought to act, at least in part, by depriving the malignant cells of the amino-acid L-asparagine. Resistance to L-asparaginase which develops during or following treatment has been associated with increased levels of asparagine synthetase activity within the leukaemic cell (S. M. Haskell *et al.*, *Cancer Res.*, **29**, 974 ; 1969).

The response of acute leukaemia in man to treatment with the antimetabolite cytosine arabinoside has also shown a correlation with the rate of uptake of the drug *in vitro* and its subsequent activation by phosphorylation (M. Y. Chu and G. A. Fisher, *Biochem. Pharmacol.*, **14**, 333 ; 1965). Stewart and Burke report in next Wednesday's *Nature New Biology* that leukaemic cells from patients who respond to treatment contain six times less cytosine deaminase than is present in cells of those who do not respond. The concentration of this enzyme, which inactivates cytosine arabinoside, increases following a course of therapy and this is correlated with increased resistance to therapy.

Tests *in vitro* in man for predicting response to the initial treatment using different drugs are unfortunately unreliable—the tests pay no attention to an agent's absorption, detoxication, excretion, distribution and duration in the body. The mechanisms whereby cells can repair or circumvent damage by the agent may be very different *in vivo*. Nevertheless, in acute leukaemia it may be possible to predict the development of resistance, following one course of treatment, to a particular drug. This could well be important in the management of patients relapsing with this disease.

ENZYMES

Proprotease Prospects

from our Molecular Biology Correspondent

IT seems to be a general rule of nature that animal proteases are manufactured in a harmless packaged form, as zymogens, and are activated, generally by the intervention of some other protease, when the organism has need of them. This process may have evolved in part as a device to prevent the onset of a lethal proteolytic activity on completion of biosynthesis, and, more interestingly, as a refined control mechanism, as reflected, for example, in the role of the enzyme cocoonase. This is a serine protease, produced by the silk moth for use as a chemical crowbar to break open its cocoon. It is synthesized as a zymogen, procococoonase, in cells at the mouth of the insect, is deposited on the surface, where it forms a solid crystalline crust, is activated, and then dissolved in a specially secreted potassium bicarbonate solution, with a *p*H corresponding to the activity optimum. The solution seeps into the cocoon, and dissolves out the sericin, a proteinaceous "silk glue", and leaves the moth free to pass into the outside world.

The latest in a series of articles from the two laboratories which have uncovered this scheme (Berger, Kafatos, Felsted and Law, *J. Biol. Chem.*, **246**, 4131; 1971) now describes the nature of the zymogen, and the manner of its activation. An extract of the secretory parts of the insect, at a point in its development some days before emergence, contains no cocoonase activity. The attempts first made at activation of the procococoonase in this solution with active cocoonase resulted in rapid loss of the activity of the latter. This, it transpired, was a consequence of attack by a tyrosinase present in the blood. When this enzyme was inhibited the cocoonase was seen to bring about gradual activation. Trypsin or subtilisin, on the other hand (but not chymotrypsin), caused very rapid liberation of the full cocoonase activity, and it was therefore surmised that free cocoonase can play only a secondary role in activation. One does not, however, have to search far for a source of activating protease activity: at an early stage of adult development the region between the adult and the pupal cuticle is full of a liquid, the moulting fluid, rich in proteases, which serves to digest the pupal cuticle. When the fluid has done its work it is reabsorbed, leaving apparently a residuum of proteases to look after the procococoonase.

Estimates of molecular weights by a number of methods showed that the molecular weight of the zymogen was about 32,000, some 8–10,000 more than free cocoonase. Thus by contrast with trypsin, which it otherwise closely resembles, a large part — about one quarter—of the chain is lost on activation. Benger *et al.* point out that because the activation fragments of zymogens are in effect nothing more than covalently bound inhibitors, the structural requirements may be supposed to be much less stringent than for the active enzyme; and that large differences introduced in the course of evolution in these parts of the chain are not perhaps so very surprising.

Another new zymogen has been discovered by Harper, Bloch and Gross (*Biochemistry*, **10**, 3035; 1971). This is the precursor of the collagenase which the tail of the tadpole carries, as the means for its own annihilation when the moment of metamorphosis is at hand. Collagenases have been widely identified in animal tissue, and especially in diseased conditions, in which the elimination of collagen masses is desirable. The proenzyme was discovered through the observation that anti-collagenase antibodies were absorbed by collagenase-free extracts of tadpole tail-fin tissue. The chromatographic fraction of the extract believed to contain the zymogen failed to generate collagenase activity on treatment with trypsin or chymotrypsin. When, however, the fraction was mixed with a medium in which living tail-fin tissue had been maintained for some days, and incubated at 37° C, activation ensued. The collagenase could be detected by its effect on the viscosity of the substrate, and by the appearance of tropocollagen fibrils with characteristic periodicities in the electron microscope. The collagenase produced under these controlled conditions was identified with the usual preparations by its inhibition by anti-collagenase antibodies. The collagenase has apparently the remarkably high molecular weight (assuming the reliability of the detergent-gel electrophoresis method) of 110,000, or so, and the zymogen seems to be slightly larger. The activating factor is easily inactivated by heat, and is evidently a specific protease, that appears only at the right moment.

Precursors of DNA Replication

THE biochemistry of DNA replication as opposed to DNA repair is still a great enigma and, according to Werner, so it will remain if those intent on elucidating it persist in assuming that DNA replicase uses the deoxynucleoside triphosphates as immediate precursors. For in next Wednesday's *Nature New Biology*, Werner describes experiments which lead him to the conclusion that the enzyme responsible for the semi-conservative replication of DNA in *Escherichia coli* polymerizes some activated form of the deoxynucleoside monophosphates.

Earlier this year, Werner reported in *Nature* (**230**, 570; 1971) that short pulses of ^{3}H-thymidine are incorporated preferentially in small DNA chains, Okazaki pieces, whereas short pulses of ^{3}H-thymine are preferentially incorporated into larger DNA chains. From this he argued that *E. coli* can differentiate between thymidine and thymine using the former for repair DNA synthesis and the latter for replication. His latest experiments are an attempt to identify the pathway of incorporation of ^{3}H-thymine into DNA. When there is a dynamic equilibrium between the intracellular nucleotide pools—a steady-state condition—the pool of thymidine monophosphate is maximally labelled about 1 min after the cells are exposed to ^{3}H-thymine. Maximum incorporation of the thymine into DNA and maximal labelling of the pools of thymidine diphosphate and triphosphate are attained after about 10 min. TMP is therefore not itself the immediate precursor of DNA replication but either TDP or TTP might be.

When, however, the cells are fed ^{3}H-thymine and simultaneously deoxyadenine or thymidine, which act as donors of deoxyribose, the pool of TMP is greatly increased and is maximally labelled within about 10 s and the maximal rate of incorporation of the thymine into DNA is reached within 20–30 s. The pools of TDP and TTP are, however, not maximally labelled until about 2.5 min. From these observations and measurements of the specific activities of the pools of TMP, TDP and TTP Werner concludes that although TMP is not itself an immediate precursor of DNA replication it is on the direct pathway to replication while TDP and TTP are not, and has to be converted to an activated form of TMP before being incorporated into DNA.

Because the size of the pool of activated TMP precursor is much smaller than the pool of TTP, and because DNA is replicated so very rapidly Werner suggests that simple diffusion and collision would be too slow to support the selection and alignment of the activated monophosphate precursors by the DNA replicase. He speculates therefore that some protein like that described by Alberts and Frey (*Nature*, **227**, 1313; 1970) may unwind the DNA duplex ahead of the replicase. The exposed single strands can then act as templates for the preselection and alignment of the precursors and all the DNA replicase has to do is link them together by phosphodiester bonds.

MITOCHONDRIAL DNA

Symmetric Transcription

from our Cell Biology Correspondent

THE transcription of double helical DNA by DNA dependent RNA polymerase is generally assumed to be an asymmetric process with only one of the two strands of the DNA, the coding strand, being transcribed into messenger or one of the stable species of RNA. In *Escherichia coli,* as Travers, Bautz and Burgess and their associates have shown, the ability of RNA polymerase to select and transcribe the coding strand depends on the presence of sigma factor, without which transcription is symmetric.

Mitochondria which seem to have evolved from symbiotic bacteria, not only contain small double stranded and closed circular DNA molecules but also their own RNA polymerase, which is distinct from that in the nucleus of eukaryotic cells. As Küntzel and Schäfer reported recently (*Nature New Biology,* **231**, 265 ; 1971), the RNA polymerase in the mitochondria of *Neurospora crassa* is a much simpler and smaller molecule than *E. coli* RNA polymerase ; it comprises a single polypeptide chain with a molecular weight of about 64,000. On reflexion therefore, Aloni and Attardi's report (*Proc. US Nat. Acad. Sci.,* **68**, 1751; 1971) that both the strands of the mitochondrial DNA of HeLa cells are transcribed *in vitro* is perhaps not all that surprising. Symmetric transcription may perhaps be the evolutionary price mitochondria have had to pay for their simple genome and its RNA polymerase.

The two strands of HeLa cell mitochondrial DNA can be separated by appropriate centrifugation, and Attardi and his colleagues have developed methods for isolating mitochondrial RNA made *in vivo* after pulse labelling with uridine. Determining whether transcription is symmetric or asymmetric can therefore be determined by comparatively straightforward DNA. RNA hybridization experiments. Aloni and Attardi find that RNA made in the mitochondria during short (1–5 min) pulses hybridizes equally to the heavy (H) and light (L) strands, but RNA made during longer pulses hybridizes preferentially with the H strand. They have already obtained evidence which indicates that the entire H strand is transcribed, and this together with the finding that RNA made during short pulses hybridizes equally with H and L strands, and the rapid sedimentation of the L strand RNA, suggests that like the H strand the L strand is extensively and perhaps completely transcribed. The L strand transcript, however, rapidly disappears from the mitochondria either because it is degraded or possibly because it is exported to the cell cytoplasm. Why the transcription of mitochondrial DNA is an exception to the rule of asymmetric transcription remains to be seen.

Schäfer and Küntzel and their colleagues (*Europ. J. Biochem.,* **21**, 478 ; 1971), having isolated the RNA polymerase of *N. crassa* mitochondria, have now devised a method for isolating intact the DNA from these mitochondria. They find it is a circular DNA some 25 μm long, in other words some five times longer than the mitochondrial DNA of HeLa and other vertebrate cells ; this leads them to speculate that the mitochondria in fungi may represent an early stage in the reductive evolution of bacterial endosymbionts which culminated in the mitochondria of vertebrates with their very small genomes. Using mitochondrial DNA from *N. crassa,* Schäfer *et al.* have investigated its transcription *in vitro* by *E. coli* RNA polymerase with sigma factor and conclude that there are about eight to nine specific binding sites for this mitochondrial genome. Some 50 per cent of the RNA transcribed *in vitro* by the bacterial enzyme seems from hydridization experiments to be ribosomal and transfer RNA. Among the remainder of the *in vitro* product are RNA strands which self anneal and may well be the product of symmetrical transcription of regions of the genome other than those which specify the stable mitochondrial RNAs. As Schäfer and Küntzel have themselves shown, however, the mitochondrial RNA polymerase is very different from the bacterial enzyme and it would be interesting to see what RNAs are transcribed *in vitro* by the mitochondrial enzyme.

STOMATA

Response to Humidity

from a Correspondent

TRANSPIRATION in plants is controlled by adjustments in the size of minute pores, the stomata, which perforate the surface of leaves. Each stoma consists of a slit bounded by two specialized epidermal cells, or guard cells, which determine its size. When the guard cells take up water and swell their shape alters in such a way that the pore opens and when they become less turgid it closes. Stomata are normally in an open or partly-open condition during the day and closed at night. As a result, diffusion of carbon dioxide into a leaf, which follows the same pathway as

Seismic Signals from Underground Explosions

IN next Monday's *Nature Physical Science,* A. Douglas, P. D. Marshall and D. J. Corbishley, from UKAEA, Blacknest, suggest reasons why some of the seismic signals from underground explosions are more complex than others. The basis of their idea is that the direct P waves recorded in a complex signal have passed through material with a lower average value of the quality factor, *Q*, and have been alternated.

Most P signals from underground explosions are simple in character and usually comprise a single pulse—two to three cycles long—followed by a tail of lower amplitude arrivals which can be explained in terms of reflexions in the crust or upper mantle. But the less common complex signals have a tail with an amplitude that is similar to or even larger than the first arrival.

Douglas *et al.* point out that, according to their theory, the tails of complex signals should contain more high frequency energy than the direct P waves because high frequencies are selectively attenuated in materials of low *Q* ; complex signals from an underground explosion should therefore point to lower average explosion magnitudes than the simple signals because of the reduction of the amplitude of the first arrivals. To test the whole theory Douglas *et al.* have examined the P seismograms of two underground explosions—one fired near Bukhara in the Soviet Union and the other (the nuclear explosion Longshot detonated on October 29, 1965) at Amchitka in the Aleutian Islands.

The vibrations from the Russian explosion were detected at Eskdalemuir in Scotland and at Gauribidanur in India, and the records from these two stations provide good examples of simple and complex signals from the same source. The record at Gauribidanur is complex and there is clear evidence of late arrivals with a higher dominant frequency than the earlier arrivals. And the magnitude of the explosion as measured from the Eskdalemuir records is higher than that indicated by the Gauribidanur data, suggesting that the direct P waves arriving at Gauribidanur had been attentuated.

Douglas *et al.* detail two examples of complex signals from the Longshot explosion (both recorded in British Columbia) from which the magnitude of the explosion was estimated to be 4.8 and 5.4 ; this is much lower than the average estimate of 6.2 from the data of the arrays operated by the United Kingdom Atomic Energy Authority which all detected a simple signal. They also find that the beginnings of the complex signals have a dominant frequency of ~0.8 Hz compared with a dominant tail frequency of ~1.4 Hz, again in accord with the theoretical predictions.

water vapour, is facilitated during periods of photosynthesis, and water loss is curtailed at night. But stomata also close sometimes during the day where the air is dry and excessive evaporation places the plant under water stress.

It is now well established that light causes stomata to open by lowering the carbon dioxide concentration in the vicinity of the guard cells as a result of photosynthesis. By this device increased consumption of carbon dioxide by the leaf leads to stimulation of absorption and vice versa. The response to changes in the humidity of the air has been less intensively investigated, but it has been generally accepted that it depends on alterations in the water content of the leaf as a whole. Lange, of the Botanical Institute, University of Würzburg, has dissociated himself from this view and in a recent article in *Planta* (**100**, 76; 1971) he and his colleagues put forward further evidence that guard cells respond directly to changes in the humidity gradient existing between the external air and substomatal air space.

Lange *et al.* have devised an elegant technique whereby it is possible to observe microscopically the behaviour of stomata in isolated strips of leaf epidermis when subjected to rapid changes of ambient air of different moisture contents. Induction of opening and closing by alternate exposure of the outer surface of the epidermis to moist and dry air was repeated as many as fifteen times on the same stoma. When different areas of the same piece of epidermis were treated separately the stomata in each part responded independently according to the conditions to which they were subjected.

Lange *et al.* conclude that guard cells act as humidity sensors which respond directly to changes in the gradient of water potential between the air above the leaf and in the sub-stomatal air space. When this gradient increases the guard cells lose water and the pore closes and when it falls they take up water causing the pore to open. This mechanism causes a rapid adjustment in pore size in response to changes in evaporative conditions in the vicinity of each stoma irrespective of the water status of the leaf as a whole. Lange *et al.* assume that the level of carbon dioxide was unaffected by the treatments that were applied and that the changes in turgidity of the guard cells is attributable to a direct influence of the humidity gradient on absorption and loss of water by these cells. The possibility of an interaction between carbon dioxide concentration and water potential gradient in the overall control of stomatal response will no doubt be examined in the future extension of this work.

Satellite Lines in the Solar X-Ray Spectrum

In next Monday's *Nature Physical Science*, J. H. Parkinson of the University of Leicester reports observations of satellite lines in the solar X-ray spectrum. These are lines which are observed close to the resonance transition of each helium-like ion, hence the name "satellite".

During the past few years the study of helium-like ion spectra and associated satellite lines has proved to be of considerable interest to solar physicists. In addition to the resonance line, a partially forbidden "intercombination" line is observed for each ion. A further strong line at slightly longer wavelength than the resonance and intercombination lines was shown by Gabriel and Jordan (*Nature*, **221**, 947; 1969) to originate from a transition which was previously thought to be highly forbidden as a one quantum transition. These authors (*Mon. Not. Roy. Astron. Soc.*, **145**, 241; 1969) then showed that the relative intensity of the intercombination and forbidden lines can depend on the electron density of the emitting plasma.

The satellite lines are caused by transitions in the stage of ionization lower than the helium-like ions, that is, in the lithium-like ions. The resonance line of the helium-like ion is a $1s^2$–1s 2p transition. The satellite lines have a similar wavelength to this transition because they are transitions of the same type but in the presence of a further electron (that is, $1s^2\ nl$–$1s\ 2p\ nl$). Satellite lines have been observed in laboratory spectra for many years—Edlén and Tyrén first reported them in 1939, and proposed the forementioned origin.

As part of the investigation leading to the identification of the helium-like ion forbidden transition, Gabriel and Jordan classified several multiplets of satellite lines, because it was at one time suspected that the strong solar line could have a similar origin. They proposed that the excited levels giving rise to the satellite lines are populated by the process of di-electronic recombination, hitherto not reported as a process important in laboratory plasmas, but thought to be important at the lower densities in the sola corona.

Several groups of workers (at the Aerospace Corporation, the Goddard Space Flight Center, the Lebedev Institute, and the US Naval Research Laboratory) have recently reported the presence of weak satellite lines in solar spectra, chiefly during flare activity. The new observations by Parkinson were obtained with improved spatial resolution by using a collimated crystal spectrometer, and refer to a stable phase of the active region. Parkinson's intensity data indicate that, as in the laboratory, the solar satellite lines are formed by the process of di-electronic recombination; this demonstrates directly the existence of this process in the solar corona. The relative intensities of the satellite lines and helium-like resonance lines are therefore dependent on the electron temperature in the emitting plasma. Parkinson has observed the intensities of the helium-like lines and satellite lines, so both the electron density and temperature in the emitting solar active region can be studied. And because each group of lines occurs over a limited wavelength region it should be possible to obtain reliable relative intensities.

Aspirin and Human Embryonic Cells in Culture

In next Wednesday's *Nature New Biology*, Paine and Nagington report that sodium salicylate—a breakdown product of the common sodium acetylsalicylate or aspirin—inhibits the growth of some human embryonic cells in culture.

Tissue cultures from skin, lung, heart and kidney were prepared from 12, 14 and 18-week old human embryos. The cells were grown in the presence of various concentrations of sodium salicylate. Kidney cells from 12 week old embryos showed the highest sensitivity —2 mM (320 μg/ml.) salicylate completely inhibited growth and 0.5 mM (80 μg/ml.) almost reduced the growth rate by half. Lung cells were also affected, but less markedly. Heart cells were even less sensitive than kidney and lung cells; only 2 mM (320 μg/ml.) salicylate showed any significant effect at all. Skin fibroblasts were not inhibited, even by 2 mM salicylate.

The mode by which salicylates produce this inhibition is unknown. Salicylates have a wide variety of pharmacological effects. There are indications of a correlation between salicylates taken during early pregnancy and foetal defects; but a causal connexion has not been established. Analysis of the foetal blood of 272 consecutive deliveries showed that about 10 per cent had salicylate concentrations of more than 10 μg/ml.; the mean concentration in these infants was 33 μg/ml. (about 0.2 mM) (Palmisano and Cassady, *J. Amer. Med. Assoc.*, **209**, 556; 1969).

Paine and Nagington quite properly point out that one cannot infer that the action of a drug in an intact animal or human is similar to the effect of the drug on cells in tissue culture. But the evidence they present is enough to warrant further investigations.

BIOENGINEERING

Aids for the Disabled

from a Correspondent

THAT the successful design of an aid for the disabled depends on detailed knowledge of the behaviour of the normal was clearly illustrated at the conference on human locomotor engineering held at the University of Sussex from September 7 to 10. The Institution of Mechanical Engineers had assembled speakers from many disciplines and discussion was both brisk and fruitful.

From a number of contributions dealing with the characterization of individual gait patterns in the various phases of the walking cycle, using electromyographic, cine-photographic or force-plate recording, it became clear that there is a need for more data about the functional anatomy of the limbs, and in particular about the exact positions of the lines of action of the various muscles relative to the centres of rotation at the relevant joints. Without this information it is difficult to calculate the force which any specific muscle exerts at a particular time or what the stresses are on the ligaments or articular facets of a joint. Quantitative descriptions of the time courses of several parameters in the gait pattern are required both for the design of the dynamics of artificial limbs and also in assessing the fitting of particular limbs to individual patients. Some of the walkway studies, such as that described by Dr M. Milner (Groote Schuur Hospital, Cape Town) and Dr T. Kasvand (National Research Council, Ottawa), now incorporate computer-scanning of the cine-film to derive the paths of markers attached to the subject over the axes of rotation at the various joints.

The recordable electrical activity of the muscles proves disappointing as a guide to the magnitude of the forces, but it does give useful timing information and an ingenious technique of display has been devised by Dr D. S. Grieve and Mr P. R. Cavanagh (Royal Free Hospital, London). Instantaneous values of the angles at adjacent joints, say, knee and ankle or knee and hip, are plotted against one another during the walking cycle and the resulting locus is marked off to indicate the period during which a specific muscle is active. The patterns generated in this way can be recognized by eye and may prove useful in recording the progress of clinical conditions.

Knowledge of the normal patterns of muscle activity also provides the basis for programmed electrical stimulation of muscles to restore a lost function. Dr E. M. Sedgwick and Mr S. A. G. Chandler (University of Southampton) have developed a neat trick of feeding back the amplitude of the M wave of the electromyograph in a servo to give proportional control of the number of motor units stimulated. This method overcomes the difficulty that the range of intensities that lie between threshold and maximal stimulation is small and is comparable with the variation in the threshold itself.

In the design of artificial joints for implantation, there are many conflicting requirements. Dr P. S. Walker (The Hospital for Special Surgery, New York) concluded that the chief long term problem now is the reduction of wear at the surfaces of contact. New materials need to be tried. The properties of the adhesives at present in use also leave something to be desired, particularly where tensile stresses are involved.

In the field of simulations, Mr D. C. Witt and Mr J. I. Hall (University of Oxford) have made considerable progress with their automatically stabilized powered walking device. This device is about five feet tall and stands on small feet at the ends of telescopic legs a few inches apart. The leg lengths are adjusted by hydraulic actuators to give alternating support, stability being achieved by adjusting the time of dwell on the appropriate side. They use a device modelled on the semicircular canals of the inner ear as an attitude sensor to control the switch-over. In a film, the "robot" was shown walking on the spot and it was not unbalanced when a block of wood was suddenly inserted under the descending foot. Symmetrical fore-and-aft motion of the legs has now been incorporated and the device is making its first steps in forward locomotion.

Professor J. M. Nightingale and Mr R. W. Todd (University of Southampton) described a robot hand which provides complex finely-adjusted movements of the digits to perform acts initiated by electromyographic signals from the operator (patient). The control signals that can be picked up from a patient and made available to control an external device are very severely limited in their information-carrying capacity. Adjustments of the digits to take account of the shape or fragility of the object to be grasped thus need to be delegated to an automaton which can take over the "unconscious" elements in the control task.

Measuring Epstein-Barr Virus Genomes

WHETHER or not Epstein–Barr (EB) virus has any role in the aetiology of Burkitt's lymphoma or nasopharyngeal carcinoma is still a matter of controversy and there seems little prospect of the issue being decided in the next several years. On the other hand, it is now generally accepted, chiefly as a result of the hybridization experiments done by Zur Hausen and his colleagues (*Nature*, **227**, 245; and **228**, 1056; 1970), that cell lines obtained from Burkitt lymphoma biopsies may contain detectable amounts of EB virus DNA even though these cells do not produce progeny EB virus particles. That is of course a familiar situation to tumour virologists who for the past several years have argued about precisely how many polyoma virus or Simian Virus 40 genomes are associated with the genome of a transformed cell. And now the same arguments are arising among those studying EB virus, for in next Wednesday's *Nature New Biology*, Nonyama and Pagano suggest that the so-called Raji line of Burkitt lymphoma cells may contain about sixty genome equivalents of EB virus DNA rather than about six equivalents, which was the estimate of Zur Hausen's group.

Zur Hausen's estimate was based on measurements of the extent to which EB virus DNA hybridizes with DNA from Raji cells and Nonyama and Pagano have measured the extent to which hot RNA, transcribed *in vitro* off EB virus DNA by *Escherichia coli* RNA polymerase, will hybridize with Raji cell DNA. The advantage of this second technique is simply that the RNA can be made some 100-fold hotter than EB virus DNA. Using their high specific activity RNA, Nonyama and Pagano find some 700 genome equivalents of EB virus DNA in a line of Burkitt cells which produces progeny EB virus and some sixty-two genome equivalents of EB virus DNA associated with the mitotic chromosomal DNA of the non-producer Raji line. They also detect between forty-five and a hundred genome equivalents of this viral DNA in human leukaemic and normal leukocytes culture, cells which do not carry detectable amounts of EB virus antigens. By contrast the human HeLa cell does not contain detectable amounts of EB virus DNA, and Nonyama and Pagano believe that their data support the idea that infection by EB virus is required for the continuous growth in culture of human leukocytes.

What Sugawara, Mizuno and Osato have to say in next Wednesday's *Nature New Biology* should also interest anybody working with EB virus. By centrifugation through discontinuous gum acacia density gradients they have separated, by virtue of differences in buoyant density, EB virus carrier cells from cells actively supporting the replication of this virus. As the active replication of the virus in carrier cells is initiated and progresses so the buoyant density of the cells seems to decrease, and their separation becomes possible.

Reminiscences of Rutherford

from a Correspondent

AT the thirteenth international congress of the history of science held in Moscow, August 18–24, one day (August 20) was devoted to a colloquium on the Rutherford centenary and 75 years of radioactive studies. The USSR Academy of Sciences extended a generous invitation to many of Lord Rutherford's former members of staff and students of the Cavendish Laboratory to attend this colloquium and to spend many days in the Soviet Union as guests of the academy. Academician Peter Kapitza (Institute for Physical Problems, Moscow) welcomed the rather small group of his old colleagues who had been able to accept the invitation; he had asked them to include in their addresses personal reminiscences of Lord Rutherford and the Cavendish Laboratory as it was in the 1920s and 1930s.

Professor Kapitza's acquaintance with Rutherford extended over more years than that of any other speaker; he had come to England in 1922 and remained as a member of the staff until his return to Russia in 1935. He spoke of the lessons he had learned from his Cambridge days and the great debt he owed to Rutherford who was not only an outstanding scientist but was the head and organizer of the most famous school of physics at that time. The genius of Rutherford lay in the speed with which he revealed the laws of nature and also in his remarkable talent for selecting young men with creative ability. Professor Kapitza recalled that only an accident had brought Rutherford to England: there were two candidates for the one 1851 Exhibition scholarship offered to New Zealand that year and Rutherford was placed second; fortunately for the world the winner decided not to leave New Zealand. Yet reading Rutherford's earliest works, which today are of no consequence and have sunk into oblivion, even at that time his genius can be recognized; he could not be referred to as a scientist of great erudition but his creative imagination and daring in the development of hypotheses which fitted the facts were the key to his success. Professor Kapitza thought that young people ought to read more of the original works of the great pioneers in science, and that this was especially important for those who are likely to be in charge of a group of research students and who may have to select staff.

Lord Blackett was prevented by illness from attending the celebrations and his contribution was read by Professor N. Feather (University of Edinburgh). He reviewed Rutherford's remarkable career of scientific research and discovery and suggested ways by which a study of this can help science today. He reminded the audience that some of Rutherford's greatest discoveries arose from a keen observation of what may be described as chance or anomalous effects. Air draughts in his room at McGill University spoilt measurements of the strength of a thorium source but led to the discovery of the "emanation" gas escaping from the thorium and thence to the whole pattern of radioactive decay; a few alpha-particles coming back from the same side of a thin metal foil as was bombarded by a strong beam of such particles puzzled him and led him to what is generally regarded as his greatest discovery, the nuclear theory of the atom; and a few exceptionally long-range protons projected forwards from some light nuclei, but not from all, when bombarded by energetic alpha-particles led to the discovery of the artificial disintegration of nitrogen. "May every young scientist remember (these incidents) and not fail to keep his eyes open, for the possibility that an irritating failure of his apparatus to give consistent or unexpected results may . . conceal an important discovery."

Lord Blackett himself had to examine 400,000 alpha-particle tracks to discover only six which revealed the process of disintegration described earlier by Rutherford. In those eventful years Rutherford was right in believing that nuclear physics was one of the greatest if not the greatest single branch of physics and he tended to decry the importance of some of the other branches, notably theoretical physics; he did not see as clearly as he might have done the impact of the study of the electronic structure of the atom on the developing quantum theory which itself was soon to supply the explanation of the range of the alpha-particle. Rutherford was not a mathematician but he used mathematics to great effect in his two greatest experimental achievements and he sought out those fields of research in which little mathematical reasoning was needed. He would have thoroughly enjoyed the early days of the new unstable elementary particles.

Professor Feather then described his own experiences in the Cavendish Laboratory from 1926 up to the date of Rutherford's death; he began as a research student and, apart from two brief interludes, he remained as a member of the staff and was perhaps the last to talk to Rutherford about the work of the laboratory before Rutherford went to hospital for the hernia operation from which he never recovered. Feather's research work for many years involved the use of the Wilson cloud

Professor P. Kapitza addressing the Rutherford colloquium at the thirteenth international congress of the history of science. On Kapitza's right, his secretary, Dr D. Shoenberg, Dr T. E. Allibone and Dr W. B. Lewis; on his left, Professor N. Feather, Professor S. Devons, Mr G. Danin and the chairman of the congress, Professor B. M. Kedrov.

Pure Chemistry Library
SCIENCE COLLEGE
Circular Road

chamber which was operated in those days most laboriously by hand and needed the assistance of a collaborator. During some of those years Rutherford, as president of the Royal Society, had many calls upon his time so his tours of the laboratory were less frequent than formerly, but Professor Feather described an experience shared by everybody at the Cavendish, of hearing Rutherford's heavy footsteps coming along the corridor, and his voice mounting louder as, in room by room, he fired questions about the work in progress, sometimes administering a much-needed prod to "cut out the frills", sometimes half grudgingly applauding good work, always cautious in assessing results, but then when quite satisfied, ensuring prompt publication as in the case of the papers submitted to the Royal Society announcing the discovery of the neutron and its effects.

Professor S. Devons (Columbia University), a product of the last phase of Rutherford's life, gave a vivid description of the undergraduate's contacts with the great men of that age, and then, later, attendance at the meeting of the Cavendish Physical Society. "Rutherford took the chair but was no passive chairman, his vigorous and amiable personality shone as he introduced the lecturer and later opened the discussion; his attitude was always unambiguously expressed." Facts were to be respected and treated differently from theory; though Rutherford greatly respected at least some of the theoreticians he disliked a smoke-screen of verbiage. The era of simple apparatus, the "string and sealingwax" trademark of the Cavendish was slowly passing when Devons began research—it had indeed been almost Henry Cavendish's own trademark 150 years earlier—but the budding research student still had to be "broken in" in the famous attic, making apparatus without assistance, using only the scraps and junk discarded in days gone by. His own choice of work, cosmic rays, had never actively interested Rutherford, possibly, Professor Devons suggested, because they were too complex and, not being under direct control in the laboratory, may have needed apparatus too elaborate for those days, and, as Rutherford had commented, the interpretation of results could prove a trap for the unwary.

Dr D. Shoenberg (University of Cambridge) contributed some delightful asides. Rutherford had once said that science can be classified into physics and "stamp collecting", but, as Shoenberg pointed out "stamp collecting" can yield rewarding results if a general principle can be deduced from the collection of data and Moseley's idea of making a systematic study of the X-ray spectra of the elements did indeed provide an important clue to the nucleus. Dr Shoenberg reminded the audience that Rutherford won the Nobel Prize for "stamp collecting", that is, for chemistry, and on one occasion Rutherford introduced Debye with the remark "although he is a chemist he is really quite a decent sort of chap"!

Dr Shoenberg began his research as one of Professor Kapitza's pupils in the new building, the Mond Laboratory then being erected to rehouse Kapitza's intense magnetic field work and the new work on very low temperatures. Rutherford had taken a very great interest in the magnetic field work and had obtained for several years very considerable financial support for it, certainly considerable as judged by the standards prevailing in the "old" Cavendish. Although Rutherford was no expert in these two subjects studied in the Mond he had an uncanny way of getting straight to the heart of the matter under discussion, seeing what was missing, and asking for key measurements to be taken; and when Dr Shoenberg used mathematical terminology to describe the new theories of metals, Rutherford would say "now just tell me in simple language what that term is that you keep using".

Dr T. E. Allibone spoke of Rutherford's lectures at the Royal Institution and of his visits to the Royal Society Dining Club at which so many eminent Russian scientists had dined starting with Count Razumovski, the president of the Russian Academy who had dined in 1766 with Henry Cavendish, Benjamin Franklin and others. Rutherford was invited to give the Bakerian Lecture of the Royal Society in 1904, a signal honour for so young a man, he dined as a guest of the club that evening, and on the following day gave his first Friday Evening Discourse at the Royal Institution. It was there that he spoke of the energy released by the radioactive elements probably being responsible for a much slower rate of cooling of the Earth than had been calculated by Lord Kelvin—who was in the audience—a few thousand million years compared with Kelvin's estimate of a hundred million years. During the Manchester period he gave several discourses, demonstrating the counting of alpha-particles and describing the disintegration of nitrogen. His lectures were always very popular. Dr Allibone thought it doubtful that Rutherford ever believed in the theory of nuclear structure put forward by Gamow in 1928/29 which precipitated the work on positive-ion acceleration to half a million volts, because when Rutherford opened the new High Voltage Laboratory of the Metropolitan-Vickers Company in February 1930 he asked for millions and millions of volts and a discharge tube compressed into a small-sized room, whereas, if Gamow were to be believed Rutherford ought to have asked for a very large current of positive ions accelerated to only a modest voltage.

Dr W. B. Lewis (Atomic Energy of Canada Ltd) spoke as one of the small band of personal collaborators of Rutherford. Most of the Cavendish men worked either independently or in pairs, responsible to one of the senior members of staff and only a few worked with Rutherford. Dr Lewis spoke of the strict routine to which Rutherford worked and expected his assistants to copy, a routine which had probably contributed greatly to his own success; "stop work at 6 o'clock and go home and think". In a short time this group of which Dr Lewis was a member had unravelled the complexities of the long-range alpha-particles, a subject which had attracted Rutherford's interest for a long time. The success was due to the use of the first of the new generation of "expensive" pieces of apparatus, a magnet designed and made for £250 by the M-V Company. After this Rutherford switched to his last experimental work, the disintegrations caused by deuterium ions accelerated to the paltry voltage of 200-thousand volts, in deference, so to speak, to Gamow's theory. He complained to Allibone about the cost of the 100-thousand volt transformer which the Metropolitan-Vickers Company had supplied. Allibone calculated that it had only cost a farthing a volt, cheaper than the cost of flash lamp batteries in those days, and Rutherford took the repartee in good grace.

Mr G. Danin, a well known Soviet writer on the history of science, discussed the role of Rutherford in the quantum interpretation of the planetary electrons. Published letters and material still unpublished in Copenhagen reveal a complex picture of collaboration between Rutherford and Bohr between April 1912 and March 1913; Rutherford's stimulating encouragement of a man in whom he had great faith combined with a restraining touch "take your time", "don't hurry". Mr Danin spoke of Rutherford's own unpublished attempts to find a quantum solution to the problem of the stability of the planetary model and he concluded that a thorough examination of all the papers and correspondence in the Manchester period would reveal much of interest to the history of one of the most fundamental discoveries of the century.

As each speaker left the rostrum Professor Kapitza presented him with a medal which had been struck in the Soviet Mint to commemorate the centenary, and also with it the first volume of Rutherford's collected works printed in Russian. Dr Allibone also presented to Professor Kapitza on behalf of the Royal Society of New Zealand the medal struck in New Zealand.

T. E. A.

Has Science Never Had It So Good?

by our Special Correspondent

ARE science and society now in a radical confrontation or does their apparent conflict merely represent old issues in new guises? This central question ran through the three controversial themes which dominated the Roche Jubilee Symposium, "The Challenge of Life—Biomedical Progress and Human Values", which was held in Basle from August 31 to September 3. What are the ethical responsibilities which scientists and doctors should assume in response to the advance of biomedical research? Should science and technology be more closely directed, perhaps even restrained, and if so, how? And how are the difficulties of communication between scientists and between scientist and layman to be resolved?

"Humanity is at a hinge of history", said Dr Philip Handler (President, US National Academy of Sciences), when introducing the first session, on biomedical frontiers. But his insistence that the rewards of science have created a critical situation, on whose resolution will depend whether man is a blind alley in evolution, was challenged by Solly Lord Zuckerman (former Chief Scientific Adviser to the British Government) who maintained that: "This is no new issue. This is the oldest issue I know . . . I don't believe for one second that this is a hinge of history. There have been corresponding hinges before. There have been people who hated the development of science and said so before." This disagreement, which was to be repeated during later sessions, foreshadowed the quarrel between idealism and pragmatism which the symposium spotlighted.

Molecular Biology: Threat or Promise?

Assessing the prospects opened for man by the dramatic advances of the past decade in molecular biology, immunology and neurobiology was the task of the opening speakers. Although there is general concern about the implications for man's heredity of the possible development of means for genetic manipulation, Dr Joshua Lederberg (Professor of Genetics, Stanford University) felt that the prophesies of genetic engineering merely bring into sharper focus problems which have been present for some time. There was also general agreement that organ transplantations are exaggerating ethical concerns which are in any case raised by medical immunology.

Particular controversy was aroused by the fear that it may become possible to clone humans to yield many identical individuals, with Dr Salvador Luria (Sedgwick Professor of Biology, Massachussetts Institute of Technology) saying that this idea is of as little scientific interest as the exploration of space; nothing could be learnt from it, he maintained, which could not be found by using the mouse instead. But Professor Lederberg felt that cloning humans would be interesting from another viewpoint, because of how it might help distinguish the effects of environment and heredity on man. Because cloning cattle, for example, might have useful implications for agriculture, Professor Handler asked whether perhaps it would be wise to take a decision on whether to support such research before a technique is developed which could easily be extended to man. The general view of the meeting, which was sanguine that on balance there is only slight risk that genetic manipulations or neurobiological techniques will be abused to change man, was caught by Dr Seymour Kety (Professor of Psychiatry, Harvard Medical School), who noted that identical twins are not deliberately separated at birth to facilitate studies of the interaction of environment and heredity.

Gazing into crystal balls is always a hazard for the scientist, but when asked for predictions of future developments in the biomedical area, Professor Kety thought it likely that the major psychoses will be understood if not eradicated, and Dr Niels Jerne (Director, Basel Institute for Immunology) suggested that allergies and leukaemias are likely to be brought within the scope of medical treatment. Professors Luria and Lederberg agreed that molecular biology has as yet contributed little to modern medicine, but each predicted that it will prove indispensable as the basis of future treatments. Professor Luria specified the intensive research into reverse transcription, which he thinks is likely to be productive in understanding at least some forms of cancer; Professor Lederberg pointed to the extensions which seem likely in the scope of antenatal diagnoses.

The first topic on anyone's list for useful future research should be ageing, said Dr Arnold Burgen (Director, National Institute for Medical Research, Mill Hill), later during the symposium. This should include attempts both to increase the human life span and also to make the later part of life more liveable, although this latter problem is particularly difficult because of the way in which the different systems of the body age at different rates. Although endogenous diseases such as ageing seem likely to prove far more difficult to resolve than those caused by external agents, Professor Burgen feels that significant progress could be made if only a fraction of the funds supporting cancer research were put into such studies. Increases in life span which have already been achieved, however, were identified by Dr Alfred Sauvy (Professor of Social Demography, College of France) as the principal cause of the rise in world population. He is sure that future research will lead to some further abatement, even if it does not yield the dramatic lifespans of 90 or 120 years forecast by some people.

Over population was a topic which had loomed over all the sessions, said Alexander Lord Todd (Professor of Organic Chemistry, University of Cambridge) in his summary of the meeting. He took the consensus to be that Malthusian forebodings of famine are misplaced, but he supported Professor Handler's plea that although man has always had a sense of crisis, this time is different because the resources of the planet are only finite and some are beginning to run out. This contrasted with the earlier remarks of Dr Margaret Mead

Philip Handler.

Solly Lord Zuckerman.

Salvador Luria.

Joshua Lederberg.

(Professor of Anthropology, the American Museum of Natural History, New York) that some people regard population as an evil so great that almost any step should be encouraged to limit it, even if that step were drastically to change the nature of human society. The general opinion of the meeting, however, that society will in any case be changed, greatly for the worse, unless some acceptable way is found to limit population, was echoed by Professor Luria's comment that scientists and doctors have not fulfilled their responsibility to explain the consequences of population growth.

How Should Scientists Report Results?

The purposes which scientists ascribe to research were attacked by Dr Jeanne Hersch (Professor of Philosophy, University of Geneva), who argued that science is itself a vested interest, and that what this meeting really represented was the gathering of an élite of scientists to defend their interest. Promising a cure for cancer is just a ruse to keep funds flowing, she said; the molecular biologist working on "cancer" should recognize that his real motivation is not to cure the disease but rather to pursue truth for its own sake—and he should convey this attitude to others instead of using pretexts to gain funds. But the pursuit of science is in itself a human value and as such needs no further justification, insisted Professor Jerne, who extrapolated this reasoning to suggest that because social evolution may retard and perhaps even stop science, possibly quite soon, those people interested in research should press ahead as fast as possible while there is still time to do so.

The conservative view of science was taken further by Professor Jerne when he argued that scientists have already evolved the most apposite way to communicate their results and that no change should be made in this procedure. Their proper *modus operandi* is to publish original research articles; they should not try to popularize their work because it is philosophically impossible for any such attempt to avoid introducing some degree of distortion. Those who want to follow the conclusions of scientists should learn the language of science, he said. But other participants evidently felt that this attitude is symptomatic of the arrogance which tends to bring science into disrepute with society. The general consensus was with Dr Charles Berry (Director of Medical Research and Operations, NASA), who feels it is vital that scientists should explain their work to the public, and with Professor Lederberg, who stressed how essential it is to reverse the alienation of science for society. The scientist is responsible, he argued, to the community of people from whom he derives the funds which support his work.

There should be two levels of communication of scientific result, suggested Professor Burgen, one for scientists who may wish to repeat a particular set of experiments, and another to enable non-scientists to appreciate the significance of a research result if not the precise means by which it was obtained. Both Professor Burgen and Professor Lederberg think that the communication network by which scientists communicate with each other works quite well on the whole, and Professor Burgen suggested that a similar communication network should be developed to enable scientist to communicate with non-scientist. Professor Lederberg feels that one way to approach this problem might be to make use of the facilities of the professional associations of scientists; these learned societies would be in a good position to provide accurate assessments of research.

Is there a Right to Health?

The conventional view that individuals are entitled to health was challenged by Professor Hersch, who suggested that health implies the ability to withstand minor ailments, which should not be subject to exhaustive treatment as they tend to be at present. When the improvement of health becomes an obsession, she maintained, it becomes an ill in itself. Of obvious relevance to the pharmaceutical industry were the discussions by Professor Berry, Dr Kenneth Arrow (Professor of Economics, Harvard University) and Dr Axel Strøm (Professor of Social Medicine, University of Oslo) of the attitudes of doctors and of how medical care is organized. Max Lord Rosenheim (President, Royal College of Physicians of London) summed up the general view that although new treatments and improved methods of diagnosis will change much of the organization of the medical profession, it is essential that the one doctor–one patient relationship should be maintained.

"The relief of minor symptoms is not only desirable from the point of view of the sufferer but also of the pharmaceutical industry", said Lord Rosenheim when answering one of the questions raised at the beginning of the symposium by Professor Alfred Pletscher (Director of Research, Hoffman-La-Roche): how much effort should the pharmaceutical industry devote to producing drugs to cure minor ailments? Lord Rosenheim's point was that the industry derives much of its income from just such drugs, and it needs the money made in this way if it is to be able to support the development of much more expensive, although less commercially rewarding drugs such as immunosuppressive agents. But in any case, Lord Todd said during his summary, even when the industry has decided how to balance its research efforts, drugs to treat minor ills will inevitably be developed as the result of side effects noticed during research originally designed for other ends. Another problem of some concern, that of the rising extent of self diagnosis and treatment, will also inevitably continue to increase, he observed, with the rising level of education of the population.

The demanding pattern of development of medical research was the concern of Lord Zuckerman's paper. Quoting a former British Minister of Health, Enoch Powell, he pointed out that demand for medical care is for all practical purposes unlimited. "Every advance in medical science creates new

Margaret Mead.

Alexander Lord Todd.

needs that did not exist until the means of meeting them came into existence, or at least into the realm of the possible. For every heart–lung machine or artificial kidney in operation, there must be many times that number of cases to which the treatment would be applicable." As Lord Zuckerman himself argued: "If money is provided for medical research workers, the brilliant research worker will use that money successfully. He will provide some new discovery, the discovery will be applied, and then you get the pattern of demand for the particular advance he has made". Continuing this reasoning, Lord Zuckerman argued that because resources must always be inadequate to meet demand, priorities must be imposed on the provision of treatment. Lord Rosenheim added that the provision of expensive treatments in early days must be limited not only by money but also by provision of trained doctors.

The inconsistencies in the pattern of medical care were remarked by Lord Rosenheim. "Contraception and abortion are preventing the life of an infinite number of healthy people, whilst medicine is keeping alive idiots, hydrocephalics and cases of spina bifida." The medical profession will not change this paradox, he said, which can only be influenced by an informed consensus of public opinion. And as Lord Zuckerman observed: "There is an unquestioned right to health and people have been brought up to believe they have that right. However scientific a treatment may be, it cannot hold its place in the market if there is no demand for it. Nor can the greatest quackery be kept off the market if there is a demand for it."

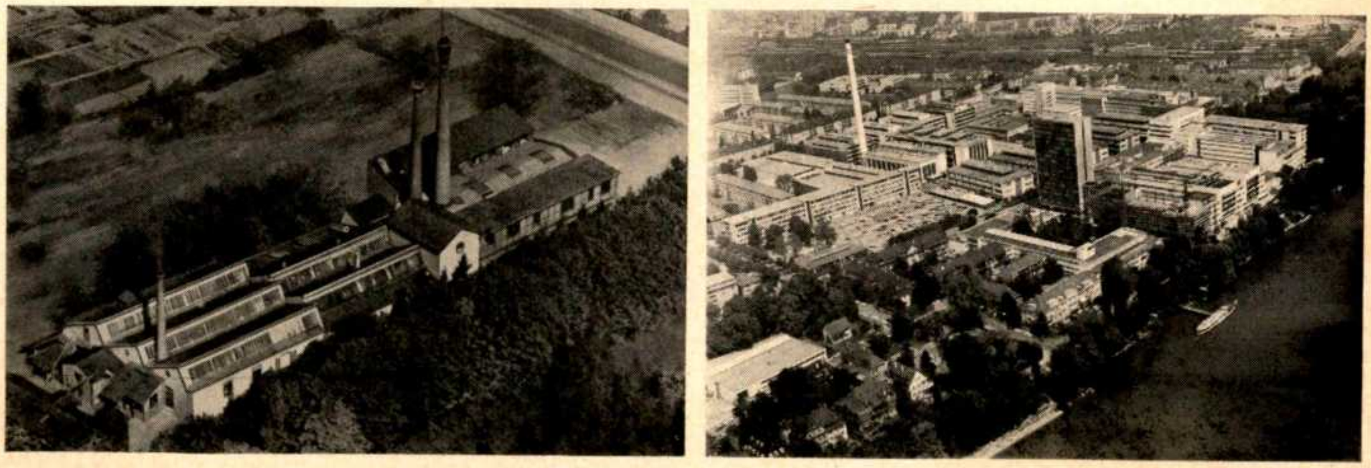
Should Biomedical Research be Curtailed?

Is it irresponsible to allow advances in biomedical research to create demands which cannot be fulfilled? Should some of the funds now allotted to basic research be redirected to support studies of more clearly defined and perhaps more urgent needs? These talking points created considerable controversy. Lord Zuckerman's view was clear: "There can be no question in my mind but that the resources available will be inadequate for the claims of researchers. I am one of those who believe that (one should) beam research funds at certain fields in relation to need."

Both the ethos and the practicality of Lord Zuckerman's ideas were fiercely challenged by Professor Handler. First of all, he pointed out, many diseases which were at one time expensive to treat have now been eliminated completely. The patients who take great resources are those who have contracted diseases of this type which should by now have been eliminated, or diseases for which only supportive rather than definitive treatments are available. The development of expensive treatments such as renal dialysis is just a stage, but a necessary one, on the way to developing a simple and inexpensive cure. The doctors present agreed that routine operations may develop from what is at first an operative technique used only at great expense in individual cases. Both Professor Handler and Professor Luria observed that, in any case, the cost of biomedical research in the United States (quoted as $2.5 billion) is so slight compared with the cost of corrective medicine (quoted as $70 billion) that it is illusory to suppose that there could be any appreciable financial saving from cutting back funds for biomedical research.

Opinions varied on whether society should control the direction of basic research in more detail, but the general consensus was that this is neither desirable nor, indeed, possible, because of the adventitious way in which knowledge advances. Because the motivation of the brilliant researcher who makes the more significant advances is intellectual curiosity, attempts to direct his efforts would be misplaced and unproductive. But the speed of change worried Professor Luria, who felt that we are engaged in a "breathless race with technology" because science and technology progress so much faster than the changes in the values of society which are needed to assimilate new living conditions. But an important distinction should be made, urged Professor Luria, between discovering new facts of nature and developing them into a technology. The critical stage at which to exercise control must be at the development of a technology, because it is not possible to control the development of research itself, nor to control the uses to which a technology is put once it has been developed. And because the acquisition of knowledge is fast in comparison with its application, even a moratorium on research would not halt the development of new medical treatments from existing knowledge for a considerable time yet.

The sense of the meeting was caught by Professor Handler when he argued that because only governments can now afford to pay the bill for research, and because resources are only limited, decisions on general priorities must be taken. Whereas it is an absolute necessity to decide how much goes into biomedical research it would be a very grave mistake to distinguish between the narrower parts of the biomedical sciences. Responding in particular to Lord Zuckerman's comments, he said that "if you mean anything narrower than that cancer research should go into the general properties of cells, you are wrong". Another point was caught by Dr Talcott Parsons (Professor of Sociology, Harvard University) who suggested that it might be of more use to control how



The first factory of Hoffman-La Roche in Basle, pictured in 1903, is shown on the left; the present buildings on the same site are shown on the right.

research is to be done, by setting standards of scientific competence and objectivity, than to control what research is performed.

Should the Sociologist Judge Science?

Although Dr Handler's eloquent assessment received vigorous applause from the meeting, the sociologists and theologians did not agree with it. Dr Franz Böckle (Professor of Moral Theology, University of Bonn) argued that the desire to knowledge must be subjected to certain limitations because it is impossible for the researcher to have a proper grasp of the overall situation. The real problem, he and Dr Jürgen Moltmann (Professor of Systematic Theology, University of Tübingen) argued, is to establish a set of ethics by which to judge biomedical progress, but for which the natural sciences alone cannot suffice and which must depend on the humanities.

This plea was echoed during a more detailed discussion of how research funds should be allocated. Dr Bernard Barber (Professor of Sociology, Columbia University), commenting on the peer review system by which panels of scientists assess for the US National Institutes of Health the values of projects in their own fields of competence, urged strongly that the sociologist, if not to be allowed to sit in judgment on the scientist, should at least play an important part in deciding the lines along which research should be directed. People who know the views of the public, he felt, should have at least some say in the allocation of funds. Professor Mead supported Professor Hersch's comment that scientists are too much an élite concerned only with their own problems, and complained that the accurate predictions which sociologists had made in the past that there would be a backlash to science had been ignored. An urgent need, she said, is for proper studies of the effects of scientific advance on the attitudes of society.

In contrast to the sociologists, Professor Luria is not too worried about the operation of the peer review system in the United States, but pointed to the discrepancy that although basic research is usually assessed by this means, its technological development is ultimately at the whim of the politician. Professor Luria intends to argue strongly at congressional hearings to be held in Washington next month that the new cancer research programme should be directed under peer review.

"I am frightened of peer review," said Lord Zuckerman, "because I know of peer reviews where there is only one peer, or two or three men." In a scathing attack on the way support for research is organized, he said he is particularly perturbed by the ways in which fashions in science can dictate the pattern of research. Professor Handler maintained that many of the options open to man have been generated by research, but according to Lord Zuckerman, "whilst science opens enormous avenues for the future, at the same time it is closing other options by setting fashions". Molecular biology is a notable example of this trend, he implied; in reference to Sir Macfarlane Burnet's recent view (in *Genes, Dreams and Reality*) that molecular biology has contributed little to medicine nor is likely to, he said that: "If six other Burnet's were to share that view, the Medical Research Council should start diverting funds to fields which represent clearly defined needs (such as) the problems of old age . . . which are receiving too little attention".

But would not setting priorities impinge on the freedom of the researcher? "We are already imposing the pattern of social justice on the pattern of medical research and it does not in any way infringe the independence of the medical research worker." Who should decide such priorities? "I dismiss (the medical research worker) because he merely creates demand regardless of need. I dismiss the public and client because he absorbs anything he is given." This left government, which, said Lord Zuckerman, was not desirable either. Referring in particular to medical research, he demanded that peers conducting reviews should hold a wider view of the field than just their own speciality. "Those people who have a sophisticated view about resources and need should choose." *Sed quis custodiet ipsos custodes?*

The problem which Professor Lederberg visualizes is how to improve the social utility of research without damaging other values. But researchers have no less social conscience and probably more compassion than politicians, he suggested, and a "shopping list" made for useful research would in any case very largely list the present pattern of activities. Centralized planning, he argued, would be very likely merely to lead to the excesses of the space programme. Both he and Lord Zuckerman observed, however, that the scientist as such has no particular political franchise; if scientists and doctors wish to be the people who determine the ends to which research is put, they must enter directly into the political arena.

How Many Cultures?

Can the life sciences be integrated with the social sciences in some way? Professor Handler, summarizing his view of the meeting, said that: "All my life I have tried to be amongst those who have maintained, somewhat defensively I guess, that there are not two cultures". But he had rarely seen a better demonstration that there are indeed two cultures. Professor Mead was among those who felt that there had been too much talking and not enough listening. "The only way in which people from different disciplines can learn to understand each other is to look at the same problem."

But Professor Parsons suggested that integration can be provided by modes of thinking which go beyond one particular science, such as the idea of coding. Might there be some parallel between the biological and the linguistic codes, he asked? Lord Todd brought the meeting to earth by asking how integration might be achieved in practical terms, suggesting that perhaps the lack of interdisciplinary thinking is a consequence of the way university courses are organized. Professor Handler, however, maintained that integration of subjects occurs only in individual human brains; the failure of packages of arbitrary multidisciplines at universities in the United States showed that there is no way to contrive integration. Professor Luria agreed that the function of a university is to give a superior training to people who can then use it to communicate effectively and to cross disciplinary boundaries.

Of what value did the conference prove to Hoffman-La-Roche? Introducing the meeting, Professor Alfred Pletscher said that "society has not considered soon and systematically enough the outcome and final consequences of biomedical advances. This omission must be remedied. The aim of the symposium should therefore be to define more clearly the problems created by biomedical progress and to find, if possible, essential facts or clues for their solution." At the end of the symposium, Professor Pletscher said that there could be no definite answer to most of the important questions, but that the symposium had succeeded in formulating more precisely the questions which should be asked. Now that a frame had been set to examine the problems, future conferences would be able to examine more specific problems.

The final discussion was directed by Lord Todd, chairman of the symposium, to answer the questions which Professor Pletscher posed at the outset. No certain way could be seen to avoid the potential harmful effects of research on mankind, but there was a consensus that fears are in general exaggerated. Basic research should not be directed, except in a very general way, and Professor Luria's point was well taken that the only option for control is at the development of a technology. An important target for biomedical research should be ageing, although this will bring other problems in train. And there was general agreement with Professor Burgen's refusal to regard the improvement of health as two edged. In spite of much talk, the most cogent comment on the ethical issues raised by biomedical progress was the lack of an answer to Lord Todd's question: "Can we harmonize biomedical science and human values?"

Science and the Common Market

BRIAN FLOWERS

State House, High Holborn, London, WC1

This article is based on a speech delivered by Professor Sir Brian Flowers, chairman of the Science Research Council, on September 6, 1971, at the meeting of the British Association in Swansea.

In the great debate on the proposed entry of the United Kingdom into the Common Market science has been mentioned hardly at all, and even technology has received only scant attention to bolster up the economic arguments. It would be strange indeed if we were not to explore the likely effects of joining our West European friends on the advancement of science in this country and in Europe. I therefore propose briefly to do so.

But first I must make a declaration. I am a public servant appointed to a non-political post under the Labour government and now serving under a Conservative government and I must therefore make clear that I do not seek to indulge in party politics, nor am I acting, in this article, as a mouthpiece for the Science Research Council, still less for the government. I had hoped—together, I am sure, with many other people—that the Common Market issue would not be one to divide the parties, but alas that was not to be. For that reason the excellent custom whereby public servants do not indulge in party political controversy are best maintained if I confine myself as far as possible to examining the issues as they seem to me to concern science and technology.

Two Freedoms

I think we all accept that the two most basic elements of international science are the free exchange of information and the free movement of scientists across national frontiers. Indeed, one recalls the unhampered travels of Davy and Faraday in continental Europe during the Napoleonic wars. The learned and professional societies, as well as institutions like the British Association, contrive to preserve these international elements in spite of limitations inevitably imposed from time to time by national security and commercial confidence. In recent years the Royal Society with its post-doctoral exchange scheme has been particularly successful in the European context.

It is, however, a matter of quite recent history that the funding of science is also becoming international. This is the consequence of the scale on which some of it must be practised if anything significant is to be achieved; I need mention only nuclear physics, space research and, perhaps in the future, molecular biology. And it is the consequence of the nature of some branches of science, which are international in content as well as in practice: there would, for example, be very little point in trying to explain the British climate using only British data. Indeed, for some branches of science it is necessary to consider the planet as a whole, and so we have bodies like the World Weather Watch and the World Health Organization.

Of course, our experiences of European scientific organizations have been far from uniformly successful. At the one extreme we have CERN, the European Organization for Nuclear Research, which is universally acknowledged not only as a successful scientific venture but as an example of successful international collaboration in general. It was created in 1952 during the post-war wave of European idealism, and its existence has enabled scientists from its member states to regain their position at the forefront of a science that is still of profound significance. Ironically, the UK did not join CERN at its inception—we waited to see whether it would be a good thing. And we hesitated in 1968 when the question arose of taking the next great step in energy to 300 GeV. But again we eventually joined, recognizing not only scientific merit but also the political will of Europe. At the other end of the spectrum we have ELDO, the European Launcher Development Organization, founded in 1962 at the instigation of the UK because we wished to find a use for our own discarded defence rocket Blue Streak; the combined European launcher which resulted has so far failed dismally to put into orbit even a Kruschevian orange. Unlike the more or less federal CERN with its efforts concentrated on its accelerators in Geneva, ELDO is much more a loose confederation of national efforts and it is in competition with the attempts of several member states to achieve a purely national launcher capability, our own included until the recent cancellation of Black Arrow.

Between these two extremes comes ESRO, the European Space Research Organization, which, in spite of acute and still unresolved conflict between its member states over the essential purposes and the scale of effort of the organization, has achieved some notable successes in scientific research in space, and by so doing has enabled a European technology to develop which may soon make its mark in satellite applications such as regional telecommunications, meteorology and navigation. Nearer to the failure end, but this time innocent of British assistance, comes Euratom, the Atomic Energy Authority of the Common Market. Both of these organizations, but especially Euratom, are again in competition with national enterprises deemed to have greater success and significance. There are those who hope that the accession of the UK to the Common Market will bring in fresh air for Euratom to breathe; others believe that asphyxiation would be a better remedy in this case. Unlike the other organizations I have mentioned, Euratom is one of the official Common Market institutions and it will require very close examination in the future.

A more recent case is that of the still embryonic European Molecular Biology Organization, dedicated to exploit on a European scale one of the most exciting developments in all of science, our recent chemical and physical understanding of the nature of biological processes at the molecular level. This began controversially not because of the quality or content of the science, but because of the scale on which it was originally proposed that it should be prosecuted. The attitude of the British scientific establishment at that time was hostile because of the scale and because we were already strong in the subject. On the scale now proposed, however, this is a case where, confident of our own achievements, we might now feel that we have something to give to the other nations of Europe in an enterprise whose effects may yet be still more significant to our understanding of the life sciences, including medicine.

Criteria for Success

The lessons to be learnt, it seems to me, from this brief survey of some of the existing European scientific and technological organizations are five-fold. First, the science must be good science and the purpose of setting up the organization must be clear; second, the initiative for creating the organization must come from the scientists working in the field; third, scientists of the highest quality in each country must want to take part; fourth, the nature of the science must render organization on a European scale either necessary or at least likely to bestow distinct benefit on all participants; and finally, each country must give its support of the organization priority over any residual national effort in the field. CERN has satisfied all these points; the other European organizations I have mentioned have not.

The internal criteria I have described for a successful European research organization seem stringent enough, but scientific research is only part of the whole social activity. There are also economic, political and social criteria which exert a powerful influence on what research is supported from public funds and on how that research is organized. Thus in fields where independent sovereign governments and business concerns believe that the results of research could give their possessor some economic or other social advantage, the pressure to support national programmes may be great enough to prevent successful international collaboration. This has been the case with space technology and with nuclear power. Indeed, attitudes towards collaboration in technology sometimes differ sharply from attitudes towards collaboration in science because of the hope of earlier gain. Even on a national scale there have been many examples of firms failing to collaborate wholeheartedly in government supported co-operative research projects, which could bring benefits to all, because they feared that their competitors might secure the greater benefit. In cases where research can be expected to lead to a fairly early economic or social benefit some obvious common interest is required to ensure real cooperation. For example, all countries are persuaded that it is in their interest to control plague, and realize that this must be tackled on an almost world-wide scale. In economic and political matters, however, our self-interest is not yet enlightened enough for the majority to see that cooperation both in research and in its applications will pay simply because, ultimately, we prosper or suffer together.

It will be a long hard slog to the millennium and, meanwhile, anything we can do to encourage real collaboration between sovereign groups will help enlightenment. Despite all the difficulties I have mentioned, scientific research is a very promising area in which to encourage international collaboration in general. Such collaboration, of course, took place in fundamental science long before the Common Market was thought up. No doubt this will continue if only to meet objectives beyond the scope of Western Europe. But the Common Market will provide a psychological as well as a financial framework within which international collaboration in Europe will become more naturally the rule than the exception.

What is Science?

To be able to discuss the effects of the Common Market upon science, we must first be clear about the nature and purposes of science.

Science in the first place is a cultural activity and, as such, peculiarly a product of European culture. It changes profoundly our comprehension of the world around us and of our place in it. We have only to remember the work of the early astronomers like Galileo and Kepler and of the impact of their ideas upon contemporary philosophical and theological thought, or of Darwin on the origin of species, or of Einstein on the nature of space and time, or of Rutherford on the structure of the atom to see that science is sedition at its best. Modern astronomy is striving for an understanding of the nature and origin of the universe, particle physics for the ultimate structure of matter and the forces which act upon it, molecular biology for the templates of heredity and the nature of biological laws. Their effect upon our understanding of the world about us is likely to be no less profound. It is even a tenable hypothesis that space travel, and the first glimpse it has given us of our world from outside, has helped to focus our attention on the problems that bedevil our sorry planet and its inhabitants. If so it will have paid for itself.

But there is more to it than that. Society demands satisfaction from its investments in science; it has goals it wishes to achieve. It wants better transport, better communications, better housing, better health, better food, more electric power; it wants to be defended, it wants more rewarding leisure, more relevant education: it wants all these things at a reasonable price, and increasingly it wants them not to intrude in unwelcome ways, not to pollute the environment with noise or filth or poison. But none of these things is possible—or at least they would be much more difficult to achieve—without science. For the great majority of people, whatever they may think of science in their leisure hours (if they think of it at all) and whatever its cultural value, science is not a thing in itself but a means to achieve some of the aims of society.

Counting the Cost

The goals I have mentioned are, by and large, recognized in every industrialized country in the world as so important that they each rate a separate government department. Many of them, indeed, can be pursued only on an international basis of some sort. They are not so much national goals as goals of the human race to be striven for independently of national politics, and in some cases in spite of national economies. It is in the hope of achieving these aims of society, and not for the sake of science itself, that the industrialized nations of the world devote something like 2% of their gross national product to research and development, including something like a tenth of that to fundamental scientific research. Scientists cannot expect support on this scale—indeed they should not have it—unless their work can be seen to form part of a pattern which as a whole produces substantial public benefits.

Of the science-based industries—those that depend especially upon automation, instrumentation, electronics, chemistry, and materials science—most are in favour of joining the Common Market. The reason is that many of them believe that a home market of 50 million poeple is insufficient as an export base, and that 300 million is nearer the mark. And, in turn, the reason for this is often that only on such a basis can the huge research and development overheads be covered in an economic manner—aerospace is the notorious example, but nuclear power and computers are others. And in some cases—transport and communications, for example—it is only by adoption of a much larger measure of standardization than is possible on a purely national basis that economic operations become possible, our recent moves towards metrication were, of course, a helpful step in this direction. As far as science is concerned, our postwar failure to achieve a steady economic growth has been due not so much to any failings, real or imagined, in our achievements in pure and applied research, but to our inability, with a home market increasingly confined to the UK, since the loss of an empire, to find the wherewithal to pay the much heavier development costs (including prototype production) required to profit from expenditure on research.

On a European basis we can expect to be able to afford our share of these development costs provided that we, and our partners, gradually give up the idea of alternative and purely national competing ventures. And in streamlining our research and development efforts within the Western European nations we can afford to devote more than we have done in the past to mitigating some of the undesirable effects of technological growth—for the humane technological society requires more, not less, research.

The six are already working towards a common energy policy, in particular for cooperation between themselves and the oil-supplying countries. This, of course, is basic to all industrial activity. They hope to widen the field of trade with the oil countries and thus improve relations generally between themselves, the oil countries, and the oil companies. They also plan to take joint action to support exploration for new supplies of oil, and to raise loans to finance the building of nuclear power stations, a matter which I imagine will be of great importance to us. But improvement of the quality of life is just as important an objective of the economic and social policy of the community as the quantitative raising of the standard of living. Because pollution does not recognize frontiers and because no country can be expected to act alone to penalize its industries in competition with the less scrupulous, action must be taken by the community as a whole. Joint action has already been proposed to control air and water pollution. The community intends, for example, to support and extend the work of the International Commission for Rhine Pollution Control in order to protect the North Sea as well as the whole of the Rhine basin.

Facing the Competition

The need for a larger home market is perhaps arguable; some would say that it exists already in spite of tariffs. But excessive competition between countries of limited industrial capability reduces the share of the world market available to each. The real competition is very often with the super-states, especially the United States and, increasingly, Japan, and it is in this context that European cooperation can be most effective. I believe that this applies to research and development as much as to production. The result of joining the Common Market can be expected to be a much larger measure of rationalization amongst European firms, some of it painful in the short term. We may complain about the common agricultural policy as a device to protect inefficient European farmers, but we should not forget how many of our own industries are at present inefficient, and depend upon protection of one sort or another in order to survive foreign competition. The multinational industrial corporation may be expected to take the place, to some extent, of national protectionism. There is also great value for both science and technology in having common tasks tackled by teams of mixed nationality, provided that one can extract the best from the varied experience and training of the different nationalities. We have surely all learned by now that British is not always best!

Especially if the social goals I have mentioned are to be achieved, an industrialized and free society like ours can neither afford to go it alone nor can it, in my view, allow itself to become utterly dependent upon a super-state, whether in the east or the west, in which it has no hope of sharing in and contributing to the government. It is better that we join a community of like-minded nations of similar size amongst which our voice must and will be heard. Nor can we allow ourselves to become politically and economically subject to the uncontrolled growth of multi-national corporations which at present owe allegiance to no nation and whose annual turnover in some cases already exceeds the individual gross national products of several European nations. Thus, regardless of whether it is international capitalism or international socialism that we wish to foster—and because we live in a mixed society it may be that we should wish to foster both—we have more hope of achieving an acceptable result within a community of like-minded nations especially when, in both respects, they have as much to offer to us as we have to them.

Watching Over Science

We are much exercised in this country at the present time with trying to match the inner logic of scientific activity to the aims of our society. In this endeavour the research councils have a vital function, for it is primarily their responsibility to look across the whole range of science from astronomy to production engineering, from molecular biology to social behaviour, from botany to meteorology, to see not only that society provides for the needs of its scientists, but that the pattern of scientific research and the training of scientists and technologists in the universities and colleges adapt themselves as time goes on to the changing needs of society. From time to time governments ask themselves whether the research councils should have as much independence as they have at present. Certainly adjustments need to be made at intervals, but the fact is that most countries decide to channel a proportion of their support for research through organizations which are not themselves responsible for the achievement of specific applications. Whatever failings our research council system may possess it is still among the best in the world; it is something in which we can take some pride and something we can offer to others.

If we join the Common Market there will, of course, in time emerge a further layer of committees and consultations in the hierarchy which determines our scientific and technological affairs. This will take time to evolve, and it will bring with it new frustrations for those who feel that the national system is already over-burdened. Be sure it will also bring frustrations to those who have to operate the system! But we can take some comfort from the fact that European nations all seem to be dealing with the same kinds of problems in the same kinds of ways in spite of different national systems. In recent years there have in particular been many discussions between the research councils and similar bodies on the continent. Correspondingly, there has been a series of meetings at ministerial level which have attempted to define priorities in fields of technology which are of immediate concern. From all this can be expected to emerge, in some form at least, a consultative assembly of the various scientific authorities of Western Europe, a new forum for scientists and for governments to discuss issues requiring, for reasons of scale or of content, organized international coordination and funding, whether it be the provision of a new and expensive scientific facility, or the war against pollution, or the rationalization of industrial research and development.

If, as a result, our administrative procedures become more cumbersome and therefore more slow moving—and I fear that this is almost bound to be the case for a time—we must set this disadvantage against the fact that it will enable us to continue to take part in, and certainly in some cases to lead, activities which are rapidly growing beyond the capabilities of individual nations. There will not necessarily be any disadvantage in the longer term, because if we have learned anything from the past few years it is that science can only benefit from a deeper and wider discussion of its content and purpose.

This, for scientists, mirrors in a small way the problem of political sovereignty that worries so many people in this country. By sacrificing a certain measure of our own sovereignty, more often than not over matters which are in any case beyond our sole control, we share in a wider sovereignty within which we can hope to achieve more. And, insofar as scientific affairs are determined somewhat differently in the various European countries, we have perhaps something to gain on that score also. Scientists, too, will have their analogues of the political and economic problems of the development regions. Each country has its scientific strengths and weaknessess and it will be important to see that, while maintaining the strengths, we do something to ameliorate the weaknessess, whether they be in scientific or in geographical areas.

More Collaboration

In recent years we have seen the advantages of university departments and other research institutions with parallel or complementary interests coordinating their activities in order to share more equitably in limited funds and to have access to facilities to which otherwise they can no longer singly aspire. This in microcosm, is the whole story. But even in microcosm it can be extended. There should be nothing to prevent the collaboration between departments of British universities and departments of other European universities, and in principle there is nothing to prevent it now. Indeed, the research councils are already trying to encourage such developments. It is clear that the only real barrier is a mental one: we are not yet, most of us, accustomed to thinking in such terms. Our work will be immeasurably enriched, both financially and intellectually when collaboration at this grass root level has become commonplace. From it will come not only advantages to science, but also a sharing of experiences between universities of different countries on a scale not yet seen. And here again we have something to offer in exchange, for there is a greal deal to admire in the British university system.

It seems to me that much the same can be said of industry. Although there is already a measure of European collaboration between companies, we are not yet accustomed to thinking of it as commonplace, especially among smaller firms. The placing of joint Common Market development contracts would help to speed the processes of collaboration and rationalization without which an economic union is unlikely to succeed at the technical level.

But we shall have to be wary of undertaking scientific or technological ventures for purely political reasons, merely in order to appear European. Building up a new Europe is indeed a worthy end in itself, and one that scientists and others must be ready to accept. But we and our partners will be foolish to forget that, however high our purpose, bad science leads to bad politics and bad technology to bad economics.

The fear has often been expressed that the Common Market is an inward-looking community. This seems to me an extremely odd view of the efforts of a group of nations who have done at least as much as ourselves to foster contacts between east and west and whose record in helping the developing nations is as good as ours. And it comes singularly amiss from individuals who simultaneously insist upon the supposed advantages of our antiquated monetary system, now fortunately buried.

Be that as it may, in science there has so far been, on the whole, a recognition that exclusiveness is not the proper path. CERN was born before the Common Market and its membership has always been open to all the West European countries. It has observers from Poland, Turkey and Yugoslavia, and it has throughout maintained the closest of international working relationships especially with the United States and the Soviet Union. This will remain the case even when its membership is chiefly that of the enlarged Common Market. And in our space affairs, too, there has been the closest relationship with, and indeed a large measure of dependence upon, the United States. Enlightened self-interest as much as idealism is likely to preserve this state of affairs in a world becoming increasingly "the space-ship Earth". Certainly we shall have to play our part in maintaining an outward-looking stance, and our world-wide bonds of common language will help us to do so. The profound historical and economic interests which the European countries, especially France, Holland and Britain, have in the developing nations of Asia and Africa should make possible an extensive attack on problems of tropical medicine and agriculture to which our contributions in this country have already been highly distinguished. The possibility of extending scientific research within the Common Market towards the solution of problems relevant to the developing world, such as the local development of natural resources, could make an important contribution to the relationship between Europe and most other countries.

Funding Science

Until about five years ago post-war science budgets had been growing at about 10 to 15% a year—at about the maximum rate, that is to say, which could be absorbed by the scientific community. It was a mark of confidence in the importance of scientific research and in the ability of trained scientists and engineers to use their talents in the service of the nation. Indeed, wartime experience had amply justified this view. Since then, and not only in this country, a certain measure of disillusion has set in. Our rapidly increasing expenditure on research has not led to the once expected increase in national wealth. We have begun to doubt whether new science leads to new technology, and even more whether new technology is synonymous with progress for the human race. We have been forced to ask whether our priorities were right, whether more effort should not be devoted to mitigating the effects of technological progress rather than to hastening it and whether an increase in the quality of life is not more important than sheer economic growth. And yet, at the end of the argument, the quality of life has to be paid for from the profits of technology, whatever the remedy may be in detail.

In our own case the process has been made more acute by our lack of many vital natural resources, by a home market insufficient by itself to support a highly technological export industry, and by the political vacuum resulting from a lost empire and a slowly deteriorating economic position. Of course we are better off than we were twenty years ago, but we have been slow to realize, in the fastness of our island state, that our competitors have been steadily gaining over us in their standard of living and in their influence in the world.

Modern science and technology can flourish only in the environment of a prosperous industrialized state; it is utterly dependent upon industry, not only for its share of the taxes contributed by firms, but for power supplies, instruments, materials and top-grade manufacture. If the nation is not prosperous, science cannot prosper; this is the immediate effect. But in the longer term, if science does not prosper the basis of a technological nation is undermined and it will never become prosperous. Scientists therefore have to be more outward looking than they have ever been except in time of war. We have to turn our minds more towards the scientific basis of the twenty-first century, more towards mitigating the effects of twentieth century technology and more towards the problems which have arisen for the human race from the technological advances we have ourselves helped to create, not least for the developing world. And we have to do it on a scale that we cannot by ourselves sustain.

If we join the Common Market we shall at least be presented with an opportunity—an opportunity to gain; an opportunity to contribute; an opportunity to refresh our culture and to regain our prosperity within a common European heritage. We shall be joining a community of nations less afflicted by disillusion, who have not lost their faith in the cultural value of science nor in the economic value of its application. It is an opportunity we must not fail to grasp.

Use of Frog Eggs and Oocytes for the Study of Messenger RNA and its Translation in Living Cells

J. B. GURDON, C. D. LANE & H. R. WOODLAND
Department of Zoology, South Parks Road, Oxford

G. MARBAIX
Department of Molecular Biology, University of Brussels, Rhode-St-Genèse

Injected frog eggs and oocytes provide a very sensitive assay system for the identification of messenger RNA and permit the study of translational control in living cells. The translation of each haemoglobin messenger RNA molecule once every 5–10 minutes for at least 24 hours makes it possible to recognize less than 1 ng of this messenger RNA.

EXPERIMENTS described elsewhere have shown that oocytes injected with haemin and purified 9S RNA from rabbit reticulocytes will synthesize haemoglobin; the effect is not obtained with other kinds of reticulocyte RNA[1,2]. This raises the possibility that the microinjection of messenger RNA (mRNA) into frog oocytes and eggs may constitute a generally useful experimental system for identifying and studying the translation of different kinds of mRNA. This system has the unique advantage of enabling purified RNA to be translated and the control of this process to be studied in a normal living cell. In this article we discuss several of the conditions associated with the successful translation of injected mRNA. In particular we are concerned with the treatment of recipient cells, with the duration and efficiency of haemoglobin (Hb) message translation, and with the potential use of this experimental system for translating messages other than that for Hb.

9S mRNA for haemoglobin has been prepared by SDS treatment of the 14S messenger ribonucleoprotein released from reticulocyte polyribosomes by EDTA[1]. For all experiments described here, oocytes and eggs were taken from *Xenopus laevis*. Except where stated, oocytes were taken from frogs which had been induced by hormone treatment to ovulate between 2 and 4 weeks previously. This was done to ensure that the larger oocytes were growing actively. The eggs used were unfertilized, but underwent the usual activation response to penetration. Oocytes were injected dry but without the removal of follicle cells[2], and eggs were irradiated with ultraviolet light to facilitate penetration of the jelly and vitelline membrane[3].

Micropipettes 10–15 μm in diameter were calibrated to deliver a volume of 50–70 mμl., the actual amount being kept constant in each related series of experiments. Samples for injection were kept on ice, as well as under oil to avoid concentration by evaporation. All samples were taken up in, or dialysed into, the following injection medium: 88 mM NaCl, 1.0 mM KCl, 15 mM Tris-HCl, *p*H 7.6. Injected cells were incubated at 19° C in the saline medium described by Gurdon[2].

Labelling of Eggs and Oocytes

Labelled amino-acids can be introduced into cells either by injection or by addition to the culture medium. The choice of labelling procedure is determined chiefly by: (*a*) the rate at which label in the culture medium penetrates incubated cells; (*b*) the rate at which injected label leaks out, and (*c*) the duration of the labelling period.

We have investigated the labelling of cells by ^{3}H-histidine, with the results shown in Fig. 1*A*–*D*. Labelled amino-acids leak out of injected oocytes rapidly, so that less than 50% remains after 1 h, but leakage from injected eggs takes place at a much lower rate (Fig. 1*A* and *C*). In contrast, labelled histidine in the medium penetrates eggs very poorly, but is rapidly taken up by oocytes (Fig. 1*A* and *C*). The incorporation of intracellular amino-acid into protein is shown in Fig. 1*B* and *D*. The general conclusion from experiments of this kind is that when use is made of short labelling periods of up to 1 h or of unfertilized eggs (which do not normally remain metabolically active for more than a few hours), the highest yield of labelled protein is obtained by injection. When oocytes are used, especially for labelling periods of more than 2 h, it is best to label by incubation (at least for ^{3}H-histidine). A minor disadvantage of labelling oocytes by incubation is that the follicle cells and very small oocytes (usually present in ovarian tissue containing large oocytes) will take up label and synthesize labelled "endogenous" protein, but not the protein coded for by added mRNA because they are too small to be conveniently injected. When it is important to eliminate totally the background contributed by follicle cells and small oocytes, unfertilized eggs (which have no follicle cells) should be used.

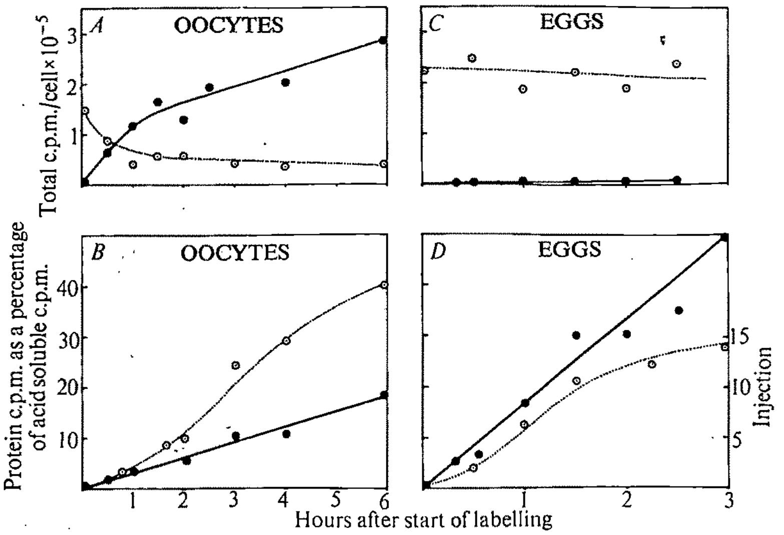


Fig. 1 *A* and *B*, Full size oocytes; *C* and *D*, ultraviolet irradiated unfertilized eggs. For incubation experiments (●) cells were injected with saline solution and incubated at 19° C in culture medium containing ^{3}H-histidine at about 1 mCi/ml. For injection experiments (⊙) each cell received 50 nl. of ^{3}H-histidine at about 6 mCi/ml. At the end of the labelling period, samples of ten cells were frozen. After homogenization in 0.5 M KCl 15 mM Tris, 0.1% unlabelled histidine, *p*H 7.6, an aliquot was dissolved in 90% formic acid and counted to give total counts per sample. The rest of the sample was precipitated in 10% trichloroacetic acid (TCA), and centrifuged. The pellet was taken up in 10% TCA, heated at 90° C for 30 min, pelleted, rewashed in cold TCA, then with alcohol and ether, and finally dissolved in 90% formic acid for counting. From this was determined the amount of labelled protein in the original sample. In *A* and *C*, it is important to note that the total radioactivity includes protein as well as acid soluble c.p.m. The proportion of total radioactivity which is protein can be determined by reference to *B* and *D*.

Translation of Hb Message

The accumulation of labelled haemoglobin in conditions of continuous labelling is shown for injected eggs in Fig. 2*A*. The slow initial rise in the rate of Hb synthesis probably reflects the time needed (10–15 min) for the injected message to become fully incorporated into the structure of the cell in such a way as to serve for protein synthesis; the reduced rate at which labelled Hb is accumulated after 2 h from the time of injection can be partially accounted for by the substantial reduction in the amount of unincorporated labelled amino-acid which remains in eggs injected 2 h before (compare Fig. 1*C* and *D*). The translation of Hb mRNA is compared with that of endogenous mRNA in continuously labelled oocytes in Fig. 2*B*. The amount of Hb mRNA injected was sufficient in these experiments for the labelled Hb to amount to about half that of the labelled "endogenous" protein. It is clear from Fig. 2*B* that both Hb and endogenous protein accumulate at approximately constant rates during the 9 h period of these experiments.

The relative rates of Hb and endogenous message translation have been further examined by pulse labelling oocytes injected with Hb mRNA. Fig. 3 shows that the ratio of Hb to endogenous protein synthesis is similar up to 21 h after injection. Only within the next 10 h is some decrease in this ratio observed. Because no steps were taken to prevent RNA synthesis which takes place in cultured injected oocytes[2], this last result does not necessarily indicate that the rate of translation or stability of Hb mRNA differs from that of the endogenous mRNA already present at the time of injection.

Table 1 summarizes some other respects in which injected and endogenous mRNA translation have been compared. Injected message translation is at least as sensitive as that of endogenous message to the inhibition of protein synthesis by puromycin. The intracellular location of injected message translation is interesting because the nucleus of normal oocytes always has a higher concentration of labelled protein than the cytoplasm, even after short labelling periods (discussed in ref. 4). It has not yet been clearly established whether proteins are synthesized in the nucleus or whether they pass from the cytoplasm to the nucleus very soon after synthesis. From the experiments summarized in Table 1, it seems that injected message is translated in the same part of the cell as most of the endogenous message. The fact that haemoglobin synthesis takes place in oocytes enucleated before RNA injection, as well as in unfertilized eggs which possess no nucleus but only ultraviolet-irradiated chromosomes, shows that Hb is synthesized largely, if not entirely, in the cytoplasm.

We conclude that injected Hb mRNA becomes rapidly associated with components of egg and oocyte cytoplasm so as to be translated in the same way as resident mRNA molecules already in use for protein synthesis. Once started, Hb

Table 1 Inhibitor Sensitivity and Intracellular Location of Hb Synthesis by Injected Oocytes

Recipient cells *	Materials injected		Protein synthesis as % of acid soluble radioactivity		Synthesis ratio: haemoglobin / endogenous
			Endogenous protein †	Haemoglobin	
Large oocytes	9S RNA at 800 μg/ml. and haemin		14.6	43.9	3.01
Small oocytes (half max. diam.)	9S RNA at 800 μg/ml. and haemin		12.5	41.6	3.33
Large oocytes	9S RNA at 800 μg/ml. and haemin	Puromycin at 700 μg/ml.	2.4	3.5	1.46
Large oocytes, enucleated after incubation ‡	9S RNA at 800 μg/ml. and haemin		10.1	33.3	3.30
Large oocytes, enucleated before injection ‡	9S RNA at 800 μg/ml. and haemin		1.5	6.4	4.27

* Each sample, which consisted of thirty oocytes, was labelled by incubation in ^{3}H-histidine for 6 h at 19° C.

† Calculated from 'Sephadex G-100' fractionations as described by Moar *et al.*[13].

‡ Enucleation involved manual removal of the germinal vesicle, which is squeezed out of oocytes after an animal pole incision. The oocytes injected after enucleation did not heal satisfactorily, and incorporation into protein was very low.

mRNA and endogenous mRNA are translated at the same relative rate for at least 21 h; Hb mRNA continues to be translated, possibly at a lower rate, for several hours after this.

Efficiency of Hb mRNA Translation

We wish to know the frequency with which each injected mRNA molecule is translated per minute; this information will permit an estimate of the total number of times an mRNA molecule is translated during its life in an egg or oocyte. The frequency of message translation can be determined from a knowledge of the number of mRNA molecules present in a host cell, and of the specific activity of the intracellular histidine pool (hence the number of Hb molecules synthesized).

The size of the histidine pool in eggs and oocytes has been determined by fractionating on an automatic amino-acid analyser the supernatant of cells homogenized in 0.5 N perchloric acid. One sample of 300 oocytes, from which the follicle cells and ovarian tissue were not removed, contained 36 pmol of histidine per large oocyte. Another sample of 400 oocytes, from which were removed all small oocytes and ovarian tissue except the follicle cells immediately surrounding each large oocyte, contained 23 pmol of histidine per oocyte. A sample of 350 unfertilized eggs, analysed in the same way, contained 91 pmol of histidine per cell. These values for the histidine pool may be compared with the figure of 146 pmol per egg obtained by Kutsky *et al.*[5], using unfertilized eggs of *Rana pipiens*, and 330–700 pmol per egg obtained by Ecker and Smith[6] for eggs of this same species. Since *Rana pipiens* eggs are three times the volume of *Xenopus laevis* eggs, our measurements suggest that a similar or slightly smaller intracellular concentration of histidine exists in *Xenopus* than in the only other amphibian species tested.

Oocytes from the same frogs as those used for estimating the histidine pool size have been injected with Hb mRNA, and the efficiency of message translation has been calculated according to the procedure outlined in Table 2.

To test whether the results summarized in Table 2 are representative of our normal experience, we have calculated in Table 3 the efficiency of translation of the same sample of mRNA used in fifteen other experiments with cells whose histidine pool size was not measured but assumed to be similar to that of other oocytes or eggs. The two samples of oocytes which gave the results in Table 2 were evidently worse in one case, and better in the other case, than is typical. As might be expected, the efficiency of translation is greater at the lowest intracellular concentrations of injected mRNA. This effect is clearly seen in Fig. 4 and is consistent with the observation, reported elsewhere[13], that there is a substantial decrease in translational efficiency at intracellular concentrations of message which exceed 8 μg/ml.

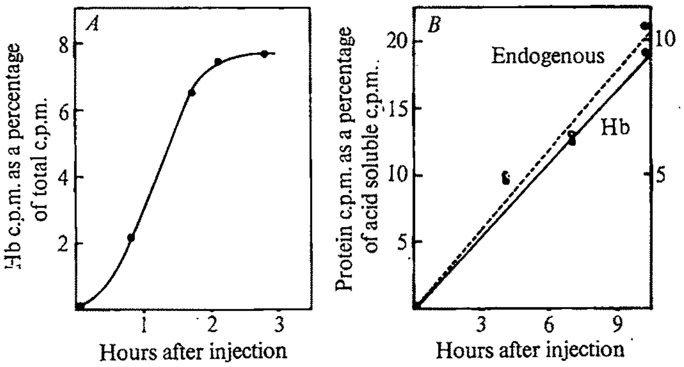


Fig. 2 *A*, Each point is based on a 'Sephadex' analysis of thirty unfertilized eggs injected with 9S RNA at about 800 μg/ml., haemin, and ^{3}H-histidine, and frozen at the times shown. *B*, Each point is based on a 'Sephadex' analysis of twenty large oocytes injected with 9S RNA at about 800 μg/ml. and haemin, incubated in medium containing ^{3}H-histidine, and frozen at the times shown. "Endogenous" refers to protein synthesized from endogenous mRNA, as opposed to the injected 9S mRNA. The ordinate on the left refers to endogenous and that on the right to Hb radioactivity.

In estimating the efficiency of translation, we have made certain assumptions which affect the accuracy of our calculations in the following ways. In determining the specific activity of the histidine pool, our measurements of the extractable free histidine constitute an upper limit to the size of the pool that is actually used for protein synthesis; this could have been

Table 2 Efficiency of Hb mRNA Translation in Injected Oocytes of Measured Histidine Pool Size

	Oocytes of frog A	Oocytes of frog B			
1 Concentration of Hb mRNA in sample for injection (μg/ml.)	200	250	125	62.5	31.25
2 Injected Hb mRNA (ng/cell)*	14,000	17,500	8,760	4,360	2,180
3 ,, ,, ,, (pmol/cell)†	0.070	0.087	0.043	0.022	0.011
4 Number cells/sample	30	20			
5 c.p.m. ^{3}H-histidine incorporated into Hb/cell/h‡	4,480	6,428	8,490	6,439	3,155
6 Pool size of histidine (pmol/cell)	23.2	35.9			
7 Specific activity of intracellular histidine pool (c.p.m./pmol)§	2,845	758	1,392	2,654	1,186
8 Globin chain synthesis‖ (pmol globin/cell/h)	0.157	0.848	0.610	0.243	0.266
9 Number globin molecules synthesized/h/9 S RNA molecule (row 8 ÷ row 3)	2.24	9.77	14.0	11.1	24.35

* These values disregard leakage from injected oocytes, though this is believed to occur (see text).

† Assumes that the molecular weight of 9S mRNA is 200,000. Various published and unpublished measurements by different methods have estimated it to be between 175,000 and 225,000 (for example, ref. 8).

‡ All samples were labelled by incubation for 10 h at 19° C in ^{3}H-histidine of specific activity 52.1 Ci/mmol.

§ Calculated from the histidine pool size and the acid soluble c.p.m./cell recovered from columns of 'Sephadex'.

‖ Assumes 9.5 histidine residues per average (α and β) globin chain.

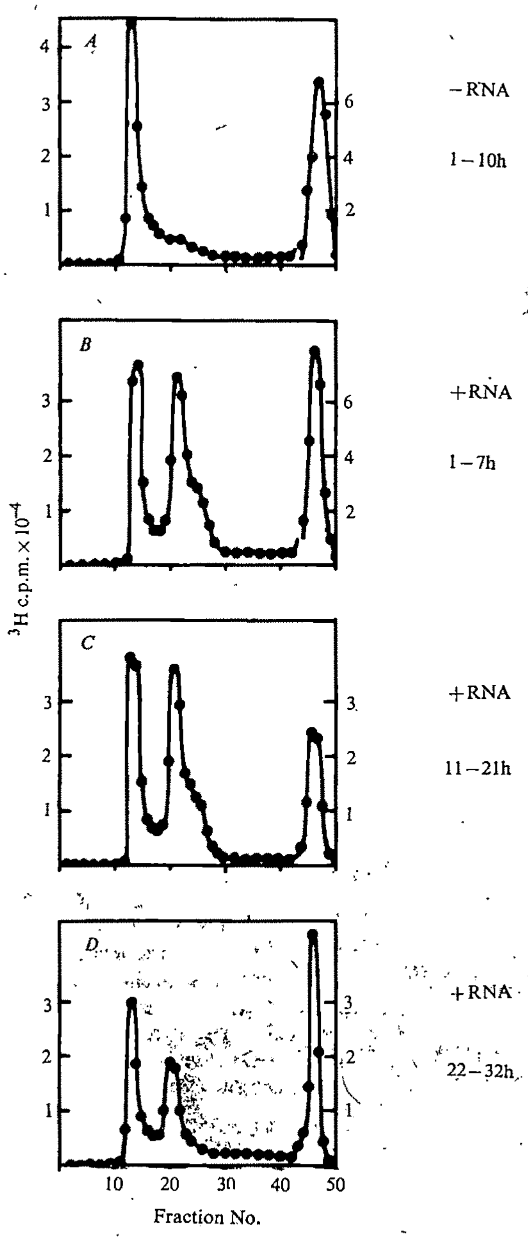


Fig. 3 'Sephadex' analysis of injected oocytes. Each sample of twenty oocytes was injected with haemin, and in the cases of *B*, *C* and *D* with 9S RNA at 400 μg/ml. (about the saturating concentration for these oocytes). Samples were labelled for the periods shown, and frozen at the end of the labelling period; samples *C* and *D* were incubated in unlabelled culture medium until the beginning of the labelling period. The radioactivity on the left hand ordinate of each figure refers to the first two 'Sephadex' peaks; that on the right hand ordinate refers to the right hand 'Sephadex' peak of ^{3}H-histidine.

much smaller, and if negligible would mean that we have overestimated the efficiency of translation by about six times. On the other hand, we have assumed that the same amount of acid-soluble radioactivity as was extracted from oocytes at the end of the labelling period was present throughout the incubation. It is clear from our study of histidine penetration (Fig. 1*A*) that the cell content of ^{3}H-histidine increases during the labelling period, and this will have led to our underestimating the efficiency of message translation. Another factor which could have led to a two-fold underestimate of translational efficiency is the leakage of injected mRNA. We know that about half of all injected ^{3}H-histidine leaks out of oocytes within an hour (Fig. 1*A*). Finally, it is not certain that every RNA molecule within the 9S preparation injected was a potentially functional message. By assuming that every molecule within the injected sample is able to serve as a normal Hb message, we can only have underestimated the efficiency of translation.

In Table 4 we compare the efficiency of Hb mRNA translation in different conditions. It is evident that Hb mRNA is translated 100–1,000 times more efficiently in frog oocytes than in a good cell-free system, and if, as seems possible from the above arguments, we have underestimated the efficiency of injected message translation, then the translation of Hb mRNA in frog oocytes compares very favourably with that in normal living reticulocytes.

Can Other mRNAs be Translated?

The results so far reported do not permit the conclusion that every mRNA could be translated in frog oocytes. The successful translation of rabbit 9S RNA in these cells might depend on its possession of one or both of the following properties: (*a*) it is prepared from a species different from that of the translational system; (*b*) it codes for a protein never normally synthesized (in detectable amounts) by the host cells.

In the absence of a purified mRNA which codes for proteins normally synthesized in *Xenopus* eggs and oocytes, we have not been able to test the importance of these conditions. But the results of injecting three other types of mRNA into frog eggs and oocytes permit us to conclude that (i) rabbit 9S RNA is not the only heterologous RNA capable of being translated in frog oocytes; (ii) some kinds of artificial polymer cannot be translated, (iii) at least one kind of heterologous mRNA cannot be translated. The three types of mRNA referred to are the mRNA for mouse myeloma protein, AUGpolyU, and f2 bacteriophage virus mRNA.

A 9–13S fraction of RNA extracted from the K-41 mouse myeloma promotes the synthesis of the expected myeloma

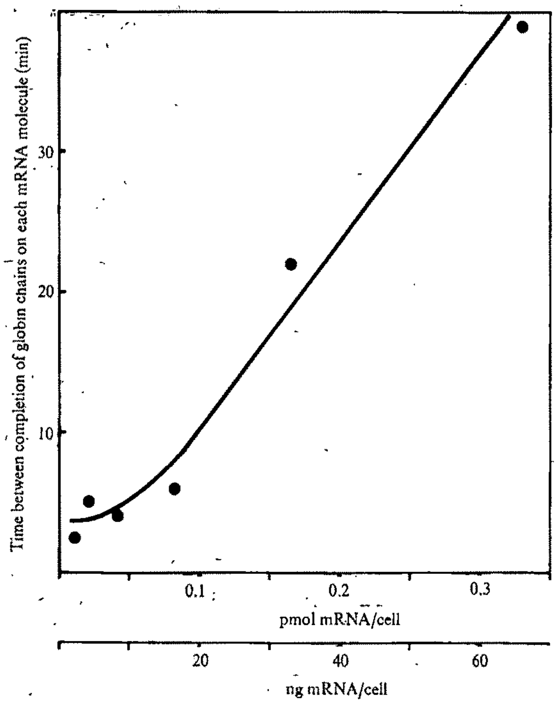


Fig. 4 Each point is based on the 'Sephadex' analysis of twenty oocytes injected with 9S mRNA and haemin, and incubated in medium containing ^{3}H-histidine for 10 h. The various assumptions made in the calculations in Table 2 are also made here. It is assumed that all molecules in the injected RNA sample were translated with the same efficiency. If it is assumed that there is only one ribosome working on each mRNA molecule at a time, then the ordinate scale shows the time taken to synthesize one globin chain.

light chain immunoglobulins when added to a cell-free system[7]. When this RNA is injected into frog oocytes, 2.5–4% of the proteins labelled by ^{3}H-serine in one experiment, and by ^{3}H-threonine in another experiment, were precipitated by K-41 antiserum; this constitutes a four to six-fold increase above the 0.5% precipitation observed in controls. When the precipitated material was dissociated and passed through a column of 'Sephadex', the experimental but not control samples contained radioactivity which migrated with carrier light chain monomer (Stavnezer, Huang, Gurdon and Lane, in preparation).

Table 3 Efficiency of Hb mRNA Translation in Injected Cells of Unknown Histidine Pool Size

Intracellular concentration of injected mRNA (μg/ml. or ng/cell)*	c.p.m. ^{3}H-haemoglobin synthesized/cell /h × 10^{-3} †	Duration of labelling after injection (h)	No. molecules Hb synthesized/h/mRNA molecule ‡
Oocytes			
25	13,340	10	3.4
13	16,940	10	4.8
12.5	8,170	10	4.6
11.8	15,310	10	5.4
11.0	4,250	3	4.8
6.3	5,500	10	4.4
6.3	6,590	10	7.6
6.2	7,050	10	10.4
5.0	15,310	10	5.4
3.12	8,200	10	12.0
1.66	4,690	10	5.8
0.56	4,520	10	8.6
	Average of concentrations up to 13 μg/ml.		6.7
Eggs			
20	1,390	2	9.9
10	1,360	2	17.7
5	660	2	14.6
		Average	14.1

This table includes the results of five experimental series, carried out on oocytes or eggs of different frogs.

* Calculated from the known concentration of RNA in samples for injection and from the known volume injected into each cell. Leakage, which may occur on a substantial scale, is ignored. 1 egg or oocyte is assumed to contain 1 μl. of solution.

† Based on 'Sephadex G-100' analyses.

‡ The calculations for oocytes assume a pool size of 30 pmol per cell (average of the two values in Table 2), and those for eggs assume a pool size of 91.2 pmol per cell, this being the value obtained from an automatic amino-acid analyis of 350 unfertilized eggs. In other respects calculations were carried out as in Table 2.

The effect of injecting AUGpolyU and ^{3}H-phenylalanine into oocytes has been examined by Woodland and Browne (unpublished). AUGpolyU seems not to stimulate incorporation of ^{3}H-phenylalanine into polyphenylalanine, but it drastically inhibits the synthesis of proteins characteristic of the host cells.

The possibility that bacterial virus mRNA can be translated in frog cells has been examined in collaboration with Dr A. Garen. RNA was prepared from f2 phage by two cold phenol extractions. Immediately after alcohol precipitation it was injected into oocytes at a concentration of about 1 mg/ml. ^{3}H-tyrosine-labelled proteins were extracted from RNA-injected and control eggs and analysed by acrylamide gel electrophoresis. No difference between the two samples was observed, and in neither case was a peak of radioactive protein seen in the region of the gels that contained ^{14}C-labelled carrier coat protein and replicase. Total protein synthesis was unaffected. Although we have not determined the reason why f2 RNA fails to be translated in frog oocytes, this may be because it requires the translational machinery of bacterial as opposed to animal cells.

Identification of Unknown mRNAs

The opportunity which injected frog cells offer for translating purified mRNA with high efficiency in a normal living cell suggests the application of this system to the identification of unknown mRNAs and their products. The identification of the unknown message for a known protein can be undertaken by separating total RNA into different fractions, injecting these into oocytes or eggs, and assaying for the synthesis of the protein. It is essential to know that the message product is not degraded in oocyte cytoplasm or rendered unrecognizable by complexing with oocyte materials. We have failed to find such effects with several different proteins which had been labelled with ^{125}I.

Table 4 Comparative Efficiency of Hb·mRNA Translation

Translational systems	Temperature of incubation (°C)	No. globin chains synthesized per mRNA molecule/h	Half-life of translational system	Total No. translations/half-life of system
Cell-free system (lysate using added mRNA)	25*	0.8*	60 min*	0.8*
Rabbit reticulocytes in culture (using endogenous mRNA)	37	800†	2–3 h‡ (in culture)	1,840
	20	80†	?	?
Injected frog cells:				
Oocytes (typical)	19	7§	26¶	180
Oocytes (best)	19	24‖	26¶	625
Eggs	19	14§	?	?

The estimates for the efficiency in frog cells are based on various assumptions, one of which is that the amino-acid pool functional in protein synthesis is the same as the total extractable amino-acid. If this were not so, our estimate would be a maximum of six times too high (see the text for the other assumptions made).

* From ref. 9.

† From ref. 10.

‡ From ref. 11.

§ See Table 3.

‖ See Table 2.

¶ See Fig. 3.

If it is required to identify an mRNA and its product when neither have so far been identified, two procedures are available. The first is to look for an increase in the total proportion of intracellular labelled amino-acid that is incorporated into protein. mRNA as pure as our 9S RNA for Hb raises the overall rate of protein synthesis of recipient cells about two-fold when injected at saturating concentrations[13]. The second procedure is to test the ability of a potential message-containing fraction of RNA to compete with a known message (such as Hb mRNA) for a limited supply of the host cells' translational apparatus. Hb mRNA, at a saturating concentration and supplemented by increasing amounts of potential messenger RNA, is injected into a series of test cells; to keep the total RNA injected per cell constant, the increasing amounts of potential mRNA are supplemented with proportionately decreasing amounts of a translationally inert RNA such as 28S rRNA (see below). The existence of mRNA in the potential message fraction is indicated by a reduction in the amount of Hb synthesis when related to acid-soluble radioactivity in the injected cells. Table 5 illustrates the use of this procedure in respect of cultured mouse cell polysomal RNA. Further experiments would be required to prove that the cultured cell RNA reduces Hb synthesis by competition for translational apparatus rather than by some unspecific inhibitory effect; however, this design of experiment can provide a useful means of screening the potential message content of RNA fractions.

For either of these tests to work satisfactorily it is necessary to know that none of the major classes of cellular RNA (that is, 28S, 18S, 4+5S) with which mRNA is likely to be contaminated will affect Hb mRNA translation. The results in Table 5 and other unpublished experiments have shown that 28S rRNA does not inhibit Hb mRNA translation, and it has been demonstrated elsewhere[12] that neither 28S, 18S nor 4+5S RNA from reticulocytes promotes Hb synthesis when injected on their own. We have found that very high concentrations of RNA, especially of 4+5S RNA, are toxic to some samples of eggs and oocytes; therefore the total amount of injected RNA should, whenever possible, be kept below 50 ng per cell, and the total amount of RNA in all samples to be compared should be kept constant. By attention to these details, we believe that oocytes and eggs can provide a valuable assay system for identifying different kinds of mRNA and their products.

Advantages of Oocyte System

For the study of messenger RNA and its translation, the microinjection of *Xenopus* eggs and oocytes has three special merits. First, the translation of purified mRNA is undertaken in a normal living cell, and is therefore less likely to be affected by artefacts than a cell-free system. Second, injected oocytes are capable of translating mRNA with a high efficiency for long periods. Using our normal labelling procedures, we have obtained, over 24 h, as much as 10^6 d.p.m. of labelled Hb from 1 ng of injected mRNA. By increasing the amount and range of labelled amino-acids, it would be possible with this system to recognize as little as 1 pg of Hb mRNA. Finally, *Xenopus* oocytes seem to show very little species specificity with respect to the type of mRNA which they can translate. It seems possible that all kinds of eukaryotic and animal virus mRNA may be capable of translation in *Xenopus* oocytes. The large size, resistance to microinjection, and easy availability of *Xenopus* oocytes have relieved us of the incentive to test the translational usefulness of eggs and oocytes from other animal species.

This work was supported by the Medical Research Council. We thank V. A. Moar, C. Gregory, and S. Ayers for technical assistance. G. M. is "Chargé de Recherches" of the Belgian FNRS, and part of his contribution to this work has been made possible by an EMBO fellowship to work in Oxford.

Table 5 Messenger RNA Competition Experiment

Intracellular concentration of injected RNA (ng/cell)			Protein synthesis as % of acid-soluble c.p.m.	
Hb mRNA	28S rRNA	3T6 RNA	Hb	Endogenous
10	30	0	61.5	111
10	27.5	2.5	35.8	80.1
10	25	5	20.4	35.4
10	17.5	12.5	19.8	45.5
10	10	20	18.4	25.3
10	0	30	12.6	33.2
10	0	0	55.3*	103.1*

The injected cells were large oocytes, which were incubated for 9 h in ^{3}H-histidine before freezing. Analysis was by 'Sephadex'. 3T6 RNA was obtained from polysomes of cultured mouse cells. The variation in values for endogenous protein synthesis is probably due to the variable amounts of uninjected ovarian material in each sample (see text).

* These are average values for three experiments of this kind.

Received May 14, 1971.

[1] Chantrenne, H., Burny, A., and Marbaix, G., *Prog. Nucleic Acid Res. and Mol. Biol.*, **7**, 173 (1967).

[2] Gurdon, J. B., *J. Embryol. Exp. Morphol.*, **20**, 401 (1968).

[3] Gurdon, J. B., *Quart. J. Microsc. Sci.*, **101**, 299 (1960).

[4] Smith, L. D., and Ecker, R. E., *Current Topics in Devel. Biol.*, **5**, 1 (1970).

[5] Kutsky, P. B., Eakin, R. M., Berg, W. E., and Kavanau, J. L., *J. Exp. Zool.*, **124**, 263 (1953).

[6] Ecker, R. E., and Smith, L. D., *Develop. Biol.*, **18**, 232 (1968).

[7] Stavnezer, J., and Huang, R.-C. C., *Nature*, **230**, 172 (1971).

[8] Labrie, F., *Nature*, **221**, 1217 (1969).

[9] Lingrel, J. B., in *Methods in Protein Synthesis* (edit. by Laskin, A. E., and Last, J. A.) (Marcel Dekker, New York, in the press).

[10] Hunt, T., Hunter, T., and Munro, A., *J. Mol. Biol.*, **43**, 123 (1969).

[11] Armentrout, S. A., Schinkel, R. D., and Simmons, L. R., *Arch. Biochem. Biophys.*, **112**, 304 (1965).

[12] Lane, C. D., Marbaix, G., and Gurdon, J. B., *J. Mol. Biol.* (in the press).

[13] Moar, V. A., Gurdon, J. B., and Marbaix, G., *J. Mol. Biol.* (in the press).

Formation of Antibiotics

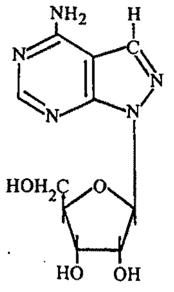
M. M. DHAR & A. W. KHAN

Central Drug Research Institute, Lucknow

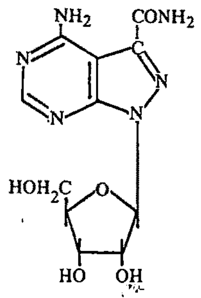
Antibiotics could owe their origin to detoxication of a metabolite that is detrimental to the culture producing the antibiotic.

It has been suggested[1] that antibiotics, the structures of which consist of heterocyclic moieties attached to peptide sequences, might owe their origin to detoxication of the heterocyclic moieties by peptidation. A consequence of this hypothesis, that introduction of analogues of the heterocycle into the antibiotic synthesizing system should yield analogues of the antibiotic, led to the isolation of quinazolone analogues of the quinoxaline–peptide antibiotic echinomycin both *in vivo*[1] and in a cell-free system[2]. We now suggest that other antibiotics are also microbial metabolites that might owe their origin to detoxication of other metabolites that are detrimental to the culture. It is implied that for its survival, the culture must

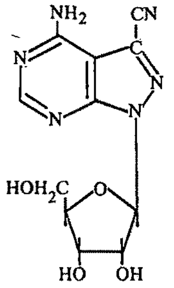
Pure Che [illegible] SCIENCE COLLEGE [illegible] Upper Circular Road CALCUTTA



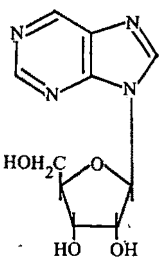
I



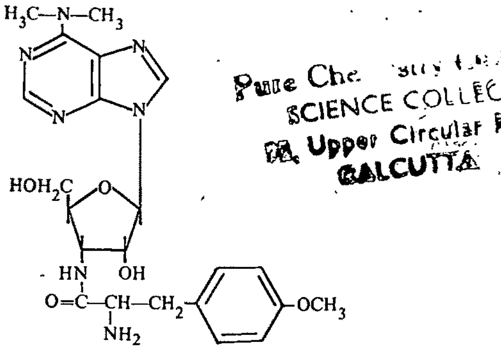
II



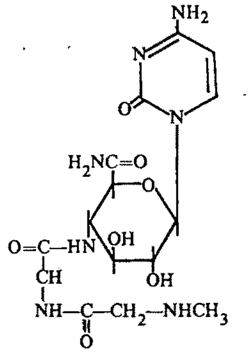
III



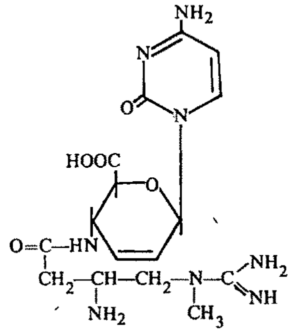
IV



XI



XII



XIII

have a mechanism for detoxicating this metabolite or that the metabolite can induce the formation of one or more enzymes required for its detoxication.

Apart from the group of antibiotics that have a peptide sequence attached to a heterocycle, several have structures made up of two types of chemical entity; for example, the nucleosides (heterocycles attached to carbohydrate residues); the macrolides (macrocycles attached to carbohydrate residues); the rifamycins (macrocycle attached to an aromatic system) and the penicillins (carboxylic acid attached to a peptide). We suggest that these antibiotics owe their origin to detoxication of one of the chemical entities by attachment to the other.

Nucleoside Antibiotics

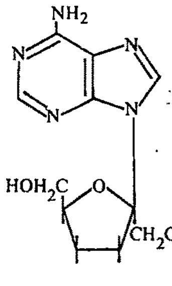
If nucleoside antibiotics are formed as a consequence of a detoxication process, two types of structure ought to be encountered. The first, containing unusual heterocycles detoxicated by glycosidation, should contain the usual nucleoside sugars ribose or 2-deoxyribose, and a second, containing unusual sugars, should be glycosides of one of the usual bases: adenine, guanine, cytosine, uracil or thymine. An examination of the structures of known nucleoside antibiotics reveals that they are indeed of these two types.

The antibiotics tubercidin (I), sangivamycin (II), toyocamycin[3] (III) and nebularin[4] (IV) all contain unusual heterocyclic moieties related to adenine. The sugar in every case is D-ribose. On the other hand, psicofuranine (V), decoyinine[5] (VI), 3′-amino-3-deoxyadenosine[6] (VII), cordycepin[7] (VIII), the fluorine containing nucleocidin[8] (IX) and homocitrullylamino adenosine[9] (X) are all adenine nucleosides containing unusual sugars.

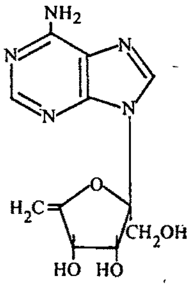
The related puromycin[10] (XI), however, seems to be a methylated product of a corresponding tyrosyl adenine intermediate.

The pyrimidine antibiotics gougerotin[11] (XII) and blasticidin[12,13] (XIII) contain unusual peptidyl aminohexoses, but the base in both cases is cytosine. The other cytosine antibiotic amicetin[14] (XIV) also fits the pattern.

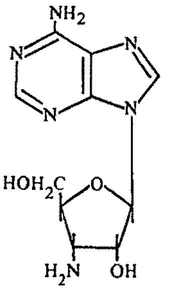
The C-nucleoside antibiotics also fit this pattern. The four known ones: formycin[15] (XV); showdomycin[16] (XVI); pyrazomycin[17] (XVII) and oxazinomycin[18] (XVIII) have unusual aglycones but in every case the sugar residue is ribose.



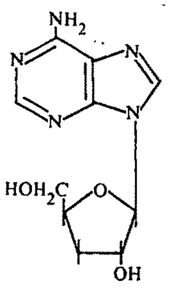
V



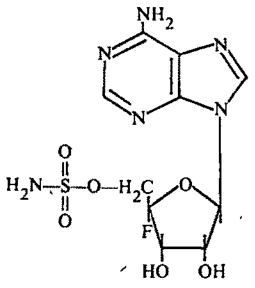
VI



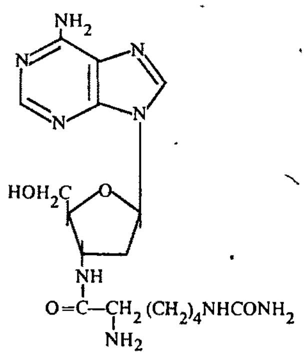
VII



VIII



IX



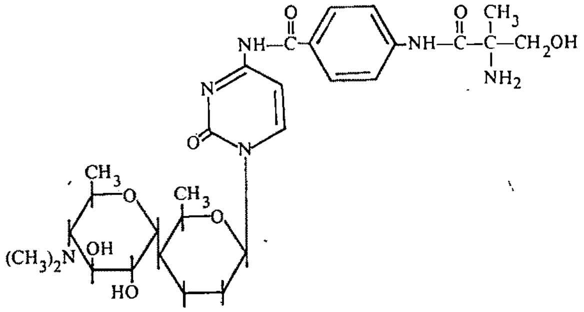
X

Rifamycin Group and the Macrolides

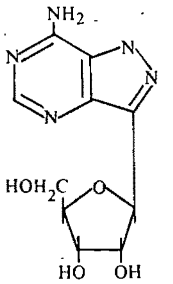
An examination of the structure of rifamycin B[19] (XIX) suggests that the molecule is derived from a polyketide chain derived from 2-methylmalonate and a 2-methyl-polyhydroxynaphthalene, which must be derived from mavelonate and a hydroquinone or phenol precursor. The fact that these two parts of the antibiotic molecule are probably derived differently suggests that the naphthalene moiety is detoxicated by attachment to the polyketide derived chain.

More than fifty macrolide antibiotics have been isolated from various actinomycetes and their structures found to possess unique correlations with regard to their stereochemistry[20].

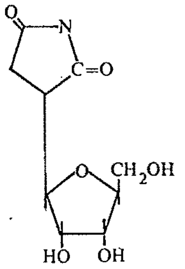
It has also been found that the twelve membered lactones are attached to only one sugar residue, a D-4,6-dideoxy or D-6-deoxypyranose, while the fourteen membered lactones are aglycones of one such sugar as well as a L-2-dideoxypyranose. Sixteen membered lactones are attached to a disaccharide: a L-2,6-dideoxypyranose attached to a D-6-deoxy or D-2,6-dideoxypyranose, which as in the twelve and fourteen membered macrolides is attached to form a β-glycoside of the lactone. In view of the unusual nature of the sugars found in these antibiotics, it seems more likely that the formation of such antibiotics may be initiated by a need to detoxicate one of these sugars, the most common one being desosamine. Studies on the biosynthesis of erythromycin[21] indicate that the fourteen membered lactone erythronolide B is first attached to the neutral sugar and the mycarosyl erythronolide B converted to erythromycin by formation of a β-glycoside of desosamine and conversion of the mycarose residue to a cladinose.



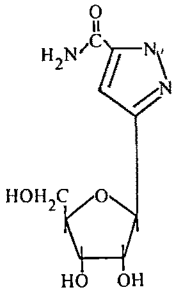
XIV



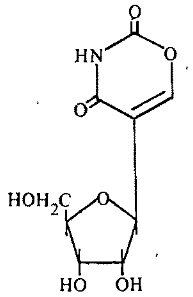
XV



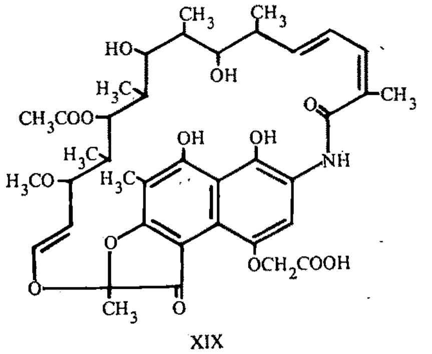
XVI



XVII



XVIII



XIX

Corollaries

There are three corollaries to this hypothesis. (1) The toxic metabolite can be used for strain selection. For example, in the case of the penicillins, it seems that the carboxylic acid is detoxicated by attachment to a 6-aminopenicillanic acid residue. It has been found that only strains which produce penicillin can grow in the presence of significant amounts of phenylacetic acid and that the most efficient penicillin producer can flourish in the highest concentration of phenylacetic acid (P. K. Bhattacharyya, personal communication). Penicillin producers are distinguished by possessing a specific acyl transferase that transfers acyl functions from acyl-coenzyme A to 6-aminopenicillanic acid[22]. Phenylacetic acid, however, has not been found to induce the formation of this enzyme. (2) The toxic metabolite should in some cases be able to induce the formation of enzymes involved in the synthesis of the antibiotic. (3) The introduction of an analogue of the moiety being detoxicated into the antibiotic synthesizing system, should lead to the formation of an analogue of the antibiotic. This has been demonstrated both *in vivo*[1,23] and in a cell-free system in the case of echinomycin, in the case of the penicillins and recently most elegantly in the case of the bleomycins[24].

The hypothesis outlined here is in agreement with two earlier important proposals on the formation of antibiotics[25,26], that antibiotics are secondary metabolites formed by normal biogenetic operations; that antibiotics constitute only a small fraction of secondary metabolites that happen to have been found to possess biological activities and that the formation of an antibiotic requires the accumulation of an initiator. It differs from those earlier views in suggesting that the initiators are toxic to their respective cultures and that a large number of antibiotics are from the group of secondary metabolites that are detoxication products of such initiators.

Received June 2; revised July 6, 1971

1. Khan, A. W., Bhaduri, A. P., Gupta, C. M., and Dhar, M. M., *Indian J. Biochem.*, **6**, 220 (1969).
2. Arif, A. J., Singh, C., Bhaduri, A. P., Gupta, C. M., Khan, A. W., and Dhar, M. M., *Indian J. Biochem.*, **7**, 193 (1970).
3. Tolman, R. L., Robins, R. K., and Townsend, L. B., *J. Amer. Chem. Soc.*, **91**, 2102 (1969).
4. Nakamura, G., *J. Antibiotics*, **14A**, 94 (1961).
5. McCarthy, J. R., Robins, R. K., and Robins, M. J., *J. Amer. Chem. Soc.*, **90**, 4993 (1968).
6. Guarino, A. J., and Kredrich, N. M., *Biochim. Biophys. Acta*, **68**, 317 (1963).
7. Kaczka, E. A., Dulney, E. L., Gitterman, C. D., Woodruff, H. B., and Folkers, K., *Biochem. Biophys. Res. Commun.*, **14**, 452 (1964).
8. Morton, G. O., Lancaster, J. E., Van Lear, G. E., Fulmer, W., and Meyer, W. E., *J. Amer. Chem. Soc.*, **91**, 1535 (1969).
9. Kredrich, N. M., and Guarino, A. J., *J. Biol. Chem.*, **236**, 3300 (1961).
10. Baker, B. R., Schaub, R. E., Joseph, J. P., and Williams, J. H., *J. Amer. Chem. Soc.*, **77**, 12 (1955).
11. Fox, J. J., Kuwada, Y., Watanabe, K. A., Ueda, T., and Whipple, E. B., *Antimicrobiol Agents and Chemotherapy*, 518 (1964).
12. Otake, N., Takeuchi, S., Endo, T., and Yonehara, H., *Tetrahedron Lett.*, 1405 (1965).
13. Fox, J. J., and Watanabe, K. A., *Tetrahedron Lett.*, 897 (1966).
14. Haskell, T. H., *J. Amer. Chem. Soc.*, **80**, 747 (1958).
15. Koyama, G., Maeda, K., and Umezawa, H., *Tetrahedron Lett.*, 597 (1966).
16. Darnall, K. R., Townsend, L. B., and Robins, R. K., *Proc. US Nat. Acad. Sci.*, **57**, 548 (1967).
17. Streighthoff, F. J., Nelson, J. A., Cline, J. C., Gerzon, K., Hoehn, M., Williams, R. H., Gorman, M., and Delong, D. C., *Ninth Conference of Antimicrobial Agents and Chemotherapy*, Abstract No. 18 (Washington DC, 1969).
18. Haneishi, T., Nomura, M., Okazaki, T., Naito, A., Seki, I., Arai, M., Hata, R., and Tamura, C., Meeting of Japanese Antibiotic Research Association, Tokyo, July 1970.
19. Sensi, P., Maggi, N., Furesz, S., and Maffii, G., *Antimicrobial Agents and Chemotherapy*, 699 (1967).
20. Celmer, W. D., *J. Amer. Chem. Soc.*, **88**, 5028 (1966).
21. Martin, J. R., and Rosenbrook, W., *Biochemistry*, **6**, 435 (1967).
22. Pruess, D. L., and Johnson, M. J., *J. Bact.*, **94**, 1502 (1967).
23. Yoshida, T., Kumura, Y., and Katagiri, K., *J. Antibiotics*, **21**, 465 (1968).
24. Umezawa, H., Antibiotics Symp., Ste Marguerite, Quebec March 1971.
25. Bullock, J. D., *Adv. Appl. Microbiol.*, **3**, 293 (1961).
26. Woodruff, H. B., *Sixteenth Symp. Soc. Gen. Microbiol.*, 22 (1966).

LETTERS TO NATURE

PHYSICAL SCIENCES

Formation of Glass Spheres on the Moon

I WISH to draw attention to a remarkable morphological similarity between lunar glass spheres and the glass shot which is formed during the manufacture of mineral wool.

Commercial mineral wool is made by pouring a controlled stream of molten slag and silica from a cupola onto a rapidly turning disk, which throws the melt out as a thin horizontal sheet. Just beyond the periphery of the disk the melt encounters an annular steam blast which breaks it up into small droplets and the resultant mixture of approximately equal parts of fibre and shot is discharged into a large chamber where it cools and settles out. The mineral wool mat formed in this way is used in several applications in which its thermal insulation and fire resistant properties are of value. Some of the shot particles remain in the wool but they can be separated in several ways.

Small amounts of a larger diameter shot fraction accumulate in certain positions in the settling chamber and I have measured the size and shape of several hundred of the shot particles from both fractions with a projection microscope, characterizing them by the parameters measured by Mueller and Hinsch[1].

The mineral wool shot, like the lunar spheres, consist chiefly of almost spherical particles, many of which have a "stalk-like appendage"[1], which is the remnant of the attached fibre. Nearly all the forms found in the lunar shot are also present, including "prolate and oblate spheroids, ellipsoids, dumb-bells with and without a central bulge, filaments, and teardrops"[1]. Rings are evident only occasionally, but I have not identified "crescents, tops, and spindles" because I am not sure how to distinguish these shapes.

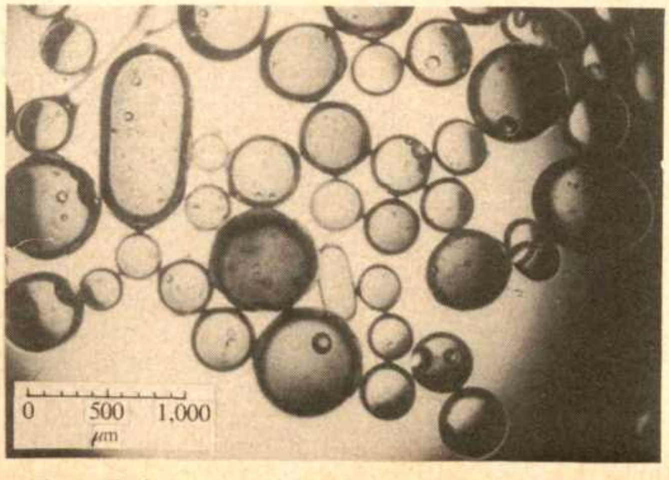


Fig. 1 Typical sample of mineral wool shot showing spheres, ellipsoids and dumb-bell forms (amber to dark brown glass with gas bubble inclusions).

Fig. 1 is a photomicrograph of a typical sample of the mineral wool shot, which may be compared with an example of the Apollo 11 lunar spheres (Fig. 2).

In Table 1 the proportions of the different shapes for both the mineral wool fractions are compared with those found by Mueller and Hinsch for the coarsest fraction of lunar shot. Diameters of the mineral wool shot range from 200 to 800 μm and 100 to 400 μm in the two fractions examined, whereas the lunar shot is from 1 to 500 μm and 70% of the particles are in the 1 to 2 μm range.

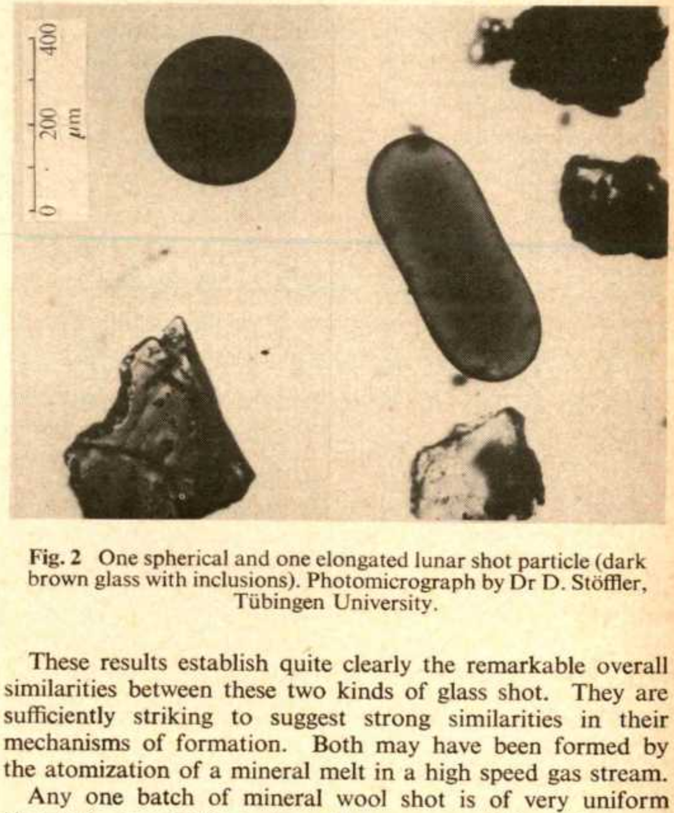


Fig. 2 One spherical and one elongated lunar shot particle (dark brown glass with inclusions). Photomicrograph by Dr D. Stöffler, Tübingen University.

These results establish quite clearly the remarkable overall similarities between these two kinds of glass shot. They are sufficiently striking to suggest strong similarities in their mechanisms of formation. Both may have been formed by the atomization of a mineral melt in a high speed gas stream.

Any one batch of mineral wool shot is of very uniform chemical composition, but there are variations between batches, and between those produced by different manufac-

Table 1 Comparative Morphology of Lunar and Mineral Wool Shot

Sample	Diameter (μm)	No. of spheroids with differing eccentricities 0 to 10%	10 to 50%	50% +	Dumb-bells	Rings	Teardrops
Mineral wool, coarse shot	457	57	29	7	6	0	1
Mineral wool, finer shot	276	39	42	7	8	0	4
Lunar shot	350	64	18	11	4	1	2

turers, according to the nature of the raw materials used in the melt. The chemical composition of the samples described in this communication is compared in Table 2 with analyses for lunar shot[2].

Table 2 Composition of Lunar and Mineral Wool Shot

Constituent	Mineral wool shot	Lunar glass spheres of colour indicated				
		Clear	Pale green	Amber	Dark brown	Black
SiO_2	35.4	49.5	55.4	45.5	41.9	38.1
Al_2O_3	18.3	28.0	1.4	14.0	8.5	4.5
Fe_2O_3/FeO	0.8	2.5	10.4	12.0	17.4	23.8
CaO	34.9	14.0	20.1	12.0	11.0	7.8
MgO	7.9	2.0	16.7	8.0	6.9	11.9
Na_2O	0.9	<0.1	0	<0.1	0.4	0.9

It has been suggested (Mueller, private communication) that the morphology may be influenced by the composition. Although the lunar shot shows marked and highly significant interparticle variation, it is clear that the mineral wool shot contains much more lime than any of the lunar glasses.

The comparison of lunar spheres and mineral wool shot lends additional support to the views put forward by authors such as McKay *et al.*[3], who believe that the lunar shot is produced by high speed primary meteorite impacts, presumably as the intensely hot plasma expands through the surrounding solid particles, causing both melting and atomization. The maintenance of geometrical similarity over the whole range from micrometeorites to relatively large impacts explains the approximately constant proportion of elliptical and elongated forms observed over the whole size range by Mueller and Hinsch[1]. The smallest particles are formed in very large numbers by the very much more frequent impacts of small meteorites at places where the plasma expands from virtually a point source. Large impacts would produce larger shot because of the less sharply divergent flows.

We may suppose that still larger impacts might produce such large molten particles that they would not have time to solidify before returning to the surface. These "meteoric bombs" would give rise to glassy patches splashed on the surface, or at the centres of small craters if the bomb had sufficient kinetic energy. These glassy patches were explained by Gold[4] in a different way. More recent work by Anders *et al.*[5] has, however, shown that they are indeed splashes of foreign molten rock, in which case their identification as secondary impact sites for the molten ejecta from much larger primary impact craters avoids the difficulties encountered in Greenwood's suggestion[6] that they are themselves primary impact sites.

The fact that mineral wool and lunar shot morphologies are so similar should at least inhibit suggestions that the lunar processes must necessarily have taken place at low ambient pressure and great dilution. Mineral wool is made at one atmosphere in a very intensive process, but the shot so formed does not contain either "grape clusters" or changes in shape obviously attributable to the surrounding gas.

One obvious difference is the absence on the Moon of fine glass fibres. One might suppose that these would be formed in substantial quantity at the same time as the shot. Because of their large surface area and fragile nature it is reasonable to suppose that these fibres would be rapidly degraded in the lunar environment. Their broken fragments should form part of the most finely divided solids fraction in the lunar regolith, and it would seem worth attempting to identify these fragments in the soil.

C. A. Cross

225 London Road,
Northwich

Received July 27, 1971.

[1] Mueller, G., and Hinsch, G. W., *Nature*, **228**, 254 (1970).
[2] Duke, M. B., Woo, C. C., Bird, M. L., Sellers, G. A., and Finkelman, R. B., *Science*, **167**, 648 (1970).
[3] McKay, D. S., Greenwood, W. R., and Morrison, D. A., *Science*, **167**, 654 (1970).
[4] Gold, T., in *Apollo 11 Preliminary Science Report* (NASA SP 214).
[5] Morgan, J. W., Laul, J. C., Ganapathy, R., and Anders, E., *Science*, **172**, 556 (1971).
[6] Greenwood, W. R., and Heiken, G., *Science*, **168**, 610 (1970).

Eddy Viscosity and the Solar Radiation

Dicke[1], although not the first to do so, has proposed that the deep interior of the Sun rotates faster than the visible exterior (see also Roxburgh[2], Sakurai[3]). One result is that the departure of the mass distribution from spherical symmetry would, through its quadrupole moment, cause a contribution to the advance of the perihelion of the planet Mercury, on the basis of Newtonian mechanics, which represents a sufficiently good approximation for this purpose. Unless the fast interior rotation and corresponding interior mass distribution is known and correct allowances made, no precise check of the success of general relativity theory can be made on the basis of the astronomically observed perihelion advance. The main question is whether some such situation concerning the interior rotation can be established, or at least made plausible.

Hide[4,5,6] has stated that, aside from plausible magnetic complications, an equatorial acceleration in planetary and solar atmospheres cannot be maintained by axisymmetric systems of motion (see also Ward[7], Starr and Gilman[8]), but requires what actually amounts to a negative eddy viscosity due to nonzonal disturbances in the motion and pressure. I have studied such phenomena observationally for many systems, including laboratory experiments, and their reality cannot be much doubted[9]. The effect is also manifest in numerically integrated models of the Earth's atmosphere (see, for example, Smagorinsky, Manabe and Holloway[10]). A requirement is that individual eddies possess a systematic asymmetry usually apparent as a "tilt" with respect to normals drawn across the basic or mean flow, in order to result in the needed covariance of downstream and cross-current velocity components.

Thus, as an illustration, in the instance of the Earth's atmosphere there is a broad maximum of zonally averaged eastward angular velocity around 35°–40° latitude in each hemisphere. Superposed on this mean rotation there are nonzonal eddy components of motion, giving the flow an irregular wavelike appearance at any instant. These eddy components of motion show a special type of asymmetry with respect to the meridians, so that their northward and eastward velocities are instantaneously correlated along the length of individual closed latitude circles at a given height. This correlation is positive so that transport of angular momentum occurs horizontally northward across vertical walls of constant latitude in the vicinity of 30° N, into the zone of maximum mean angular velocity. Around 60° N, on the other hand, the correlation is negative, indicating a tendency (weaker in this case) again to transport angular momentum across latitude walls into the zone of maximum mean rotation. Because the effect is exactly contrary to a normal viscous action—tending to maintain the fast rotation through the horizontal exchange of equal masses of eddying fluid parcels across latitude walls, instead of decreasing it—we may call the process a manifestation of negative eddy viscosity. Some fluid dynamicists prefer to call it pseudoviscosity. The whole process requires energy sources within the structure of the eddies.

In view of the fact that the several published theoretical treatments of the solar circulation problem like that of Gilman[11] and of Durney[12], in addition to the observational studies of Ward[7,13], all give evidence of the presence of negative horizontal eddy viscous effects, it is also possible that many stellar interiors have a fast rotation sustained by a vertical inward angular momentum transport due to eddies (see also Starr and Gaut[14], Starr[9]). If the main action of this kind is located in the convective zone, the shear in mean angular velocity would itself probably induce the proper tilt in the upward and downward convective eddy currents to perpetuate the regime against viscosity effects and against possible Maxwell stresses leading ultimately to joule-heating energy losses. I suggest that theoretical and numerical mathematical models should be constructed, which would allow for this possiblity.

V. P. STARR

Department of Meteorology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139.

Received June 7; revised August 4, 1971.

[1] Dicke, R. H., *Nature*, **202**, 432 (1964).
[2] Roxburgh, I. W., *Nature*, **213**, 107 (1967).
[3] Sakurai, T., *Stellar Rotation* (Ohio State Univ., Columbus, Ohio, 1970).
[4] Hide, R., *Planet. Space Sci.*, **14**, 669 (1966).
[5] Hide, R., *J. Atmos. Sci.*, **26**, 841 (1969).
[6] Hide, R., *Nature*, **225**, 254 (1970).
[7] Ward, F., *Pure and Appl. Geoph.*, **58**, 157 (1964).
[8] Starr, V. P., and Gilman, P. A., *Scientific American*, **218**, 100 (1968).
[9] Starr, V. P., *Physics of Negative Viscosity Phenomena* (McGraw-Hill, New York, 1968).
[10] Smagorinsky, J., Manabe, S., and Holloway, jun., J. L., *Mon. Weather Rev.*, **93**, 727 (1965).
[11] Gilman, P. A., *J. Atmos. Sci.*, **24**, 101 (1967).
[12] Durney, B., *Astrophys. J.*, **161**, 1115 (1970).
[13] Ward, F., *Astrophys. J.*, **141**, 534 (1965).
[14] Starr, V. P., and Gaut, N. E., *Scientific American*, **223**, 72 (1970).

Natural Sources of Atmospheric CO

METHANE is produced at the Earth's surface by various processes, chiefly biological in origin. Koyama[1] estimated an annual source of 3×10^{14} g, with rice paddy fields a major contributor, but did not consider the contribution from natural swamps, and his value for the total source may be low by as much as a factor 3. The data of Bainbridge and Heidt[2] suggest that they may have observed substantial methane production from the coastal marshes of Louisiana. The important atmospheric sinks for methane are[3]

$$OH + CH_4 \rightarrow H_2O + CH_3 \qquad (1)$$

$$O(^1D) + CH_4 \rightarrow OH + CH_3 \qquad (2)$$

and

$$\begin{aligned} h\nu + CH_4 &\rightarrow CH_2 + H_2 \\ &\rightarrow CH_3 + H \end{aligned} \qquad (3)$$

These reactions constitute permanent destruction of CH_4. Subsequent chemistry leads to formation of CO and CO_2, and we shall argue that CO is more likely. The source of atmospheric CO due to CH_4 is comparable with, and may be larger than, that associated with the internal combustion engine.

Reactions (1) and (2) dominate below 80 km. The metastable O atom in (2) is formed by photolysis of ozone and its concentration can be readily determined with available data on reaction rates[3,4]. Removal of CH_4 in the troposphere is dominated by (1) with OH produced chiefly by

$$O(^1D) + H_2O \rightarrow OH + OH \qquad (4)$$

The tropospheric chemistry of OH was discussed recently by Levy[5] and our chemical model reflects his discussion. (The rate coefficient for (1) was taken from Greiner[6]. For (4) we used relative measurements by Biedenkapp *et al.*[7] in combination with recent data[8] for the reaction of $O(^1D)$ with O_3.) The reaction

$$NO + HO_2 \rightarrow NO_2 + OH \qquad (5)$$

is an important means for recycling odd hydrogen and we adopt Levy's estimate for the rate coefficient, 5×10^{-13} cm^3 s^{-1}. Computed altitude profiles for OH and HO_2 and $O(^1D)$ are illustrated in Fig. 1.

The methyl radical is removed by

$$CH_3 + O_2 \rightarrow CH_3O_2 + M \qquad (6)$$

and subsequent chemistry leads to production of H_2CO and HCO. The important sink for H_2CO is photolysis[9],

$$\begin{aligned} h\nu + H_2CO &\rightarrow H_2 + CO \\ &\rightarrow H + HCO \end{aligned} \qquad (7)$$

although loss by rain out may be comparable. Removal of HCO is mainly by

$$\begin{aligned} HCO + O_2 &\rightarrow OH + CO_2 \\ &\rightarrow HO_2 + CO \end{aligned} \qquad (8)$$

and elementary considerations suggest that the second path, which requires a minimum rearrangement of chemical bonds, should be favoured. The rate for (8) has not been measured, but HCO is also photolysed[10] to yield H and CO, and we estimate the photolysis lifetime to be of the order of 1 h.

A computed altitude profile for CH_4 is illustrated in Fig. 2. We followed procedures described elsewhere[4] and derived values for the upward flux of CH_4 which are also included in Fig. 2. The computed CH_4 profile is in good agreement with experimental data from a variety of sources. Our calculations imply that reaction (1) is an important sink for CH_4

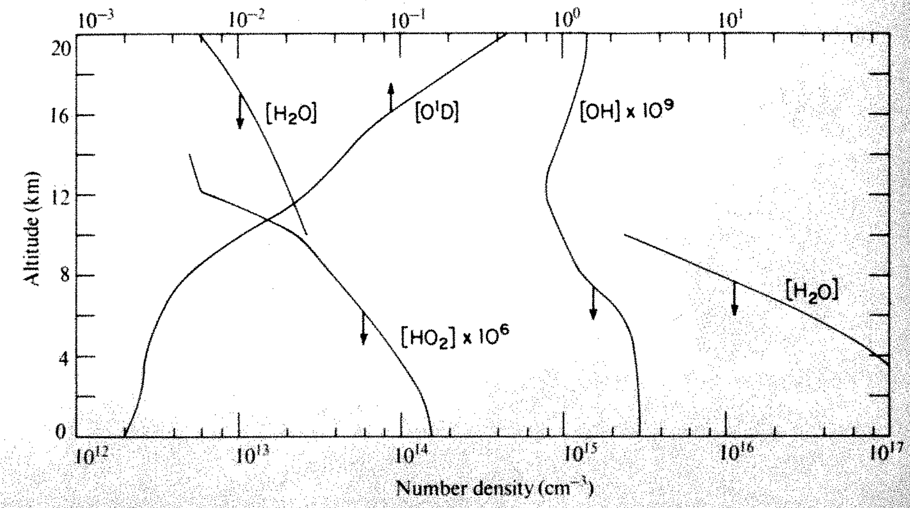


Fig. 1 Average number densities of OH, HO_2 and $O(^1D)$ caluclated for H_2O densities equal to half the dew point values in the troposphere. The tropopause was taken at 10 km and the stratospheric H_2O content corresponds to a mixing ratio 3×10^{-6} by volume. A profile for ozone appropriate for mid-latitudes was used.

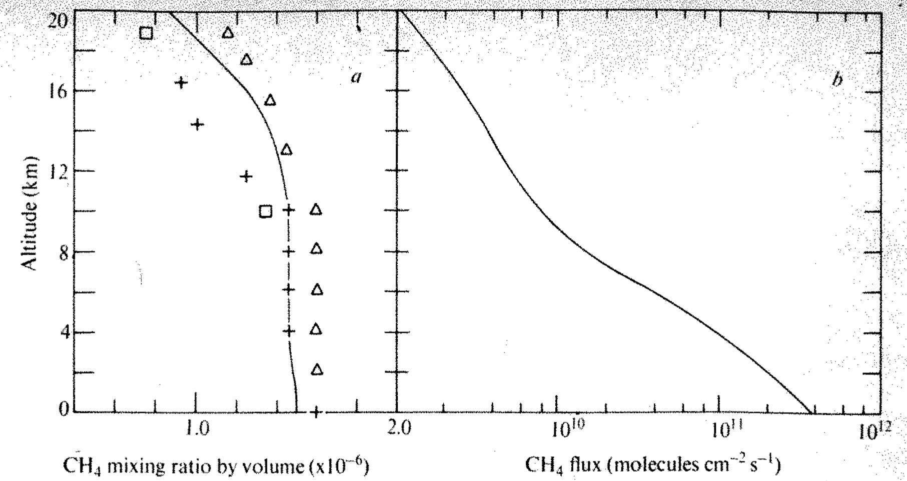


Fig. 2 *a*, Densities of CH_4 calculated by solution of the eddy diffusion equation with diffusion coefficients derived from tracer studies[4]. The solution was constrained to give the observed mixing ratio at the surface and vanishing flux at high altitudes. Measurements by Bainbridge and Heidt[2] are indicated by △ (over Texas) and + (over Louisiana). Measurements by Kyle *et al.*[17] increased by a factor 2 to allow for the reduced stratospheric scale height, are indicated by *b*, globally averaged upward fluxes for CH_4 consistent with densities in *a*. An average flux of 10^{11} molecules cm^{-2} s^{-1} is equivalent to a total flux of 4×10^{14} g yr^{-1}.

and presumably also an important source of CO below 30 km. The need for a significant tropospheric sink for CH_4 was emphasized earlier by Bainbridge and Heidt[2].

The detailed reaction scheme employed here is somewhat uncertain. A mechanism for recycling HO_2 to OH is provided by reaction (5) but the rate coefficient is not well known. Our choice agrees with Westberg and Cohen[5] and is consistent with a lower limit (2×10^{-13} cm^3 s^{-1}) derived by Niki[5]. A preliminary measurement by Johnston[5], however, suggests a somewhat smaller value (2×10^{-15} cm^3 s^{-1}). Other possibilities for recycling exist, including the reaction of HO_2 with Ozone[3] which would be important if the rate coefficient were of order 10^{-14} cm^3 s^{-1}. In addition the reaction

$$CH_3O_2 + HO_2 \rightarrow CH_3OOH + O_2 \qquad (9)$$

followed by photolysis of methylhydroperoxide to yield CH_3O and OH also gives good agreement with the results shown in the figures. It should be noted, however, that even without recycling the OH density at the surface is of order 5×10^5 cm^{-3}. The measurements of atmospheric CH_4, in combination with the known source strength, demonstrate that there must be an efficient tropospheric sink for the gas. The ultimate product is likely to be CO and the yield should exceed the anthropogenic source (2×10^{14} g yr^{-1} or 3×10^{10} molecules cm^{-2} s^{-1}).

The model offers ready explanations for a number of puzzling phenomena. It allows one to reconcile diverse estimates for the lifetime of atmospheric CO. Weinstock[11], as modified by Junge *et al.*[12], derived a lifetime of 0.2 yr using measurements of ambient atmospheric ^{14}CO and calculations for the corresponding source strength. Junge *et al.*[12] performed a similar analysis for ^{12}CO. Assuming the internal combustion engine to be the major source, they derived a lifetime of 1.3 yr. With the additional source discussed here, CH_4, the lifetime is reduced to 0.3 yr, in harmony with the ^{14}CO value. Further, using measurements by Greiner[13] for the reaction

$$CO + OH \rightarrow CO_2 + H \qquad (10)$$

and using the OH profile in Fig. 1, we calculate the absolute value of this lifetime to be 0.3 yr. The predominance of the natural source explains the failure to detect any significant increase in atmospheric CO during the past 20 yr[14], even though production by internal combustion engines has increased by about a factor 2. It further accounts for the similarity of CO mixing ratios observed in the high tropospheres of both northern and southern hemispheres[12]. Some asymmetry would be expected if the car were the dominant source, because its contribution should be concentrated in the northern hemisphere and also because the time constant for interhemispheric exchange is about 5 yr[14]. Measurements in the high troposphere are especially useful in that the influence of local surface conditions, which can act either as sources or sinks of CO, are minimized.

Our model is supported also by measurements of formaldehyde in rain[15]. This gas exhibits the same T/H ratio as atmospheric methane[15]. The rain measurements imply a gas phase mixing ratio, H_2CO to air, of 1.3×10^{-9} by volume at 5° C[16]. The corresponding photolysis rate to yield CO is 5×10^{11} molecules cm^{-2} s^{-1} if we assume a mixed distribution of the H_2CO and adopt mean isolation conditions. Our model predicts a total CO production of 3.7×10^{11} cm^{-2} s^{-1}, in satisfactory agreement with this result.

The internal combustion engine may locally determine the atmospheric CO abundance. There is strong evidence, however, that the global level is governed by natural sources and oxidation of methane is likely to be a major contributor.

J. C. McConnell
M. B. McElroy

Center for Earth and Planetary Physics,
Harvard University

S. C. Wofsy

Department of Chemistry,
Harvard University

Received July 28, 1971.

[1] Koyama, T., *J. Geophys. Res.*, **68**, 3971 (1963).
[2] Bainbridge, A. E., and Heidt, L. E., *Tellus*, **18**, 221 (1966).
[3] Nicolet, M., *Ann. Geophys.*, **26**, 531 (1970).
[4] McElroy, M. B., and McConnell, J. C., *J. Atmos. Sci.* (in the press).
[5] Levy, II, H., *Science*, **173**, 141 (1971).
[6] Greiner, N. R., *J. Chem. Phys.*, **53**, 1070 (1970).
[7] Biedenkapp, D., Hartshorn, L. G., and Bair, E. J., *Chem. Phys. Lett.*, **5**, 379 (1970).
[8] Gauthier, M., and Snelling, D. R., *J. Chem. Phys.*, **54**, 4317 (1971).
[9] McQuigg, R. C., and Calvert, J. G., *J. Amer. Chem. Soc.*, **91**, 159 (1969).
[10] Johns, J. W. C., Priddle, S. H., and Ramsay, D. A., *Diss. Faraday Soc.*, **35**, 90 (1963).
[11] Weinstock, B., *Science*, **166**, 224 (1969).
[12] Junge, C., Seiler, W., and Warneck, P., *J. Geophys. Res.*, **75**, 2217 (1971).
[13] Greiner, N. R., *J. Chem. Phys.*, **51**, 5049 (1969).
[14] Pressman, J., and Warneck, P., *J. Atmos. Sci.*, **27**, 155 (1970).
[15] Shearer, E. C., *Relationships among Atmospheric Formaldehyde, Methane and Krypton*, thesis, Univ. Arkansas (1969).
[16] Credali, L., Mortillaro, L., Galizzo, G., Russo, M., and Checchi, C. de., *Chim. Ind.* (Milan), **47**, 732 (1965).
[17] Kyle, T. G., Murcray, D. G., Murcray, F. H., and Williams, W. J., *J. Geophys. Res.*, **74**, 3421 (1969).

Early Precambrian Impact Structure and Associated Hyalomylonites near Agto, West Greenland

WORK on the Isortoq granulite facies rocks of the Nagssugtôqidian mobile belt[1] in West Greenland has revealed an area where the rocks show features believed to be caused by shock metamorphism, while in neighbouring areas small

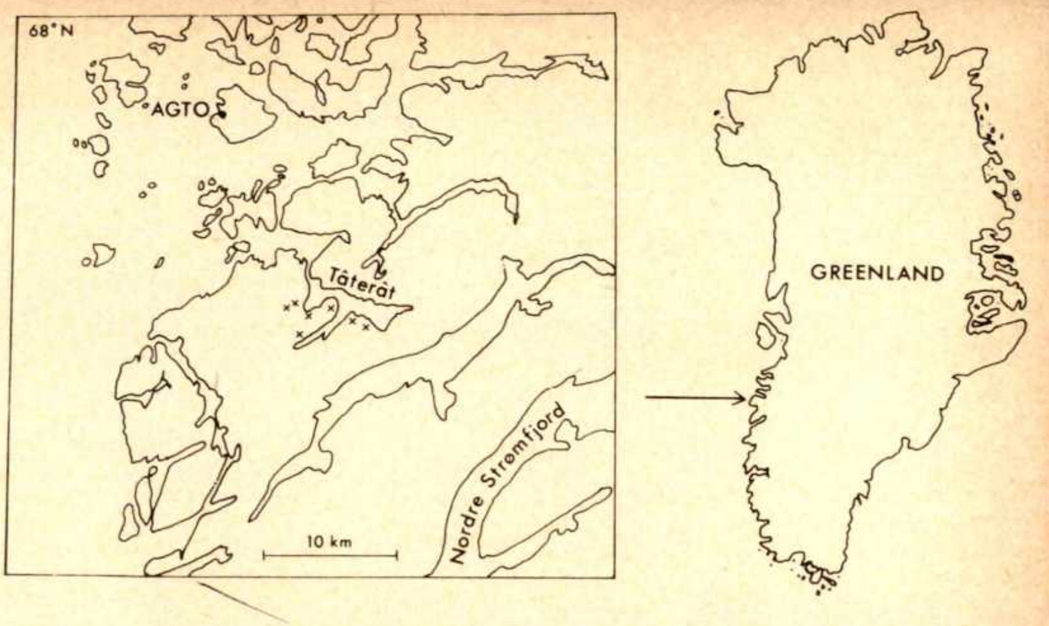


Fig. 1 Crosses indicate locations of shock-metamorphic rock-types.

veins of glass-mylonite (hyalomylonite) have been generated in pre-existing regional thrust zones.

The most convincing evidence of shock metamorphism was found about 20 km south-east of Agto along the western branches of the Tâterât fjord. Although no features suggesting an impact structure can be seen on topographic maps, the enderbitic gneisses at this locality are more or less texturally destroyed and locally more than half of the rocks consist of a very irregular pattern of small black veins embracing gneiss fragments—"pseudotachylytes". The ground mass in these veins is black and aphanitic, and decorated and non-decorated planar elements[2] are seen in quartz, plagioclase and *K*-feldspar both in the gneiss and in the fragments in the pseudotachylyte.

Apart from diaplectic glasses[3] of the above mentioned minerals every transition from diaplectic to normal fused glass seems to be present. Felsic grains in the pseudotachylyte as well as in the surrounding gneiss locally have a rim of fused material shown by gently twisting colourless flow lines in their border zones.

Pyroxenes and amphiboles are filled with additional planar elements, deformation lamellae and deformation twins. The pleochroism is no longer present and the birefringence is reduced to low values[2]. Locally the mafics are destroyed and only black–brown isotropic areas (oxidized pyroxene and amphibole[4]) are present. In the garnets up to four systems of very regular planar features are observed. Apatite also has planar features.

The texture of the gneiss varies between normal hemigranoblastic (with planar elements) to almost completely destroyed mortar-like textures.

Strong shock metamorphic effects were observed within an area of only a few square kilometres but effects have been found locally within an area of at least 30 km².

A hyalomylonite occurs in a thrust zone about 1.5 km from the area showing the most intense shock metamorphism. The thrust zone can be followed over a distance of roughly 130 km from the Inland Ice to the Davis Strait and belongs to an extensive system of fault and thrust zones in West Greenland[5], probably of pre-Nagssugtôqidian initiation. Different types of normal crush-rocks are found in these zones.

The hyalomylonite vein which occurs in a conformably enclosed relic gneiss lens in the thrust zone can be followed about 10 m and measures a maximum 5 cm across. This flat ellipsoidal vein has knife-sharp margins and exactly the same orientation as the thrust zone and the foliation in the gneiss lens. The vein consists of about 85% homogeneous glass and about 15% fragments, which, except for one zircon grain, are entirely of quartz and feldspar, often with planar elements. The glass is in contact with the gneiss, which is highly crushed with planar elements and the mafics locally in an oxidized state.

The glass contains many spherulites a few microns in diameter with dark nuclei. Flow lines are only seen in the glass around the felsic inclusions. Minute, secondary calcite-filled cracks are observed in the glass.

Chemical analysis of the hyalomylonite and the surrounding gneiss both for major and trace elements shows a very close similarity between the two. The refractive index of the glass is 1.530 ± 0.001.

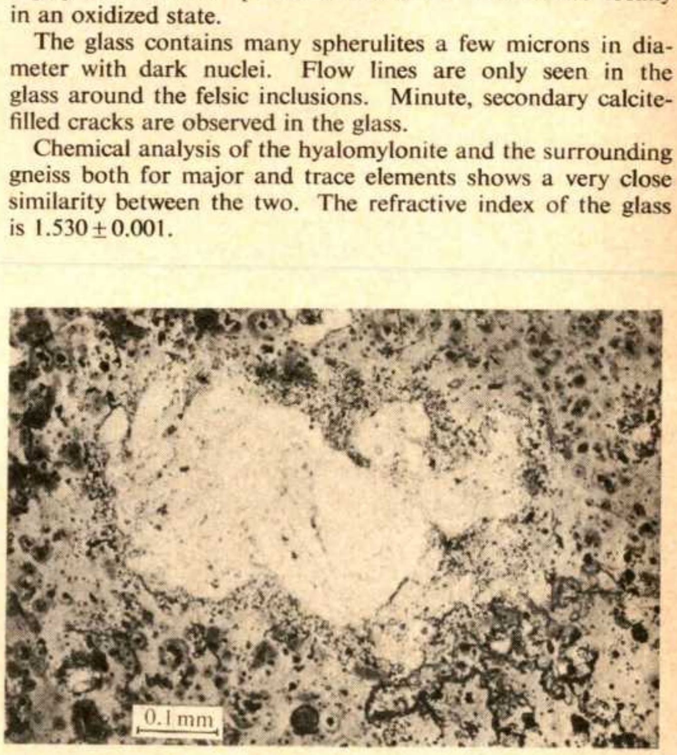


Fig. 2 Felsic grain enclosed in glass, viewed with plane-polarized light.

A K/Ar determination carried out on the glass gave an age of 3,070 ± 70 m.y.[6]. Only a very weak devitrification is observed locally, probably because of the dry conditions in the Isortoq complex as a whole[7], which would allow only thermal reconstruction, a very slow process, to occur[8].

The generally accepted age of the Nagssugtôqidian mobile belt is 1,790–1,650 m.y.[9], but several authors[5,10] believe that pre-Nagssugtôqidian rocks and structures are common in the area mentioned.

There is also a possibility that excess Ar is present, but because glasses are generally believed to be only weakly retentive to Ar and usually give low ages[11], further work is necessary to clarify the meaning of this unexpectedly old age.

The formation of the hyalomylonite vein is considered to be due to instantaneous movements in cold and rigid surroundings.

About 6 km from the most shocked area other hyalomylonites are found. Here the glass is enclosed in a mylonitic rim in contact with the gneiss, which is also severely crushed. Different types of recrystallization, partly devitrification and partly crystallization from a melt, are observed.

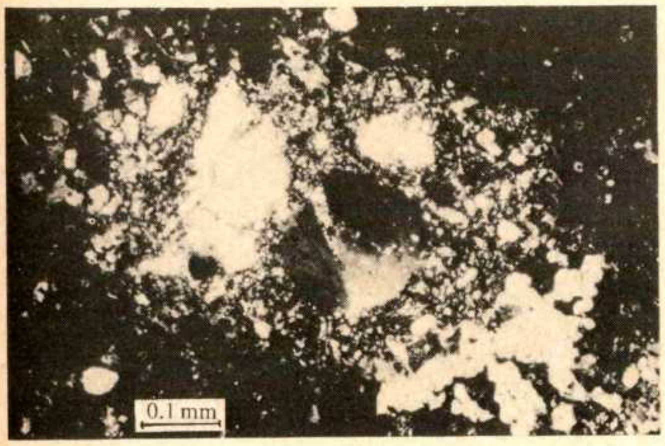


Fig. 3 Same as Fig. 2, but viewed through crossed nicols.

Different crush-rocks from tectonics are present in the Agto area, but the features discussed here are only seen in the vicinity of the shock metamorphic locality. Metamorphism giving rise to such features seems possible only as a result of meteorite impact (astroblemes[12]) and the observations around Tâterât seem indicative of an impact in early Pre-Cambrian time.

Detailed mineralogical investigations which have been carried out on the shocked minerals and hyalomylonites will be presented later.

The work was carried out under the auspices of the Geological Survey of Greenland (GGU) and Institute of General Geology, Copenhagen. Dr C. K. Brooks assisted with the preparation of the manuscript.

VAGN JENSEN

Institut for Almen Geologi,
Østervoldgade 5–7,
1350 *Copenhagen K*

Received March 12; revised July 30, 1971.

[1] Noe-Nygaard, A., *Rep. Eighteenth Intern. Geol. Cong. GB*, Part 13, 199 (1948).
[2] v. Engelhardt, W., Stöffler, D., and Schneider, W., *Geologica Bavarica*, No. 61, 229 (1969).
[3] v. Engelhardt, W., and Stöffler, D., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 159 (Mono Book Corp., 1968).
[4] Chao, E. C. T., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 135 (Mono Book Corp., 1968).
[5] Escher, A., *Rapp. Grønlands Geol. Unders.*, No. 11, 24 (1966).
[6] Larsen, O., *Rapp. Grønlands Geol. Unders.*, No. 19, 62 (1969).
[7] Ramberg, H., *Medr. Dansk. Geol. Foren.*, **12**, 27 (1951).
[8] Marshall, R. R., *Bull. Geol. Soc. Amer.*, **72**, 1493 (1961).
[9] Pulvertaft, T. C. R., *Rep. Twenty-third Intern. Geol. Congr. Czechoslovakia*, Sect. 4, 89 (1968).
[10] Sørensen, K., *Rapp. Grønlands Geol. Unders.*, No. 27 (1970).
[11] Webb, A. W., and McDougall, I., *Earth Planet. Sci. Lett.*, **3**, 41 (1967).
[12] Dietz, R., S. *Sci. Amer.*, **205**, 50 (1961).

BIOLOGICAL SCIENCES

Quinacrine Staining of Chromosomes and Evolutionary Studies in *Drosophila*

PARTICULAR chromosome regions have been found to fluoresce intensely after staining with quinacrine and related compounds[1–16], and we have been studying the pattern of distribution of these regions in the chromosomes of various species and laboratory stocks from the genus *Drosophila*. We find that these patterns vary in interesting ways and that they are a characteristic of chromosomes that can be used in studies of evolutionary cytogenetics in much the same way as structural rearrangements[17–19]. The following are among our observations.

(1) *Drosophila melanogaster* differs consistently from its sibling species *D. simulans* in the pattern of the brightly fluorescent regions. The two species can be distinguished by the patterns of bright fluorescence of either the mitotic chromosomes of the neuroblasts or the polytene chromosomes of the salivary gland cells. In neuroblast metaphases of *D. melanogaster*, every chromosome has brightly fluorescing regions. In *D. simulans*, such regions are found on only the *Y* and fourth chromosomes of metaphase cells. In salivary gland cells of *D. melanogaster* (Oregon-R) there is always an intensely fluorescent region near the base of chromosome IIIR at approximately 81F; and, in some individuals, there is also a second brightly fluorescent region more distally at approximately 83D (see (5) below). *D. simulans* salivary gland cells have no brightly fluorescent regions on the third chromosome. In *D. melanogaster* (Oregon-R) there are two intensely fluorescent regions on chromosome IV: one near the base in region 101 and one in the middle of IVR at approximately 102D. In *D. simulans* salivary gland cells there is always a brightly fluorescent region at the base of the fourth chromosome near the chromocentre; there may be a second intensely fluorescent region at the tip of chromosome IV depending on the stock (see (2)). Dots of brightly fluorescent material not obviously associated with any chromosome are frequent within the chromocentre of both species. These observations suggest the usefulness of quinacrine staining for analysing genetic and evolutionary relationships among sibling species[20–23].

(2) Certain laboratory stocks of *D. simulans* differ with regard to the intensely fluorescent regions of the fourth chromosome. In individuals from three laboratory stocks (wild type stocks derived from populations in Honduras and Madison and a *vermilion* stock from the California Institute of Technology) such regions are found at both the base of the fourth chromosome adjacent to the chromocentre and at its tip. These two regions usually pair ectopically, and the whole chromosome then takes on a U shape. In cells from larvae of the fourth stock (*yellow*, *white* from the California Institute of Technology) only the base of the fourth chromosome fluoresces brightly after quinacrine staining. In this stock there is no noticeable ectopic pairing of the base with the tip of the chromosome, and the fourth chromosome is straight[24]. Because *D. simulans* is one of the three widespread species of *Drosophila* considered to be monomorphic with regard to chromosome structure[25], our observations indicate that quinacrine staining can reveal chromosome polymorphisms among and within populations that contain no gross rearrangements and are therefore classified as chromosomally monomorphic. All four stocks are classifiable as *D. simulans* on the basis of their male genitalia[26], and breeding experiments indicate no genetic isolation among them.

(3) A survey of a few of the principal species groups within the genus reveals that not all species have chromosome regions which fluoresce brightly after quinacrine staining and that the distribution of such species follows generally what is known of the taxonomic relations within the genus. Thus, *D. melanogaster*, *D. simulans* and *D. virilis* have fluorescent bands, while

D. hydei, *D. melanopalpa*, *D. repleta*, *D. peninsularis* and *D. tumiditarsus* do not. It will be interesting to correlate such cytological observations with information about the composition of DNA, especially satellite DNA, of the various species[27].

(4) A band that fluoresces intensely after quinacrine staining is found on the dot chromosome of the Hawaiian species, *D. hemipeza* but not in *D. grimshawi*, another Hawaiian species.

These observations open the possibility of comparing closely related species, including homosequential or nearly homosequential ones, found on these islands. Homosequential species[28-29] are morphologically distinct, reproductively isolated species with identical banding patterns along their polytene chromosomes. Differences in the fluorescence patterns of homologous loci may therefore indicate alleles which differ to an evolutionarily significant degree.

(5) In some individuals of one laboratory stock of *D. melanogaster* (Oregon-R) one of the salivary gland chromosome regions (approximately 83D) fluoresces brightly after quinacrine staining; in others it does not. In still other individuals only a small amount of brightly fluorescent material is seen at this region. The analysable salivary gland cells from any one larva are all the same in this regard. It is not clear that the gene or genes responsible for bright fluorescence after staining with quinacrine are located at those chromosome regions where it is observed. To classify the phenomenon as a polymorphism, we should therefore like to be able to demonstrate heterozygous individuals within the population. In several different salivary gland preparations from this stock we have observed a few cells in which there is asynapsis for an appreciable length of chromosome IIIR, including region 83D. In such cells large portions of each homologue of IIIR can be examined separately. In some individuals we have found cells that fluoresce brightly at region 83D in one homologue but not in the other. In other individuals we have found cells that have bright fluorescence at region 83D on each of the asynapsed homologues. In still others we have found cells that show no bright fluorescence at this region in either asynapsed homologue. There must therefore be some individuals in this population which are heterozygous for bright fluorescence at region 83D, and they are probably those with a small amount of brightly fluorescent material at this region.

Taken together, these findings, which we shall report in detail later[30,31], indicate that the property of fluorescing brightly after staining with quinacrine is a phenotypic character observable at the level of specific chromosome regions in evolutionarily interesting situations. Chromosome differences have until now been observable within and among populations chiefly on the basis of gross structural rearrangements which *de facto* involve changes in gene order. The quinacrine technique has the advantage of being able to reveal chromosome differences within and among populations in the absence of gross changes in gene order. We suggest that this technique is promising as a tool in the analysis of chromosome differences between, and chromosome polymorphisms within, both natural and experimental populations of *Drosophila* and quite possibly other plant and animal taxa as well.

We thank Dr Lynn H. Throckmorton for confirming our classification of all *D. melanogaster* and *D. simulans* stocks used in this study, and Dr Harvey A. Bender and Dr Ronald S. Ostrowski for confirming our identification of salivary gland chromosome positions. Dr Barr is the recipient of a US National Institutes of Health Research Career Development Award; Dr Ellison is a US National Science Foundation postdoctoral fellow. This work is supported by a grant from the American Cancer Society and by funds administered by the Graduate and Medical Schools of this university.

H. J. BARR
J. R. ELLISON

Department of Anatomy,
University of Wisconsin,
Madison, Wisconsin 53706

Received April 15, 1971.

[1] Caspersson, T., Farber, S., Foley, G. E., Kudynowsky, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., *Exp. Cell. Res.*, **49**, 219 (1968).
[2] Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell. Res.*, **58**, 128 (1969).
[3] Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell. Res.*, **58**, 141 (1969).
[4] Zech, L., *Exp. Cell. Res.*, **58**, 463 (1969).
[5] Barlow, P., and Vosa, C. G., *Nature*, **226**, 961 (1970).
[6] Caspersson, T., Zech, L., and Johansson, C., *Exp. Cell. Res.*, **62**, 490 (1970).
[7] Caspersson, T., Zech, L., and Johansson, C., *Exp. Cell. Res.*, **60**, 315 (1970).
[8] Caspersson, T., Zech, L., Johansson, C., and Modest, E. J., *Chromosoma*, **30**, 215 (1970).
[9] George, K. P., *Nature*, **226**, 80 (1970).
[10] Pearson, P. L., and Bobrow, M., *Nature*, **226**, 959 (1970).
[11] Pearson, P. L., Bobrow, M., and Vosa, C. G., *Nature*, **226**, 78 (1970).
[12] Vosa, C. G., *Chromosoma*, **30**, 366 (1970).
[13] Vosa, C. G., *Chromosoma*, **31**, 446 (1970).
[14] Borgaonkar, D. S., and Hollander, D. H., *Nature*, **230**, 52 (1971).
[15] Shettles, L. B., *Nature*, **230**, 52 (1971).
[16] Sumner, A. T., Robinson, J. A., and Evans, H. J., *Nature New Biology*, **229**, 231 (1971).
[17] Patterson, J. T., and Stone, W. S., *Evolution in the Genus Drosophila* (Macmillan, New York, 1952).
[18] Ford, E. B., *Genetic Polymorphism* (Faber and Faber, London, 1965).
[19] Dobzhansky, T., *Genetics and the Evolutionary Process* (Columbia University Press, New York, 1970).
[20] Hubby, J. L., and Throckmorton, L. H., *Amer. Naturalist*, **102**, 193 (1968).
[21] O'Brien, S. J., and MacIntyre, R., *Amer. Naturalist*, **103**, 97 (1969).
[22] Ayala, F. J., Mourão, C. A., Pérez-Salas, S., Richmond, R., and Dobhzansky, T., *Proc. US Nat. Acad. Sci.*, **67**, 225 (1970).
[23] Entingh, T. D., *Genetics*, **66**, 55 (1970).
[24] Horton, I. H., *Genetics*, **24**, 234 (1939).
[25] Carson, H. L., in *The Genetics of Colonizing Species* (edit. by Baker, H. G., and Stebbins, G. L.), 503 (Academic Press, New York, 1965).
[26] Sturtevant, A. H., *Genetics*, **5**, 488 (1920).
[27] Hennig, W., Hennig, I., and Stein, H., *Chromosoma*, **32**, 31 (1970).
[28] Carson, H. L., Clayton, F. E., and Stalker, H. D., *Proc. US Nat. Acad. Sci.*, **57**, 1280 (1967).
[29] Carson, H. L., Hardy, D. E., Spieth, H. T., and Stone, W. S., in *Essays in Evolution and Genetics* (edit. by Hecht, M. K., and Steere, W. C.), 437 (Appleton-Century-Crofts, New York, 1970).
[30] Ellison, J. R., and Barr, H. J., *Chromosoma* (in the press).
[31] Ellison, J. R., and Barr, H. J., *Genetics* (in the press).

Chromatid Disjunction in Unfertilized Ageing Oocytes

AGEING mammalian oocytes, which have spent longer than the optimum time in the oviduct without being fertilized, may, after fertilization, give rise to abnormal embryos in which aneuploidy is frequent[1]. We have now observed a change in the organization of the chromosomes of ageing secondary oocytes of mice, which may account for the occurrence of aneuploidy at the second meiotic division.

As in most mammals, the first meiotic division is completed in the ovary, and the ovulated oocytes are propelled along the oviduct in a state of arrested meiosis at second metaphase until—and if—they are fertilized. In the second meiotic division, which is activated by sperm entry at fertilization, the chromatid pairs which constitute each chromosome of the haploid oocyte disjoin and move towards opposite poles, one of which is destined to become the second polar body. We have evidence that the disjunction of chromatid pairs sometimes occurs before fertilization, but after prolonged sojourn in the oviduct. Subsequent fertilization of an oocyte in such circumstances may lead to a viable zygote in which, however, the synchrony of the partitioning of the chromosomal elements is so disturbed that aneuploidy is more probable.

When we induced virgin mice, 5–8 weeks old, to superovulate by the administration of hormones, we noted after they had been killed, that the contents of their oviducts were variable, particularly with respect to cohesiveness and location of the cumulus-oocyte mass. The occurrence of premature chromatid disjunction, observed in the meiotic chromosome preparations[2] was more frequent when the ductal contents were characterized by dispersed cumulus, location of most of the oocytes in the subampullar region and the presence of some oocytes devoid of follicle cells (Table 1). Those conditions may be more accurately indicative of "ageing" oocytes than the lapse of time after administration of the ovulatory hormone because (1) the effects of exogenous hormones are superimposed on the endogenous hormonal state, variable in mature female mice, and (2) although the precise role of the cumulus mass in which the oocytes are ovulated has not been defined, it is known that the cumulus is dispersed and the coronae of follicle cells fall away as the oocytes are propelled along the oviduct[3].

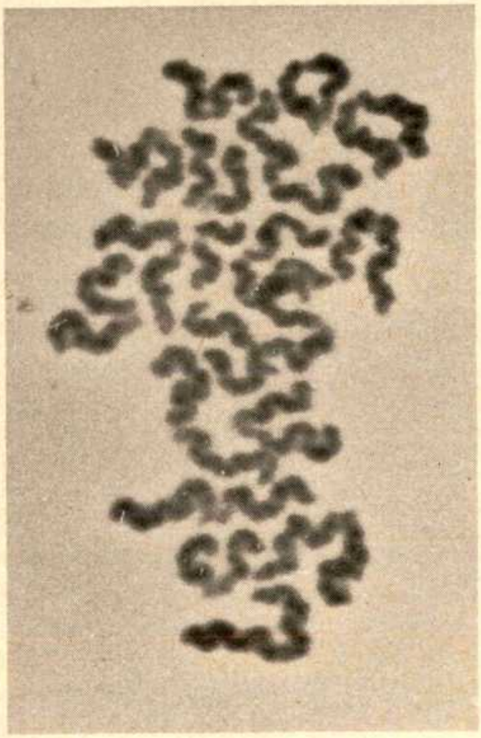


Fig. 1 Chromosome complement of secondary oocyte at second metaphase. All chromatid pairs are joined at their centromeres.

The relationship between anomalous development and experimentally induced delay in fertilization is well established[1]. Our observations, consonant with those of another investigator[4] who described morphological changes in the spindle assembly of mouse oocytes that had a prolonged stay in the oviduct, provide a cytologic basis for attributing those developmental abnormalities to impairment of the machinery of the second meiotic division. More important, they present a mechanism whereby the increased frequency of trisomy in offspring of older women may, as proposed, be due to the greater probability of delayed fertilizations.

It is significant that the type of chromosome alteration described here is not seen in the pathway of degeneration of the first polar body. Even in an advanced state of degradation, the polar body chromosomes remain as chromatid pairs, joined at their centromeres[2]. The disjunction of sister chromatids in unfertilized secondary oocytes appears, therefore, to be a characteristic of intraductal ageing rather than a non-specific type of change in degenerating meiotic chromosomes.

Table 1 Occurrence of Chromatid Disjunction in Unfertilized Secondary Oocytes in Relation to the State of the Intraductal Contents

Fresh cumulus *			Aged cumulus †		
Oviduct	No. of oocytes ‡	No. with disjoined chromatids	Oviduct	No. of oocytes ‡	No. with disjoined chromatids
2a	15	2	1a	12	3
3a	8	0	1b	2	1
3b	9	0	2b	9	2
5b	8	0	4a	7	2
7a	8	0	4b	2	1
7b	8	0	5b	12	2
10a	7	0	6a	15	1
10b	4	0	6b	7	1
11a	11	0	8a	10	2
11b	2	0	8b	9	1
12a	11	0	9a	8	0
12b	1	0	9b	0	0
15a	5	0	13a	4	0
15b	5	0	13b	0	0
16a	7	0	14a	4	0
16b	2	0	14b	4	0
17a	8	0	18a	6	0
17b	7	0	18b	1	0
20a	9	0	19a	1	0
20b	8	0	19b	0	0
21a	9	1	22a	8	2
21b	0	0	22b	2	0
24a	6	0	23a	6	2
24b	6	0	23b	2	0
24	164	3	24	131	20

* Oocyte-cumulus mass was cohesive, located in the ampullar region of the duct, and there were no separate oocytes, free of follicle cells. Chromatid disjunction was observed in 1.8% of the chromosome preparations.

† Cumulus mass fell apart in the saline solution in which the oviduct was sectioned, most of the oocytes were located in the subampullar region, and some oocytes, free of follicle cells, were present. Chromosome disjunction was observed in 15% of the preparations.

‡ Number of chromosome preparations in which the centromeres could be seen as either intact or divided in at least ten chromatid pairs.

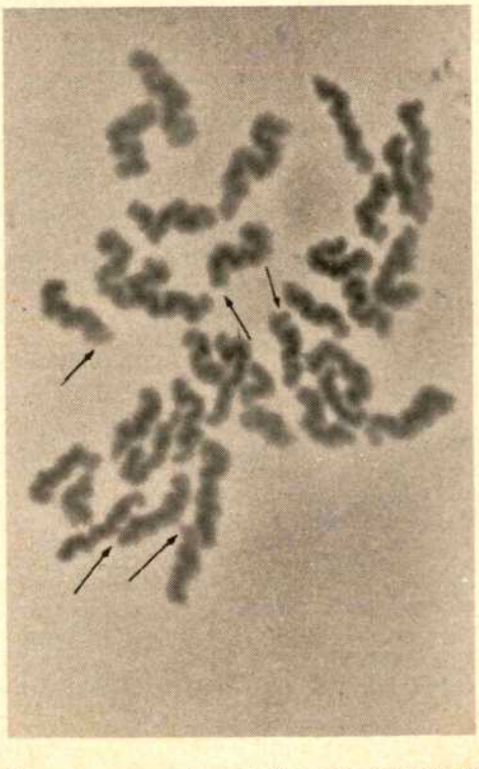


Fig. 2 Chromosome complement of secondary oocyte obtained from oviduct with "aged cumulus" (Table 1). Some sister chromatids have disjoined (arrows); others are still joined.

This work was supported by a grant from the Damon Runyan Memorial Fund Inc.

TOBY C. RODMAN

Department of Anatomy,
Cornell University Medical College,
1300 *York Avenue,*
New York,
New York 10021

Received April 2; revised June 7, 1971.

[1] Austin, C. R., *J. Reprod. Fert.*, Suppl. 12, 39 (1970).
[2] Rodman, T. C., *Exp. Cell Res.*, **66** (1971).
[3] Blandeau, R. J., in *Progress in Gynecology* (edit. by Sturgis, S. H., and Taylor, M. L.), **5**, 58 (Grune and Stratton, New York, 1970).
[4] Szollosi, D., *Amer. J. Anat.*, **130**, 209 (1971).
[5] German, J., *Nature*, **217**, 516 (1968).

Sequential Activation of Clotting Factors during Blood Coagulation

THE existence of activated forms of blood clotting factors was suggested more than twenty years ago. Ware and Seegers[1], pointing out a difference between the plasma and the so-called serum factor V, postulated a partial modification of the clotting factor by thrombin, which resulted in more potent activity. After activation, procoagulant action rapidly declines[2]. Rapaport[3] described the activation of the anti-haemophilic factor VIII by traces of thrombin. Thus, it is likely that the first traces of thrombin, produced during coagulation, convert factors V and VIII into more active clotting factors (Va and VIIIa). These thrombin-activated factors accelerate the intermediate phases of coagulation, leading to a rapid generation of more thrombin, and subsequent formation of fibrin. One of the intermediate phases is the reaction of factor VIIIa with the activated form of factor IX (IXa). In the presence of phospholipid and calcium ions factors VIIIa and IXa convert factor X into an activated form[4], which in turn reacts with factor Va, phospholipids and calcium ions to give prothrombin-converting activity[5,6]. Factor IX is activated during the initial phases of blood coagulation, which involves activation of the Hageman factor (factor XII) as a result of contact with a foreign surface, and the activating action of the latter on factor XI[7].

So far the activation of clotting factors by thrombin has been studied only with more or less purified preparations. It seemed to us that a more physiological way of studying the activation of clotting factors would be to investigate the dynamic process of activation and inactivation in slowly coagulating, recalcified citrated plasma. In this way we were able to determine the thrombin activation of factors VIII and V, the generation of activated factors IX, X and of thrombin, the disappearance of the activated factors and the sequence of the reactions involved.

Normal human plasma was obtained by centrifuging citrated blood (1 volume of trisodium citrate 3.8%+9 volumes of blood), immediately after venepuncture, for 4 min at 12,000*g* at room temperature. The platelet-poor supernatant plasma was then centrifuged at 40,000*g* for 30 min at 0° C. To obtain plasma with a very slow and low thrombin generation, we had to work very quickly so as not to damage the platelets. Otherwise the activation of the clotting factors would have been too fast to be determined and the measurement of their activities would have been disturbed by the thrombin that was generated. The activities of clotting factors were measured by one-stage procedures[8–10].

We introduced an important modification to the assays for factors VIII and IX, because of the great lability of activated factor VIII. The diluted sample was omitted from the incubation mixture, but was added immediately after dilution, simultaneously with $CaCl_2$. The presence of fibrin monomers in solution was tested by the ethanol gelation test according to Godal[11]. The thrombin generated was tested with a normal plasma sample (0.1 ml. of incubation mixture added to 0.1 ml. of citrated plasma), the clotting time of this being a measure for the thrombin concentration. The amount of thrombin generated was calculated on a calibration curve, for the determination of which we used NIH thrombin and the same normal plasma as substrate.

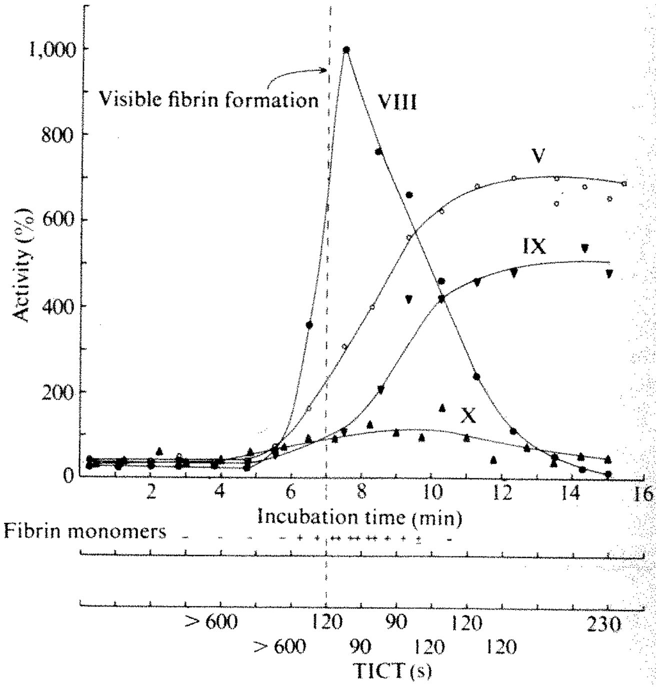


Fig. 1 Activation of clotting factors in a system consisting of equal amounts of practically platelet-free plasma, saline and $CaCl_2$, measured from the moment of recalcification. The formation of fibrin monomers, the appearance of a visible clot and the generation of thrombin, measured as the clotting time of normal plasma, are shown. □ TICT, Thrombin induced clotting time.

We investigated the activation of factors VIII, V, IX and X, the presence of fibrin monomers in solution, the appearance of the visible fibrin polymer (clotting of the mixture) and thrombin generation in a mixture of equal amounts of normal plasma, saline and $CaCl_2$ M/30, incubated at 37° C. The moment of recalcification was taken as zero time. At different times after recalcification, a sample was pipetted off, diluted 1 : 40 in Michaëlis buffer (pH $7.25 \mu = 0.15$) and simultaneously assayed for factor VIII, IX and V activity by three people. Thrombin generation was assayed in an undiluted sample. Immediately afterwards another sample of the incubation mixture was recalcified and assayed for factor X activity after dilution 1 : 10 in the same buffer. This procedure was repeated for the detection of fibrin monomers. The clotting time and thrombin generation curve were the same for all three incubation mixtures (combined results are shown in Fig. 1). The activity of the clotting factors was normal until 5 min after recalcification, when there was a large and rapid activation of factor VIII. We noted an activation from 28% to 1,000%, corresponding to a change in clotting time of the assay from 100 s to 71 s for a dilution of 1 : 40. Factor V was activated simultaneously; at the maximum the clotting time was shortened from 43 s to 20 s, corresponding to an activation from 42% to 700%. For the third factor which was activated, factor IX, there was a shortening of clotting time from 134 s to 92 s for a dilution of 1 : 40, corresponding to an activation from 40% to 500%. One minute before the coagulation of the mixture, fibrin monomers were detectable; visible fibrin formation lasted for 3 min, during which the test for fibrin monomers remained positive. Factor VIII activity disappeared from the clotted plasma very quickly, while high factor V and factor IX activities could be determined 2 h after clotting. In this clotting system the activity of factor X does not increase remarkably, the clotting time of a 1 : 10 dilution being shortened from 50 s to 40 s. The thrombin generation in this almost platelet-free plasma is very low. At the maximum of the thrombin generation curve only 0.3 U of thrombin/ml. are generated. This quantity of thrombin does not influence the determination of clotting factors, as blank tests proved.

The problem how the very first trace of thrombin, which initiates the autocatalytic activation of factors VIII and V, is generated, remains to be solved.

This work was supported by the Netherlands Foundation for Chemical Research with financial aid from the Netherlands Organization for the Advancement of Pure Research.

CATHERINE L. JANSSEN
WILHELMINA H. VAN LEEUWEN

Central Laboratory of the Netherlands Red Cross,
Blood Transfusion Service,
Amsterdam

Received February 23; revised April 19, 1971.

[1] Ware, A. G., Murphy, R. C., and Seegers, W. H., *Science*, **106**, 618 (1947).
[2] Lewis, M. L., and Ware, A. G., *Blood*, **9**, 520 (1954).
[3] Rapaport, S. I., Schiffman, S., Patch, M. J., and Ames, S. B., *Blood*, **21**, 221 (1963).
[4] Hemker, H. C., and Kahn, M. J. P., *Nature*, **215**, 1201 (1967).
[5] Hemker, H. C., Esnouf, M. P., Hemker, P. W., Swart, A. C. W., and Macfarlane, R. G., *Nature*, **215**, 248 (1967).
[6] Deggeller, K., and Vreeken, J., *Thromb. Diath. Haem.*, **22**, 45 (1969).
[7] Ratnoff, O. D., Davie, E. W., and Mallett, D. L., *J. Clin. Invest.*, **40**, 803 (1961).
[8] Stormorken, H., Sc. *J. Clin. Lab. Invest.*, **9**, 273 (1957).
[9] Bachmann, F., Duckert, F., and Koller, F., *Thromb. Diath. Haem.*, **2**, 24 (1958).
[10] Veltkamp, J. J., Drion, E. F., and Loeliger, E. A., *Thromb. Diath. Haem.*, **19**, 279 (1968).
[11] Godal, H. C., and Abildgaard, U., *Scand. J. Haematol.*, **3**, 342 (1966).

Interruption of SV40 Oncogenesis with Human Foetal Antigen

IMMUNIZATION of adult hamsters with irradiated foetal hamster or mouse cells produces strong immunity to SV40 cell challenge and simultaneously induces cytostatic (or C) antibody formation[1]. Furthermore, adenovirus 31 tumorigenesis can be interrupted by a single injection of hamster foetal cells if administered to neonatally infected hamsters 3 weeks after birth and before the appearance of the tumour[2]. SV40 viral oncogenesis could be similarly interrupted in male but not female hamsters[2]. This work provides support for the view that foetal and tumour cells share common antigens and that oncogenesis involves retrogenic activation of early foetal antigen genes in adult cells[3,4].

If the concept that ontogeny repeats phylogeny is applicable at the molecular level, early foetal proteins from different vertebrates should resemble each other more than adult proteins do. This would not be surprising, for the organizational problems of early foetal life are quite similar in different species, whereas the adaptation required for adult life may differ widely. Thus if viral oncogenesis involves activation of specific foetal genes, these may be expected to be immunologically similar in cells of widely different origin. Conversely, foetal cells of different species might be expected to immunize across species barriers against tumours produced by the same virus. This is important if conclusions drawn from animal model studies are to be applied to man and if vaccination or immunotherapy involving specific antigens is to be attempted.

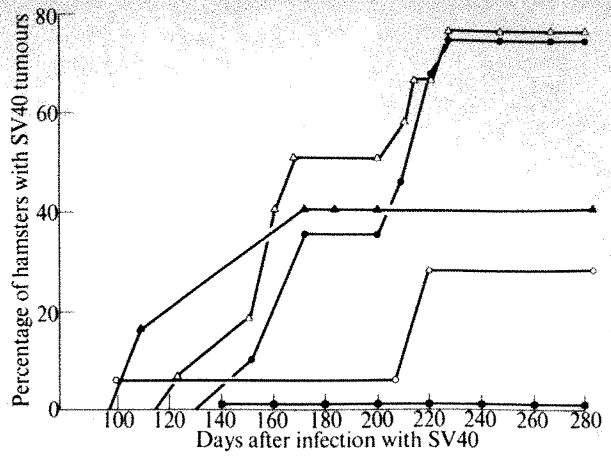


Fig. 1 Interruption of SV40 tumorigenesis by immunization with human embryonic kidney cells. △, No vaccine; ●, adult human kidney (5,000 r.); ▲, human embryonic kidney (5,000 r.); ○, SV40; ■, F5-1 SV40 tumour cells (5,000 r.).

Hamster, mouse, and human cells transformed by SV40 virus all exhibit a similar antigen capable of stimulating specific transplantation immunity against SV40-induced tumours in hamsters[5,6], supporting the concept of foetal antigen similarity. We now report cross immunization of adult hamsters against SV40 virus tumorigenesis by human foetal cell homogenates.

SV40 hamster tumour cells (F5-1 line) were cultured as previously described[7], and all experiments were done with the LVG hamster strain. C-Antibody levels were determined using implanted diffusion chambers containing SV40 tumour target cells[1,2,7,8].

SV40 virus (VA-45-54) was prepared in green monkey kidney cells (GMK) and had a titre of $10^{7.6}$ $TCID_{50}$/0.2 ml. in GMK. Hamsters were infected with 0.2 ml. of SV40 in the right subscapular space within 24 h of birth and palpated weekly for tumours after 4 weeks of age. Hamster foetal cell suspensions were prepared as previously described[1]. Lyophilized cells were prepared from washed suspensions of freshly harvested (trypsin) F5-1 tumour cells in HBSS. The cells were reconstituted in sterile distilled water to the original volume of liquid which had originally contained 5×10^6 viable cells/ml. Primary human embryonic kidney cells were purchased from Flow Laboratories, Rockville, Maryland. Foetal immunogens and control materials were exposed to 5,000 r. of X-irradiation before injection[1]. Five million trypan blue excluding cells were used for each immunization with all preparations except the lyophilized F5-1 cell vaccine.

Cytostatic antibody against SV40 tumour cells appears within 4 weeks after infection of neonate hamsters with SV40, persists in the peritoneal fluid and blood until several weeks before palpable tumour formation, then disappears[9]. SV40 tumours rarely appear in less than 90–100 days after infection. Neonatally infected hamsters were immunized with three injections of either SV40, irradiated SV40 hamster tumour cells, human embryo kidney cells, adult kidney cells, lyophilized SV40 tumour cells, 10-day gestation hamster foetal cells, or nothing. The results in Table 1 show that at 56–60 days after infection, non-immunized SV40-infected animals possessed a significant level of C-antibody, as previously reported[9]. Immunization of infected hamsters with SV40 virus, irradiated F5-1 tumour cells, primary human embryo kidney cells, or hamster foetal cells always increased the C-antibody level over that observed in control animals receiving no immunization. Adult primary human kidney cells, lyophilized SV40 tumour cells, or non-irradiated hamster foetal cells always diminished the C-antibody level below that observed in non-immunized SV40 virus infected control animals (Table 1). Lyophilized SV40 tumour cells, non-irradiated hamster foetal cells, or adult human cells failed to induce either immunity to SV40 tumour cell challenge or to interrupt SV40 oncogenesis. Fifty per cent protection against SV40 tumour induction was observed among hamsters immunized with irradiated human embryo kidney cells, and 62% protection was afforded by immunization of adult hamsters with SV40 (Fig. 1). Irradiated SV40 tumour cells gave complete protection. We have previously demonstrated that irradiated hamster foetal cells protect male hamsters against SV40 tumorigenesis[2].

Table 1 Potentiation of Diminution of Cytostatic Antibody Level to SV40 Hamster Tumour Cells using Human Foetal Cells as Immunogen in SV40-Infected Male Hamsters

Neonatal inoculation *	Immunogen †	Average No. of viable cells/ chamber‡	Inhibition§ (%)
Passage control fluid	None	626,000	Control
SV40	None	441,000	30
SV40	SV40	387,000	39
SV40	F5–1 (5,000 r.)	344,300	45
SV40	Adult human kidney cells	476,400	23
SV40	Primary human embryonic kidney cells	309,700	51
SV40	Lyophilized F5–1 (5,000 r.)	594,700	5
SV40	10 day hamster foetus (5,000 r.)	344,400	45
SV40	10 day hamster foetus (0 r.)	565,800	10

* Newborn hamsters were infected with SV40 within 24 h after birth or given noninfected cell culture control fluid.

† SV40 or irradiated cells (5,000 r.) administered intraperitoneally at 3 weeks of age, weekly for 3 weeks.

‡ Fifteen chambers originally inoculated with 18,000 viable SV40 tumour cells, implanted in immunized 56 day old hamsters for 5 days, and enumerated by trypan blue exclusion test.

§ Percentage inhibition of SV40 target cell growth in chambers observed between immunized control animals. All values given were significantly different from control at 1% confidence level as determined by Wilcoxon test.

The results show that injection of control tissues, lyophilized SV40 tumour cells, or non-irradiated hamster embryo tissues diminishes detectable levels of circulating antibody to the SV40 tumour target cells in the diffusion chambers. In contrast, human foetal cells and irradiated hamster foetal cells increase the level of circulating C-antibody, supporting the view that SV40-induced TSTA is the result of the retrogenic expression of a foetal or embryonic antigen gene.

Research was conducted under the joint NIH-AEC Molecular Anatomy (MAN) Program supported by the National Cancer Institute, the National Institute of General Medical Sciences, the National Institute of Allergy and Infectious Diseases, and the US Atomic Energy Commission.

K. R. Ambrose
N. G. Anderson
J. H. Coggin

Molecular Anatomy (MAN) Program,
Oak Ridge National Laboratory,
Oak Ridge,
Tennessee 37830, *and*
Department of Microbiology,
University of Tennessee,
Knoxville,
Tennessee

Received April 16, 1971.

[1] Coggin, J. H., Ambrose, K. R., and Anderson, N. G., *J. Immunol.*, **105**, 524 (1970).
[2] Coggin, J. H., Ambrose, K. R., Bellomy, B. B., and Anderson, N. G., *J. Immunol.* (in the press).
[3] Anderson, N. G., and Coggin, J. H., *Science* (in the press).
[4] Potter, V. R., *Proc. Eighth Canadian Cancer Res. Cong.* (in the press).
[5] Girardi, A. J., *Proc. US Nat. Acad. Sci.*, **54**, 445 (1965).
[6] Kit, S., Kurimura, T., and Dubbs, D. R., *Intern. J. Cancer*, **4**, 384 (1969).
[7] Ambrose, K. R., Candler, E. L., and Coggin, J. H., *Proc. Soc. Exp. Biol. Med.*, **132**, 1013 (1969).
[8] Coggin, J. H., and Ambrose, K. R., *Proc. Soc. Exp. Biol. Med.*, **130**, 246 (1969).
[9] Ambrose, K. R., and Coggin, J. H., *Bact. Proc.*, 187 (1970).

Correlation between Humoral Antibody and Regression of Tumours induced by Feline Sarcoma Virus

Feline sarcoma virus (FSV) induces malignant tumours in cats[1–4], dogs[1,2,4], rabbits[1,4] and monkeys[4,5], and transforms cultured cells from several species including man[6,7]. Spontaneous regression of tumours induced by FSV has been reported[3]. We describe here a correlation between the presence of humoral antibodies to virus-associated cell membrane antigens and the failure of cats injected with FSV to develop progressive malignant tumours.

An indirect membrane immunofluorescence technique similar to that described for the detection of membrane antigens in murine leukaemia cells was used[8]. A lymphoblastoid cell culture derived from a feline leukaemia virus (FeLV)-induced tumour provided target cells. The derivation and maintenance of this culture, which produces FeLV in a steady state manner, has been described before[9]. Fluorescein-conjugated antiserum to cat IgG prepared in a goat (obtained from Dr Robert Holdenreid, National Cancer Institute, Bethesda), or rabbit (obtained from Dr Edwin Lennette, California State Department of Public Health, Berkeley) was used at a 1 : 20 dilution.

Bright and dense sectorial or ring fluorescence was observed in 90–100% of the cells at serum dilutions up to 1 : 128 with serum from certain regressor cats (Fig. 1). Two-fold dilutions of each serum sample were prepared, and fifty cells were examined at each dilution until fluorescence was no longer observed. The serum dilution at which the number of fluorescing cells had decreased to 50–75% usually was the same dilution at which punctate rather than ring or sectorial fluorescence was observed. We decided to designate end-point titres as the reciprocal of the highest dilution where 50% or more of the cells had distinct spots of fluorescence. The negative serum samples caused no such fluorescence even when undiluted. All samples were coded and read blind.

Serum samples were taken from cats before the injection of FSV and between 3 and 7 weeks later. Cats with progressive tumours usually died within 3–5 weeks after FSV inoculation and so later samples could only be obtained from regressor cats or those that failed to develop palpable tumours. Cats which were 0–1, 4, 13, 26, and 52 weeks old received 1.0 g equivalents of FSV prepared as previously described[3]. The inverse relationship between increasing age at the time of FSV injection and the incidence of progressive malignant tumours has been previously observed (S. P. S., manuscript in preparation), and the gross morphology and histopathology of progressive and regressive tumours has been described[3].

A distinct negative correlation was seen between the antibody titre 3–7 weeks after FSV inoculation and the development of progressive tumours (Table 1). Of three cats which were injected with FSV at 1 yr of age, two failed to develop palpable tumours and one developed a tumour which subsequently

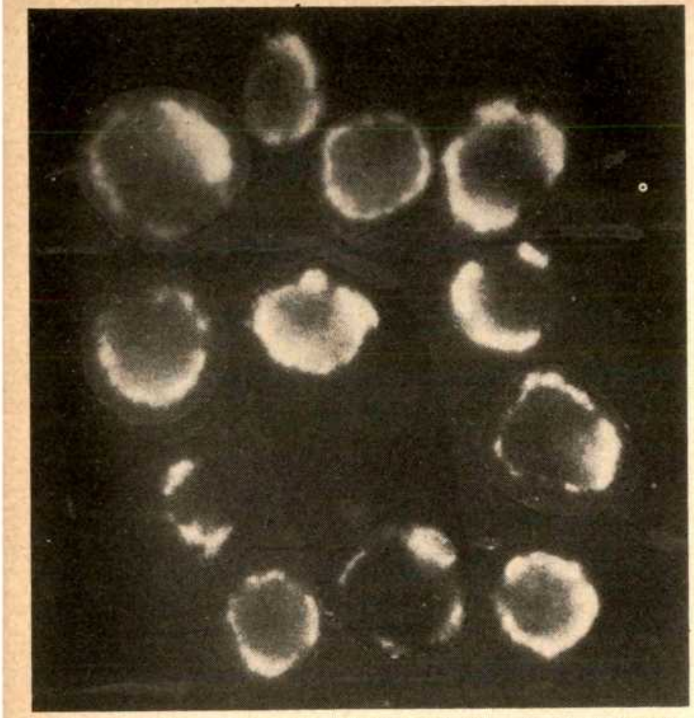


Fig. 1 Feline leukaemia virus-producing lymphoblastoid cells showing ring membrane fluorescence. The cells were exposed to serum (1 : 16 dilution) from a cat with a regressing FSV-induced sarcoma and sarcoma and subsequently to fluorescein labelled goat anti-cat IgG (1 : 20 dilution).

regressed. All three developed antibody titres which ranged from 16 to 256. The only kitten given FSV at 0–1 week of age which manifested tumour regression was also the only one in this age group to develop a significant humoral antibody titre. When all age groups are considered, nine cats developed tumours that were classified as progressive. Of these nine, none developed antibody titres above 2, and five had no demonstrable antibody. Twelve cats either developed no tumour or developed tumours which subsequently regressed. All twelve developed antibody titres of 4 or higher and eight had titres of at least 16 (Tables 1 and 2). Regressor tumours are defined as those which show a visible reduction in size before 7 weeks after FSV injection. Progressor tumours are those which show no visible or palpable regression, and which debilitate the host to the point of death. Kittens with progressive tumours were terminally exsanguinated to obtain serum. The reason for the presence of antibody titres in three of the twenty-one cats before FSV inoculation is not known, but the horizontal transmission of oncornaviruses in laboratory cat colonies has been reported[10], and since these three cats were either 13 or 26 weeks old at the time of testing, it is possible that they had been exposed to either FSV or FeLV.

Table 1 Titres of Antibody Detected by Indirect Immunofluorescence in Serum Samples from Cats of Different Ages Inoculated with FSV

Cat No.	Age in weeks*	Antibody titres-weeks† 0	3	4	5	7	Type of tumour response‡
1	52	0	128			128	regressor
2	52	0	4			128	no tumour
3	52	0	0			16	no tumour
4	26	0	16				regressor
5	26	16	64				regressor
6	26	0	2				progressor
7	26	0	32				regressor
8	13	0		0			progressor
9	13	8		64	256	256	regressor
10	13	0		2			progressor
11	13	8		8	32	16	regressor
12	13	0		0			progressor
13	13	0		1			progressor
14	4	0	4				regressor
15	4	0	4				regressor
16	4	0	4				regressor
17	0–1	0	0				progressor
18	0–1	0	1				progressor
19	0–1	0	4				regressor
20	0–1	0	0				progressor
21	0–1	0	0				progressor

* Age at time of FSV inoculation.

† Weeks following FSV inoculation.

‡ See text for definition of regressor and progressor.

Table 2 Summary of Antibody Response of Cats of All Ages According to Tumour Response and Antibody Titres

Type of tumour response	No. of cats with titres of 4 or over	No. of cats with titres under 4
No tumour	2	0
Regressors	10	0
Progressors	0	9
Total	12	9

The findings reported here clearly demonstrate an association between the development of antibody titres and either tumour regression or the lack of palpable tumour development. They also demonstrate a reaction between antibody associated with injections of FSV and a cell membrane antigen of cells that produce FeLV.

We thank Drs Edwin Lennette and Robert Holdenreid for fluorescein-conjugated antiserum, Harriet Blomquist and Sharon Beall for technical assistance, and Dr Leo Bustad for encouragement and criticism. This work was supported by fellowship awards from the Leukemia Society of America (M. E.) and the American Cancer Society (S. P. S.) as well as general support from the Special Virus-Cancer programme of the National Cancer Institute, the US National Institutes of Health and Public Health Service, the Swedish Cancer Society, the Jane Coffin Child's Memorial Fund for Medical Research, and the Harald and Greta Jeansson Foundation.

MYRON ESSEX
GEORGE KLEIN

Department of Tumour Biology,
Karolinska Institute School of Medicine,
Stockholm

STANLEY P. SNYDER
J. BOYD HARROLD

Comparative Oncology Laboratory,
University of California,
Davis

Received March 31; revised May 28, 1971.

[1] Snyder, S. P., and Theilen, G. H., *Nature*, **221**, 1074 (1969).
[2] Gardner, M. B., Rongey, R. W., Arnstein, P., Estes, J., Sarma, P., Huebner, R. J., and Rickard, C. G., *Nature*, **226**, 807 (1970).
[3] Snyder, S. P., Theilen, G. H., and Richards, W. P. C., *Cancer Res.*, **30**, 1658 (1970).
[4] Theilen, G. H., Snyder, S. P., Wolfe, L. G., and Landon, J. C., *Comparative Leukemia Research* (edit. by Dutcher, R. M.), (Karger, New York, in the press).
[5] Deinhardt, F., Wolfe, L. G., Theilen, G. H., and Snyder, S. P., *Science*, **167**, 881 (1970).
[6] Sarma, P. S., Huebner, R. J., Basker, J. F., Vernon, L., and Gilden, R. V., *Science*, **168**, 1098 (1970).
[7] Chang, R. S., Golden, H. D., and Harrold, B., *J. Virol.*, **6**, 599 (1970).
[8] Klein, E., and Klein, G., *J. Nat. Cancer Inst.*, **32**, 547 (1964).
[9] Theilen, G. H., Kawakami, T. G., Rush, J. D., and Munn, R. J., *Nature*, **222**, 589 (1969).
[10] Rickard, C. G., *J. Small Animal Practice*, **10**, 615 (1969).

Inhibiting Effect of some Antimalarial Substances on Glucose-6-phosphate Dehydrogenase

COMPOUNDS with antimalarial activity are used as therapeutic agents in a number of disorders other than malarial infections. Our dermatological interest in these substances is two-fold. First, they are specific therapeutics for a number of autoimmune disorders with cutaneous manifestations[1] and, second, the use of these substances in malarial therapy or prophylaxis often causes adverse cutaneous side effects[1].

It has been known for some time that individuals with an erythrocytic glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) deficiency (favism) have an increased resistance to malaria[2]. These individuals also have a high incidence of haemolytic anaemia when given antimalarial drugs prophylactically[2]. These observations suggest that antimalarial drugs inhibit erythrocytic glucose-6-phosphate dehydrogenase (G6PDH) in malaria. Analogously, they may inhibit the G6PDH activity of the reticulo-endothelial system in their capacity as immunosuppressants, and that of the skin in their production of cutaneous manifestations. An essential first test of such an hypothesis is to see if these compounds are capable of such inhibition *in vitro*. We have therefore tested a range of antimalarial substances on purified yeast G6PDH from Sigma and on whole tissue extracts of erythrocytes and skin.

The usual commercial forms of the antimalarial substances were dissolved and filtered before use. Enzyme was assayed at room temperature by following the formation of NADPH fluorimetrically (exitation 340 nm, fluorescence 460 nm), or spectrophotometrically when difficulties from quenching were experienced. The general details of the assay have been described by Halprin and Ohkawara[3]. The reaction mixtures used are given in Table 1.

Table 1 Reaction Mixture for Testing Inhibitors of Glucose-6-phosphate dehydrogenase

	NADP 0.002 M in buffer (ml.)	G6PDH 10^{-3} mg/ml. in buffer (ml.)	Buffer 0.05M Tris (*p*H 7.5) $+5\times10^{-4}$ M EDTA $+0.03$ M $MgCl_2$ (ml.)	Inhibitor 10^{-3} M in buffer (ml.)	G6P (diNa) 0.014 M in buffer (ml.)
1	0.10	0.10	1.60	0.10	0.10
2	0.10	0.10	1.69	0.10	0.10
3	0.10	0.10	1.69	0.10	0.01

Before calculating the results it was observed that the inhibition could be reversed completely in most cases by excess NADP, that is, the inhibition was competitive for NADP. Therefore the following equation was used to calculate the K_I values

$$K_I = \frac{V_I}{V_0 - V_I} \times \frac{Km \quad [I]}{Km + [S]}$$

where V_I is the initial reaction velocity in the presence of the inhibitor, V_0 the initial velocity of the reaction in the absence of inhibitor, $[I]$ the final concentration of inhibitor, Km the Michaelis–Menton constant, K_I the inhibition constant and $[S]$ the concentration of substrate (NADP). This equation was derived by dividing the normal Michaelis–Menton equation by the equation for a competitively inhibited reaction and rearranging to give K_I.

In normal Lineweaver–Burk plots, the Km for glucose-6-phosphate and NADP were found to be 6.9×10^{-5} M and 1.43×10^{-5} M respectively. The enzyme was specific for NADP, which could not be replaced by NAD, and the reaction was inhibited by NADPH. The results of the inhibition studies are shown in Table 2.

Table 2 Inhibition of G6PDH by Antimalarials

Inhibitor	% inhibition at low [NADP]	% inhibition at low [G-6-P]	% inhibition at high [NADP] + [G-6-P]	Approximate K_I	Method used
Paludrine	30	—	—	1.1×10^{-4}	Fluorimetric
Daraprime	36.4	—	—	8×10^{-5}	Fluorimetric
Mepacrine	17.2	—	—	1.8×10^{-3}	Spectrophotometric
Primaquine	28.1	—	—	1.2×10^{-3}	Spectrophotometric
Chloroquine	47.5	—	—	5.14×10^{-4}	Spectrophotometric

The antimalarial compounds also inhibited the G6PDH reaction in skin and erythrocyte extracts to a similar extent. Gold salts were found to inhibit skin G6PDH noncompetitively and streptomycin to inhibit competitively for glucose-6-phosphate. Both of these compounds have a similar therapeutic application and range of side effects as the antimalarials proper[1].

It is interesting to consider the consequences of such an inhibition to the metabolism of the cell. G6PDH is the rate limiting point of entry into the pentose phosphate shunt which is the sole producer of extramitochondrial NADPH and also produces riboses for nucleic acid synthesis. Obviously inhibition of this enzyme and pathway will have far reaching effects, particularly in subjects such as favists who have a reduced G6PDH activity in the first place. It would also be of great interest to know if subjects who experience lichenoid skin eruptions as a side effect of antimalarial therapy have a skin-specific deficiency in this enzyme. We are currently carrying out such investigations.

We thank Professor J. W. H. Mali, Dr P. D. Mier and Dr R. C. A. Sengers for helpful discussions.

D. W. K. COTTON
A. H. M. SUTORIUS

Department of Dermatology,
Catholic University,
Javastraat 104, *Nijmegen*

Received April 6, 1971.

[1] Rook, A., Wilkinson, D. S., and Ebling, F. J. G., in *Textbook of Dermatology*, **2**, 1209 (Blackwell, Oxford/Edinburgh, 1968).
[2] Halprin, K. M., and Ohkawara, A., *J. Invest. Dermatol.*, **46**, 43 (1966).
[3] Harris, H., *The Principles of Human Biochemical Genetics* (Frontiers of Biology, series 19) (North-Holland, Amsterdam, 1970).

Effect of Infection with Trypanosomes on the Development of Experimental Allergic Neuritis in Rabbits

DATA obtained from hospitals in western Nigeria[1] have shown that rheumatoid arthritis is uncommon in that region and, when the disease occurs, it is clinically milder than in Europeans, involving few of the expected immunological changes[2,3]. Other conditions in which auto-immune processes are thought to be involved were also found to be rare, and Greenwood suggested that repeated exposure to parasitic infections might be responsible, at least in part, for the infrequent occurrence of these auto-immune diseases. Infection with the rodent malaria parasite *Plasmodium berghei yoelii* delayed the onset of haemolytic disease in 4 week old NZB mice and prevented the development of renal disease in (NZB×NZW) F_1 mice at the same age[4]. The severity of adjuvant arthritis in rats was also reduced by simultaneous inoculation of *P. berghei yoelii*, which produces a very mild infection, but not by infection with a more virulent strain of *P. berghei berghei*[5]. We are investigating the effects of trypanosome parasites on the development in rabbits of experimental allergic neuritis (EAN), a demyelinating paralytic disease[6], the genesis of which is primarily a cell-mediated type of immune response (manuscript in preparation), and the preliminary results are reported here.

Adult New Zealand white rabbits of both sexes were infected by intraperitoneal inoculation of blood from mice infected with trypanosome parasites; five received *Trypanosoma brucei brucei* (Serial No. LUMP 143) which is maintained as a stabilate[7], and seven received a *T. brucei* parasite (strain S 427 supplied by Dr L. G. Goodwin and now maintained as stabilate LUMP 231). Parasitaemia was estimated by examination of fresh blood preparations by phase contrast microscopy (×40 objective) every 2–3 days. The rabbits infected with *T. b. brucei* LUMP 143 were cured 24 days after inoculation of parasites by treatment with 'Berenil' (10 mg/kg given intramuscularly). Five of the seven animals infected with *T. brucei* S 427 were cured in the same way 59 days after inoculation; the other two developed severe EAN signs and had been killed and autopsied by this time.

Extraneous fat and connective tissue were removed from human sciatic nerve obtained at *post mortem*. The nerve was cut on a freezing microtome and the "brei" produced was homogenized with saline. An equal volume of Freund's complete adjuvant was added and a stable water-in-oil emulsion prepared by further homogenization. 0.2% of phenol was added to the antigen which was stored at −20° C. 0.2 ml. of antigen were injected intradermally into the left hind foot pad of each rabbit. Further details of antigen preparation and injection procedure are given elsewhere[8].

The antigen was injected into nine uninfected rabbits and into the twelve trypanosome-infected animals 19 or 21 days after inoculation of parasites. After the injection of EAN antigen, animals were examined by two observers at 2 day intervals for signs of functional disability. Autopsy was carried out at 55 days after antigen challenge or earlier when necessitated by the onset of severe paralysis. Rabbits were killed by intravenous injection of 'Nembutal'. For preliminary histological survey, dorsal lumbar roots were removed and fixed with osmium tetroxide. Teased preparations were made from these nerves as previously described[8].

Live parasites were seen in all rabbits during the period between inoculation of trypanosomes and injection of EAN antigen. Since the levels of infection at this stage were so low (one to ten trypanosomes per fifty microscope fields), no importance was attached to the apparent length of the latent period; the shortest was 3 days and the longest was 14 days, the average being about 7 days. *T. b. brucei* LUMP 143 infections had reached a level of one to fifteen parasites per microscope field by day 24 and were therefore treated, but *T. brucei* S 427 was less virulent.

EAN signs were assessed on a three point scale. Early and mild cases (denoted by +) showed features such as weakness of hind limb(s), poor "displacement reflex", some ataxia, some sliding of fore limbs and poor "withdrawal reflex".

In later stages (+ +) these features became more pronounced and more frequent. Severe cases (+ + +) showed additional signs such as extended flaccid limbs, great difficulty in walking, flailing of limbs, total limb paralysis, incontinence, loss of appetite and weight and laboured breathing (for more details of EAN signs see Allt, Evans and Evans[8]).

In the "EAN+trypanosomiasis" group (twelve rabbits) seven animals were alive 55 days after EAN antigen challenge. Five of these had shown no overt EAN signs. Of the "EAN alone" group (nine rabbits) only one remained alive at 55 days after injection of EAN antigen, and it had previously shown unambiguous EAN signs. The remaining rabbits of this group developed marked (+ +) or severe (+ + +) EAN signs and had to be killed (Table 1).

Spinal roots are especially vulnerable to demyelination in EAN and lumbar dorsal roots were chosen for preliminary

Table 1 EAN Signs (Severity, Duration, Onset) and Histopathology

	Animal	Severity of EAN signs§	Day of onset of overt EAN signs (after EAN antigen challenge)	Duration of EAN signs	Survival* (at 55 days after EAN antigen challenge)†	Histology‡ (lumbar dorsal roots)	
						Demyelination	Remyelination
EAN in trypanosome-infected rabbits	1	None	–	–	Yes	–	–
	2	None	–	–	Yes	t	–
	3	None	–	–	Yes	–	t
	4	None	–	–	Yes	–	t
	5	None	–	–	Yes	–	–
	6	+	28	12	Yes	+	+ +
	7	+ +	19	21	Yes	+ + +	+ +
	8	+ + +	14	2	No	+ +	–
	9	+ +	15	8	No	+	–
	10	+ + +	13	2	No	+ +	–
	11	+ + +	14	2	No	+	–
	12	+ +	35	3	No	+ + +	–
EAN in uninfected rabbits	13	+	23	17	Yes	+	t
	14	+ + +	14	2	No	+ +	–
	15	+ + +	18	5	No	+ + +	–
	16	+ + +	10	6	No	+ + +	–
	17	+ + +	18	3	No	+ +	–
	18	+ + +	15	7	No	t	–
	19	+ + +	18	12	No	+ +	–
	20	+ +	14	2	No	t	–
	21	+ + +	13	3	No	+ + +	–

* Rabbits showing (*a*) severe paralysis (+ + +), (*b*) marked paralysis (+ +), together with respiratory difficulty, were killed.

† Difference in survival between the two groups is significant, that is, greater than two standard errors (s.e. 0.22) or $P<0.05$.

‡ Four point scale for extent of demyelination/remyelination derived from teased preparations: + + +, "extensive"; + +, "moderate"; +, "occasional"; t, "trace".

§ See text for grading of EAN signs.

examination. Teased preparations of osmicated nerves from paralysed rabbits revealed focal, segmental myelin loss characteristic of EAN. Various stages of myelin breakdown could be observed, ranging from the replacement of a part of an internode by several large myelin ovoids, through many droplets of myelin, to totally denuded axon segments[8]. Remyelination, in the form of short, intercalated internodes, was evident in animals which had recovered from EAN. Unlike the adjacent undamaged myelin, such internodes had thin myelin sheaths.

Table 1 correlates the severity of EAN signs and the extent of histological changes. Generally, the greater the paralysis and ataxia the more extensive the demyelination. Occasionally marked paralysis was accompanied by only sparse demyelination. The explanation for this may be either: (*a*) inadequate histological sampling, or (*b*) that demyelination is not the only important histopathological effect. Comparisons of the time of onset and the duration of EAN in control rabbits and in those trypanosome-infected animals which were affected showed no significant differences between the two groups.

In this series of experiments, all the control animals developed unequivocal signs of the disease whereas a significantly smaller number of the trypanosome-infected animals did so. It seems probable therefore that the presence of the parasite caused a marked suppression of EAN. We cannot say at present what the mechanism of this suppression is but it might have an immunological basis since EAN induction is primarily a cell-mediated immune response (manuscript in preparation). The effects of trypanosome infections on such responses have not to our knowledge been investigated, but Goodwin *et al.*[9] have examined the antibody response to sheep erythrocytes in mice and rabbits infected with *T. brucei*. There was a pronounced depression of the anti-sheep red blood cell response in the infected mice and immunodepression was also demonstrable in some of the trypanosome-infected rabbits. The response in other rabbits was very difficult to interpret, however, because of the appearance during the infection of heterophile antibody which reacted with antigen of the sheep erythrocytes (Goodwin and Voller, personal communication). They suggested that the presence of the trypanosomes seemed to make it difficult for the rabbit to produce antibody to "large" antigens which may require to be processed by macrophages before antibody forming cells can be stimulated.

It is interesting that the trypanosome infection suppressed EAN in some of our rabbits but was ineffective in others in terms of the time of onset and duration of EAN symptoms and the histological reactions. These variations in response to EAN were not related to the strain of parasite used and could not be correlated with any observed feature of the trypanosome infections. Thus suppression was not obviously related to the overall level of parasitaemia or to "peaks" of infection such that the results might be explained in terms of antigenic competition due to the number of parasites present. It is important to remember that the trypanosome is a tissue parasite[10] and that there are likely to be many more trypanosomes in the extravascular spaces than in the peripheral circulation. We are investigating the effect of varying the time of parasite infection in relation to antigen injection on the subsequent development of EAN.

G. ALLT
E. M. E. EVANS
D. H. L. EVANS

Department of Anatomy,
University College London,
Gower Street,
London WC1

G. A. T. TARGETT

Department of Medical Protozoology,
London School of Hygiene and Tropical Medicine,
Keppel Street,
London WC1

Received June 4; revised July 2, 1971.

[1] Greenwood, B. M., *Lancet*, ii, 380 (1968).
[2] Greenwood, B. M. *Ann. Rheum. Dis.*, **28** 488 (1969).
[3] Greenwood, B. M., and Herrick, E. M., *Brit. Med. J.*, **1**, 71 (1970).
[4] Greenwood, B. M., Herrick, E. M., and Voller, A., *Nature*, **226**, 266 (1970).
[5] Greenwood, B. M., Voller A., and Herrick, E. M., *Ann. Rheum. Dis.*, **29**, 321 (1970).
[6] Waksman, B. H., and Adams, R. D., *J. Exp. Med.*, **102**, 213 (1955).
[7] Lumsden, W. H. R., and Hardy, G. J. C., *Nature*, **205**, 1032 (1965).
[8] Allt, G., Evans, E. M. E., and Evans D. H. L., *Brain Res.*, **29**, 271 (1971).
[9] Goodwin, L. G., *Trans. Roy. Soc. Trop. Med. Hyg.*, **64**, 797 (1970).
[10] Ormerod, W. E. in *The African Trypanosomiases* (edit. by Mulligan, H. W., and Potts, W. H.) (Allen and Unwin, 1970).

---

^{13}C NMR Spectra of Lecithin Vesicles and Erythrocyte Membranes

UNLIKE proton nuclear magnetic resonance (NMR) spectroscopy, ^{13}C NMR has the potential advantages in studies of macromolecular systems of a wider range of chemical shifts ($\sim$200 p.p.m.) and of reduced resonance broadening in structures with restricted molecular motion. Thus we have been able to observe ^{13}C NMR spectra of unsonicated lecithin and erythrocytes, which do not yield high resolution proton spectra. We also report T_1 relaxation times for ^{13}C nuclei in the polar headgroup of the lecithin molecule and at both ends of the alkyl chains, in sonicated vesicles in D_2O buffer and in deuterochloroform. The results are consistent with increasing chain motion towards the terminal methyl group of the alkyl chains for both systems, in agreement with relaxation data for stearic acid derivatives specifically labelled with ^{19}F nuclei in lecithin vesicles[1], and with the results of similar spin label experiments of Hubbell and McConnell[2].

Dipalmitoyl lecithin was obtained from Koch Light, and human erythrocyte membranes were prepared as described previously[3]. Lecithin (230 mM) was sonicated in D_2O buffer containing 45 mM NaCl, 30 mM Na acetate and 5 mM Na phosphates (pH 7.4) in glass vials under nitrogen at 50° C until translucent, and transferred into 12 mm NMR tubes under nitrogen. The ^{13}C NMR spectra were obtained by the Fourier transform technique[4] on a Varian XL 100–15 spectrometer at 25.1 MHz locked onto deuterium in the solvent (D_2O or $CDCl_3$), and accumulated in a Varian 620i computer. Chemical shifts were corrected to a dioxan internal reference. A pulse method similar to that of Vold *et al.*[5] (π–t–$\pi/2$ pulse sequence) and modified by Freeman and Hill (private communication) was used to measure T_1 relaxation times, where t is the delay in seconds between the π and $\pi/2$ pulses. The T_1 measurements were made on lecithin in D_2O buffer thoroughly deoxygenated with nitrogen, and $CDCl_3$ degassed by repeated freeze–pump–thaw cycles. Proton NMR spectra were obtained on a Varian HA100–15 spectrometer.

The ^{13}C spectra of dipalmitoyl lecithin dissolved in $CDCl_3$ and a fully sonicated sample in D_2O buffer are shown in Fig. 1 *a*, *b*. The alkyl chain carbons were assigned by analogy with the published spectra of fatty acids[6], and the other resonances by comparison with the spectra of the model compounds choline, choline glycerol phosphate, and phosphatidic acid. All the resonances in both systems are relatively sharp and even the signals from the alkyl chain carbons remain well resolved in the vesicle bilayers. This enabled T_1 measurements to be made on most of the observable nuclei in the molecule and the results are shown in Table 1; values shorter than 0.1 s were inaccessible in the present experiments. In both systems T_1 increases along the alkyl chains towards the terminal methyl

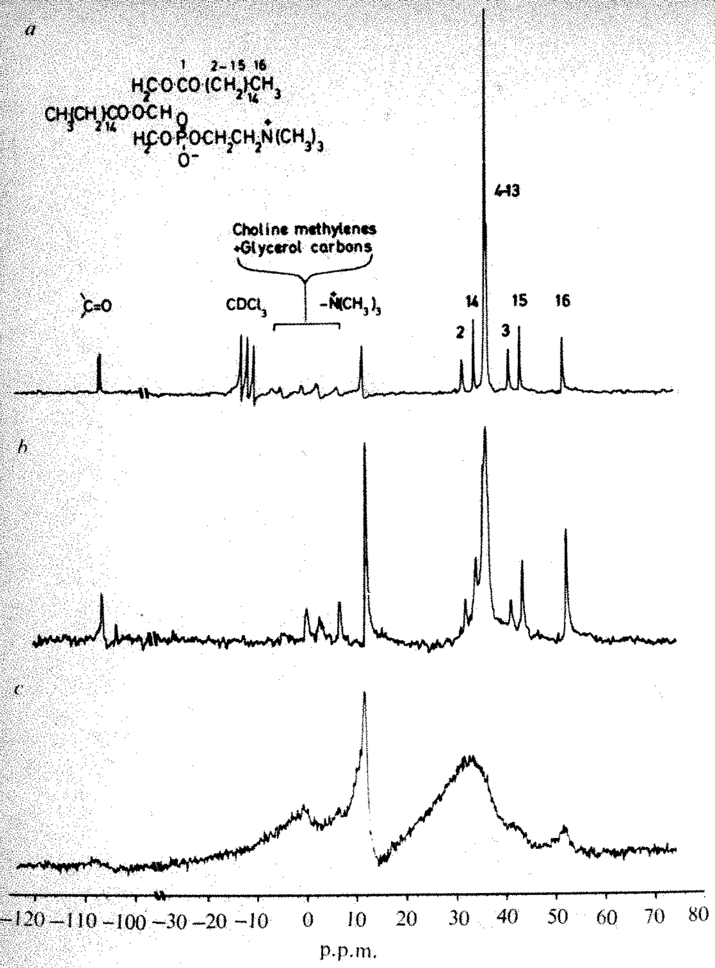


Fig. 1 The ^{13}C NMR spectra of dipalmitoyl lecithin. *a*, 290 mM lecithin in $CDCl_3$ at 28° C. Shifts are corrected to an internal dioxan reference of zero p.p.m. *b*, Fully sonicated and *c*, unsonicated lecithin (230 mM) in D_2O buffer at 52° C. (The unsonicated sample also contained 26 mM palmitic acid to prevent coagulation of the suspension; the palmitic acid did not affect the spectrum of the same sample in the sonicated state.)

group although there are significant differences in the gradation of the changes. A striking difference between the two systems is the very short T_1 value for the $\overset{+}{N}-(CH_3)_3$ carbon nuclei relative to the $(CH_2)_n$ resonance envelope in $CDCl_3$. The spectra after delays of 0.1 and 1.0 s between the π and $\pi/2$ pulses show this difference clearly in Fig. 2, in which the $-\overset{+}{N}(CH_3)_3$ resonance has almost disappeared in $CDCl_3$ after 0.1 s but is still relatively large compared with the $(CH_2)_n$ resonance in the D_2O buffer. We suggest that the short T_1 for the $-N(CH_3)_3$ nuclei in $CDCl_3$ may be due to the formation of a micellar structure with the polar headgroups restricted in motion by being at the centre of the micelle with the alkyl chains exposed to the $CDCl_3$ solvent. In contrast, in the bilayer structure formed in water where the headgroups are exposed to the D_2O solvent, we expect relatively free motion of the $-N(CH_3)_3$ group and a longer T_1 value. In both systems we attribute the changes in T_1 along the alkyl chain to increasing chain motion towards the terminal methyl group. By appropriate substitution in the alkyl chain of stearic acid, for example, with a single fluorine atom, or by the introduction of a double bond, at least twelve of the eighteen carbon nuclei can be resolved. Some of these extra resonances are resolved in egg lecithin containing unsaturated chains. Relaxation measurements on synthetic lecithins incorporating substituted and unsaturated fatty acids will be reported elsewhere.

Fig. 1*c* shows the ^{13}C spectrum of unsonicated lecithin in which both the $-N(CH_3)_3$ and terminal methyl resonances remain relatively narrow in contrast to the main methylene envelope which is broadened approximately eight-fold. In

Table 1 T_1 Relaxation Times of Carbon Nuclei in Dipalmitoyl Lecithin in D_2O and $CDCl_3$

Carbon	T_1 (s) D_2O (52° C)	T_1 (s) $CDCl_3$ (28° C)
2	0.10	0.19
3	0.52	0.28
4–13	0.55	1.04
14	1.14	2.20
15	1.76	2.65
16	3.34	2.88
$-\overset{+}{N}(CH_3)_3$	0.57	0.19

Palmitic acid carbons are numbered from the carboxyl group (1) to the terminal methyl (16).

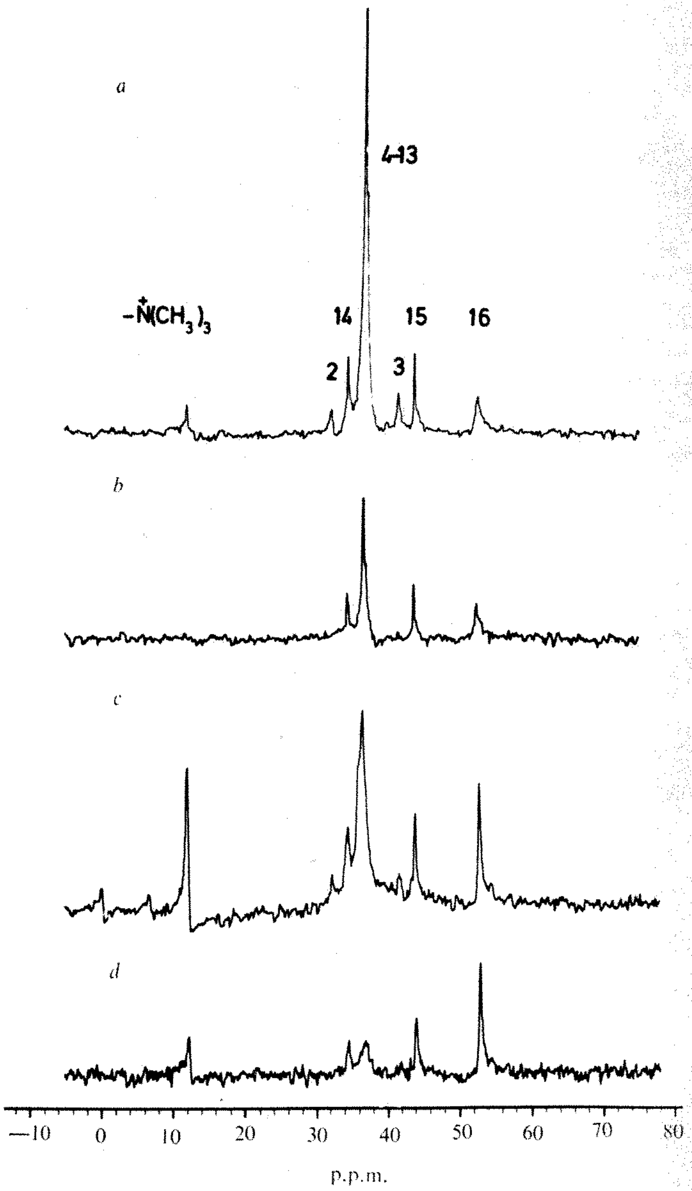


Fig. 2 The ^{13}C NMR spectra of dipalmitoyl lecithin with a pulse sequence $(\pi - t - \pi/2 - t_1 - \pi/2)$ where t is the delay between π and $\pi/2$ pulses and t_1 is a long delay (~20 s): the observed signal is the difference between the signals resulting from the $\pi/2$ pulses. With this pulse sequence, the shorter the T_1 value for a nucleus, the faster the decrease in amplitude of its resonance as t increases. 290 mM lecithin in $CDCl_3$ at 28° C: *a*, $t=0.1$ s; *b*, $t=1.0$ s. 230 mM lecithin in D_2O buffer at 52° C: *c*, $t=0.1$ s; *d*, $t=1.0$ s. Note the very short T_1 of the $-\overset{+}{N}(CH_3)_3$ resonance relative to the $(CH_2)_n$ resonance in $CDCl_3$, compared with the similar T_1 values of these two resonances in D_2O buffer (see also Table 1).

spite of this, the methylene carbons at the terminal methyl end of the chains can be detected superimposed on the main envelope. Preliminary experiments suggest that the main envelope is broadened mainly by chemical shift differences rather than by any major differences in T_1 values for the same carbon nuclei in the sonicated and unsonicated states. No high resolution proton NMR spectrum could be obtained from the same unsonicated sample.

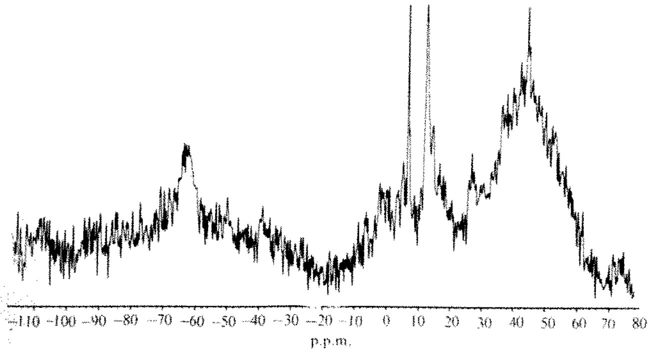


Fig. 3 The ^{13}C NMR spectrum of human erythrocyte membranes (10% w/w) in D_2O buffer at 28° C. The relatively sharp resonance at higher field (13 p.p.m.) is tentatively assigned to the $-\overset{+}{N}(CH_3)_3$ carbons of lecithin in the membrane; the sharp resonance at lower field (~7 p.p.m.) is possibly due to Trisious bound during the preparation of the membranes.

The observation of a spectrum from unsonicated lecithin suggested that biological membranes might yield ^{13}C spectra without sonication. The spectrum of 10% erythrocyte membranes in D_2O buffer at 28° C showed several broad envelopes of resonances, although the membrane suspension was extremely glutinous (Fig. 3). We will determine whether the broad envelopes of resonances result from many chemically shifted signals rather than from very short relaxation times, by selective ^{13}C enrichment of lipids biosynthetically incorporated into the membrane. If sharp resonances can be obtained from identified membrane components then ^{13}C NMR will provide detailed structural information about the membrane.

N. J. M. B. is seconded from Roche Products, Welwyn Garden City, Hertfordshire, A. G. L. holds Salters' Company and Kings College research fellowships, and Y. K. L. is a Beit Memorial Fellow.

J. C. METCALFE
N. J. M. BIRDSALL
J. FEENEY
A. G. LEE
Y. K. LEVINE
P. PARTINGTON

MRC Molecular Pharmacology Unit,
Medical School,
Hills Road,
Cambridge, CB2 2QD

Received May 25, 1971.

[1] Birdsall, N. J. M., Lee, A. G., Levine, Y. K., and Metcalfe, J. C., *Biochim. Biophys. Acta*, **241**, 693 (1971).
[2] Hubbell, W. L., and McConnell, H. M., *J. Amer. Chem. Soc.*, **93**, 314 (1971).
[3] Metcalfe, J. C., Seeman, P., and Burgen, A. S. V., *Mol. Pharmacol.*, **4**, 87 (1968).
[4] Ernst, R. R., and Anderson, W. A., *Rev. Sci. Instrum.*, **37**, 93 (1966).
[5] Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E., *J. Chem. Phys.*, **48**, 3831 (1968).
[6] Lippmaa, E., and Pehk, T., *Kem. Teollisuus*, **24**, 1001 (1967).

Release of Calcitonin from Thyroid Granules

THYROID parafollicular cells contain calcitonin in a granular form. Reduction in numbers of granules and vesicles occurs during induced calcitonin secretion *in vivo*[1], and from thyroid slices[2]. Particulate fractions with the biological activity of calcitonin have been derived from homogenates of pig, calf and rat thyroid glands[3,4]. We have prepared calcitonin containing particulate fractions from pig thyroid and have studied the properties of the isolated granules.

Sub-cellular fractions were prepared from pig thyroid glands by a modification of the method of Bauer and Teitelbaum[4]. Fresh glands were homogenized in 0.25 M sucrose with no additives. The effluent from the celite column was recentrifuged at 100,000*g* for 1 h, omitting the sucrose density gradient centrifugation, and was resuspended in barbital buffered saline (*p*H 7.2) containing 5 mg/ml. of bovine serum albumin. Calcitonin concentration was measured by radioimmunoassay. Tracer was prepared from synthetic porcine calcitonin and it was incubated with a guinea-pig antiporcine calcitonin serum prepared in this laboratory, at a final concentration of 1 : 15,000. The assay detects 50 pg of calcitonin in the 0.5 ml. incubation volume. Recoveries of calcitonin added to the granule fractions were 94–102%. Inhibition of binding by the fractions was parallel to the inhibition by standard, and there was close correlation between bioassay and immunoassay. The sub-cellular fractions were examined morphologically and biochemically. Granule fractions were comprised of dense granules and vesicles 50–200 nm diameter, some endoplasmic reticulum, ribosomes and occasional mitochondria. Beta glucuronidase[5] and malic dehydrogenase[6] concentrations relative to calcitonin were respectively 4.2 and 8.1 in the homogenate and 6.5 and 1.5 in the granule fractions.

Supernatant was separated from particulate calcitonin by centrifuging 1 ml. samples of the granule fraction at 0.8×10^6 g min. Greater centrifugal forces produced no further decrease in supernatant calcitonin concentration. Total and supernatant calcitonin were assayed in duplicate at two dilutions, and the fraction in particulate and supernatant pools was calculated.

Calcitonin was released into the supernatant with sonication, repeated freezing/thawing and with alterations of *p*H to 4.2 and 8.6 (Table 1). Filtration of granule suspensions through a 1.2 µm sartorius membrane filter did not remove particulate calcitonin. No spontaneous release of calcitonin into the supernatant occurred over 24 h at 0° C and 22° C, but at 37° C there was some release. This was not modified by the inclusion of 50 mM potassium chloride, 5 mM magnesium chloride, 5 mM calcium chloride or dibutyrylcyclic 3′5′ AMP in the incubates (Table 2).

Table 1 Calcitonin (%) in Supernatant after Various Stresses

Stress	Before	After
Sonication	33	85
Sonication	28	78
Freezing/thawing	19	48
Freezing/thawing	46	71
*p*H 3.5	46	69
*p*H 8.5	22	70

These results are consistent with the report that sonication increases calcitonin biological activity in particulate fractions of pig thyroid[4]. Freezing/thawing releases catecholamines from nerve storage granules[7], alteration of *p*H releases catecholamines from adrenal[8] and nerve granules[7], and granules containing catecholamine are heat labile at physiological temperatures[7,8]. Calcium and dibutyrylcyclic 3′5′ AMP which release porcine calcitonin *in vivo*[9,10] and from thyroid

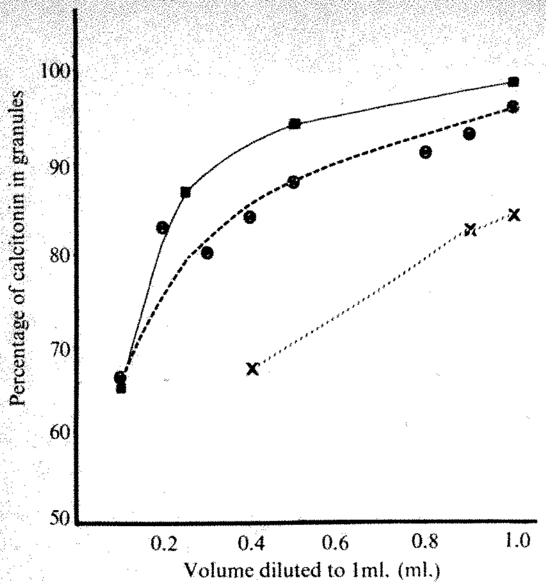


Fig. 1 Increasing volumes of granule suspension in buffer (0.025 M barbital, *p*H 7.2, 0.1 M saline, 5 mg % bovine serum albumin) were diluted in this buffer and incubated for 15 min at 4° C before separation of sedimentable from supernatant calcitonin. The percentage of calcitonin in the sedimentable fraction is shown as a function of dilution in three separate experiments.

slices[11] did not affect release from isolated granules; calcium has been shown to induce release of catecholamines from adrenal medullary granules in some electrolyte media[12].

As the granule suspensions were diluted in isotonic buffer release of calcitonin occurred (Fig. 1). For a ten-fold range of dilution, the percentage of particle-bound calcitonin decreased from 90–95% to 20–30%. This release was independent of bovine serum albumin concentration in the buffer over the range of 0.5–10 mg/ml.

Release of hormone on dilution in isotonic buffer occurs from histamine containing granules of mast cells[13] and adrenal chromaffin granules in the presence of calcium[14].

Table 2 Calcitonin (%) in Supernatant in Buffer with and without Addition of Ions and Dibutyrylcyclic 3′5′ AMP

	0	1 h
Control	22	41
Calcium 5 mM	22	28
Magnesium 5 mM	29	42
Control	48	62
Calcium 5 mM Potassium 50 mM	38	46
Calcium 5 mM Magnesium 5 mM	41	50
Calcium 5 mM Magnesium 5 mM Potassium 50 mM	36	64
Control	20	50
Dibutyrylcyclic 3′ 5′ AMP 0.2 mM	19	31
Dibutyrylcyclic 3′ 5′ AMP 0.2 mM Calcium 5 mM Magnesium 5 mM	—	46

Medium consisted of 0.025 M barbital, *p*H 7.2, 0.1 M saline, 5 mg % bovine serum albumin.

Release of calcitonin on dilution in isotonic buffer is consistent with the intra-cellular release of hormone from storage granules that von Euler has proposed for chromaffin granules[15]. Calcitonin release may be mediated at the cell membrane and intra-cellular stores repleted from the storage granules as the intracellular concentration falls.

This work was supported by the National Health and Medical Research Council. We thank Dr R. Neher (Ciba) for synthetic porcine calcitonin, and Y. Euhara for the electron microscopy.

P. B. Greenberg
T. J. Martin
R. A. Melick

University of Melbourne,
Department of Medicine,
Royal Melbourne and Austin Hospitals,
Victoria

Received April 2, 1971.

[1] Matsuzawa, T., and Kurosumi, K., *Nature*, **213**, 927 (1967).
[2] Bussolati, G., Monga, G., Navone, R., and Gasparri, G., in *Calcitonin*, 240 (Heinemann, London, 1970).
[3] Cooper, C. W., and Tashjian, A. H., *Endocrinology*, **79**, 819 (1966).
[4] Bauer, W. C., and Teitelbaum, S. L., *Lab. Invest.*, **15**, 323 (1966).
[5] Fishman, W. H., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. U.), 869 (Academic Press, New York, 1963).
[6] Bergmeyer, H. U., and Bernt, E., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. U.), 757 (Academic Press, New York, 1963).
[7] von Euler, U. S., and Lishajko, F., *Acta Physiol. Scand.*, **51**, 193 (1961).
[8] Hillarp, N. A., *Acta Physiol. Scand.*, **43**, 292 (1958).
[9] Care, A. D., Cooper, C. W., Duncan, T., and Orimo, H., *Endocrinology*, **83**, 161 (1968).
[10] Care, A. D., Bates, R. F. L., Gitelman, H. J., *J. Endocrinol.*, **48**, 1 (1970).
[11] Bell, N. H., *J. Clin. Invest.*, **49**, 1368 (1970).
[12] Greenberg, R., and Sabelli, H. C., *Proc. Soc. Exp. Biol. Med.*, **121**, 1179 (1966).
[13] Thon, I. L., and Uvnas, B., *Acta Physiol. Scand.*, **67**, 455 (1966).
[14] Lishajko, F., *Acta Physiol. Scand.*, **79**, 575 (1970).
[15] von Euler, U. S., in *Mechanisms of Release of Biogenic Amines* (edit. by von Euler, U. S., Rosell, R., and Uvnas, B.), 211 (Pergamon, London, 1966).

Significance of Surface Potential Measurements on Lipid Monolayers during Action of Phospholipase A on Lecithins

STUDIES on the action of enzymes on lipids and proteins of model membranes are aimed at identifying the relative position and orientation of the hydrophilic groups of the various membrane constituents[1,2] so that models of the molecular organization and surface topography of membrane structures can be built.

Following discovery[3] of the action of phospholipase A on films made of egg lecithin, Colacicco and Rapport described the phospholipase A action of two snake venoms on monolayers of two lecithins: phosphatidyl choline from egg and from beef heart[4]. The disappearance of lecithin and the appearance of lysolecithin in the film resulting from the action of phospholipase A are revealed by a decrease of surface potential: at neutral *p*H, the presence of fatty acid has little effect on the magnitude of the surface potential of lysolecithin[4]. It has been assumed[1-4] that the fall in surface potential is proportional to the number of lecithin molecules transformed into lysolecithin, provided that the reaction products remain in the film. This is supported by two observations[4,5]: first, thin-layer chromatography of film material showed that most of the lysolecithin was in the film; second, in conditions in which low protein concentrations (0.1 μg/ml. *Naja naja* venom) would not interfere with surface pressure and surface potential measurements, loss of reaction products would be accompanied by a considerable decrease in film pressure, which, however, was not seen[4,5]. In contrast, when *Naja naja* venom (3.6 μg/ml.) was injected under high pressure films of ^{32}P-labelled yeast lecithin, surface radioactivity was lost rapidly and more than 80% lysolecithin was found in the subphase, and Dawson argued[6] that the reaction products left the film. This has thrown doubt on the assumption of Colacicco and Rapport[7].

The conflict is traceable to basic differences in experimental conditions. Colacicco and Rapport used egg lecithin, the lysolecithin in which remains in the film at low pressure[4,5]. Dawson used yeast lecithin[6], the lysolecithin in which leaves the film readily at high pressures (near 30 dynes/cm). Some of the differences are examined here, and the surface potential measurement is shown to be an accurate method for determining the kinetics of the phospholipase A action on lecithin monolayers.

Yeast lecithin was obtained from Dr M. M. Rapport[8]. Dipalmitoyl lecithin and dimyristoyl lecithin were purchased respectively from Mann Research Laboratories, New York, and LaMotte Chemical Laboratories, Chestertown, Maryland. Preparations of egg lecithin and hydrogenated egg lecithin have already been described[2]. Before use, lipids were purified by column chromatography on 'Unisil', from which the lecithins were eluted with chloroform–methanol mixtures. Fractions which were homogenous by thin-layer chromatography and gave a satisfactory analysis for phosphorus and esters were used[9]. Chain length and unsaturation of the egg lecithin and yeast lecithin used are known[10]. The technique for bringing lecithin and enzyme into contact has already been described; the rectangular trough was used[4]. The lecithin monolayer was spread at zero pressure on 0.04 M phosphate buffer, containing 58 μM CaCl$_2$ (*p*H 7.0) 25° C, and 0.2 μg/ml. *Naja naja* venom. The film was compressed rapidly (10 s) to the desired film pressure, and the changes in film pressure and surface potential were monitored as a function of time. Details of these and other techniques have been described[1,2,4]. Surface pressure was determined by the Wilhelmy method; surface potential was measured with the use of a radioactive (^{226}Ra) air electrode[4]. Unless it is otherwise specified, the rate of fall of surface potential is identified with the rate of hydrolysis of lecithin. The identity was established by the concomitant measurement of the relative quantities of substrate and product in collected lipid films[4].

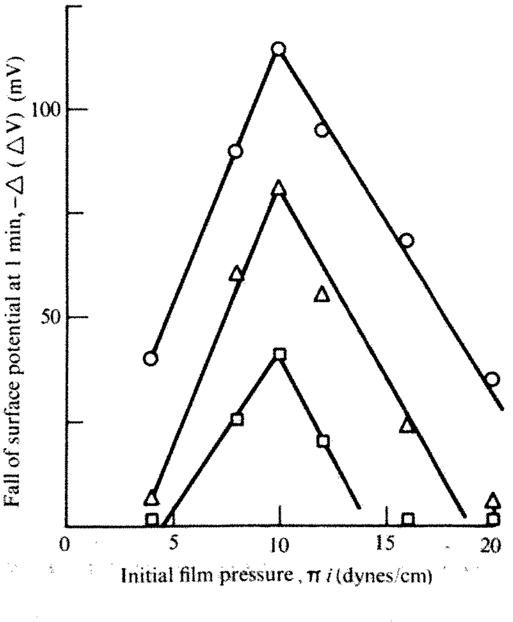


Fig. 1 Influence of initial pressure of lecithin film on decrease of surface potential, $-\Delta(\Delta V) \equiv$ hydrolysis, caused by action of phospholipase A in first minute. ○, Yeast lecithin; △, dimyristoyl lecithin; □, dipalmitoyl lecithin. Egg lecithin and hydrogenated egg lecithin are not shown: the curve of the first, with an optimum of 12 dynes/cm, was between the curves of yeast and dimyristoyl lecithins; the curve for the second was below that of dipalmitoyl lecithin. Temperature, 25° C.

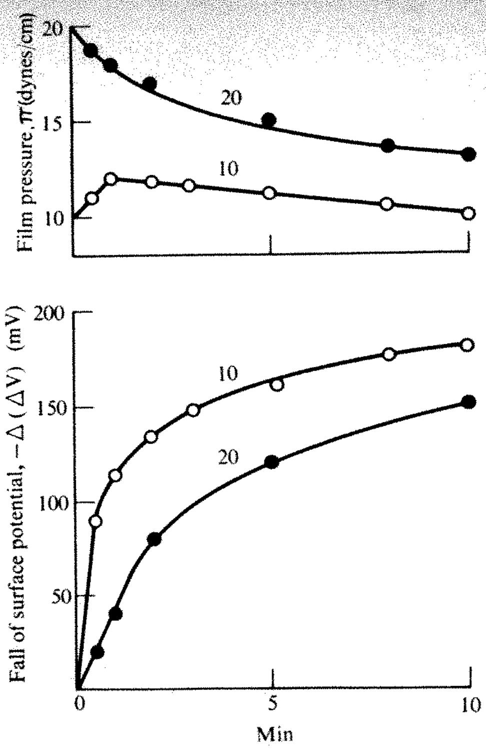


Fig. 2 Pressure-time (upper) and Δ(ΔV)-time curves during the action of *Naja naja* venom on monolayers of yeast lecithin at two initial film pressures: 10 dynes/cm and 20 dynes/cm. The temperature was 25° C.

Decrease of surface potential, $-\Delta(\Delta V)$, in the first minute is related to the initial pressure of the lipid film (Fig. 1). The data show unequivocally, first, that all three lecithins—yeast, dimyristoyl and dipalmitoyl—displayed an optimal reaction at a film pressure of 10 dynes/cm; second, that with a given chain length (C$_{16}$), phospholipase A action is favoured by unsaturation (hydrolysis of monounsaturated yeast lecithin, which consists predominantly of dipalmitoleyl lecithin[10], is markedly faster than that of fully saturated dipalmitoyl lecithin); and third, that of two saturated lecithins the one with shorter chains (dimyristoyl) is attacked more rapidly than the one with longer chain (dipalmitoyl).

The question of the conditions in which lysolecithin leaves the film is illustrated in Fig. 2 by the kinetic curves of the lipase action on monolayers of yeast lecithin. At the optimal film pressure of 10 dynes/cm, most of the lysolecithin remains in the film in the first 10 min as indicated by the little change in film pressure. Thin layer chromatography of films collected on a platinum guaze[4] confirmed that 95% of lecithin was hydrolysed and more than 90% of it was in the film. At moderate film pressures, therefore, yeast lysolecithin does not leave the interface. But when the monolayer of yeast lecithin had an initial pressure of 20 dynes/cm, loss of film pressure and escape of lysolecithin from the film are so rapid that measurements of surface potentials past 2 or 3 min have to be regarded only as qualitative. Thin-layer chromatography of materials in the film and in the aqueous phase indicated that after 10 min only 70% of lecithin was hydrolysed and about 60% of it was in the hypophase. The rapid loss of pressure following the action of the venom on high pressure films of unsaturated lecithin is consistent with the pressure-time curves reported by Shah and Schulman in the action of *Naja naja* venom (0.05 μg/ml.) on monolayers of highly unsaturated soybean lecithin[5].

The ejection of lysolecithin from the film is far from quantitative, so that the measurement of surface radioactivity loss is inadequate for the description of enzyme kinetics; it is restricted to the high pressure films and highly unsaturated and very short chain lecithins (Fig. 2 and ref. 5). We therefore conclude that surface potential is the only method which

Pure Chemistry Library

reveals instantaneously the transformation of lecithin into lysolecithin. Even in conditions in which lysolecithin is ejected from the film, the initial rate of fall of surface potential (for example in the first minute) truly reflects the enzyme kinetics. Concurrent determination of the reaction products either by surface radioactivity measurements and better by thin-layer chromatography and/or scintillation counting of aliquots from the hypophase[2] really establishes the validity of surface potential measurements. In contrast, measurement of surface radioactivity at low film pressure is useless if lysolecithin does not leave the film, and so also at high pressure in the known cases in which ejection of lysolecithin is not quantitative[4-6].

Systematic studies can shed some light on the structural requirements of the lipid for hydrolysis of film lecithin by phospholipase A (as in Fig. 1) and ejection of lysolecithins, and in turn help to define the film pressure of biological membranes. Because the criteria used are not rigorous, the arguments in favour of low film pressure[11], for example 10 dymes/cm are as valid or liable as the arguments in favour of high film pressure, for example 30 dynes/cm or more[12]. For given unsaturation and chain length of the membrane lecithins, ejection of lysolecithin after action of phospholipase A could become a criterion for establishing the film pressure of biological membranes.

This work was supported by the American Cancer Society and US National Institutes of Health Research.

GIUSEPPE COLACICCO *

Department of Biochemistry,
Albert Einstein College of Medicine,
Bronx,
New York 10461

Received January 20; revised April 19, 1971.

* Present address: Department of Pediatrics, Albert Einstein College of Medicine, Bronx, New York 10461.

[1] Colacicco, G., *J. Coll. Interface Sci.*, **29**, 345 (1969).
[2] Colacicco, G., *Lipids*, **5**, 636 (1970).
[3] Hughes, A., *Biochem. J.*, **29**, 437 (1935).
[4] Colacicco, G., and Rapport, M. M., *J. Lipid Res.*, **7**, 258 (1966).
[5] Shah, D. O., and Schulman, J. H., *J. Coll. Interface Sci.*, **25**, 107 (1967).
[6] Dawson, R. M. C., *Biochem. J.*, **98**, 35C (1966).
[7] Pethica, B., in *Structural and Functional Aspects of Lipoproteins in Living Systems* (edit. by Tria, E., and Scanu, A. M.), 37 (Academic Press, New York, 1969).
[8] Gottfried, E., and Rapport, M. M., *J. Biol. Chem.*, **237**, 329 (1966).
[9] Colacicco, G., and Rapport, M. M., *J. Lipid Res.*, **8**, 513 (1967).
[10] Shah, D. O., and Schulman, J. H., *J. Lipid Res.*, **6**, 341 (1965).
[11] Bar, R. S., Deamer, D. W., and Cornwell, D. G., *Science*, **153**, 1010 (1966).
[12] Jackson, E. M., in *Permeability and Function of Biological Membranes* (edit. by Bolis, L., Katchalsky, A., Keynes, R. D., Loewenstein, W. R., and Pethica, B. A.), 164 (North-Holland, Amsterdam, 1970).

Perceptual Fading of a Stabilized Cortical Image

AN image that is physically fixed on the retina rapidly seems to fade[1], and if the stabilization is good enough it never reappears[2,3]. We have some evidence that this process of fading is not entirely retinal, but is partly central, for we have noticed that an image free to move over the retina, but "stabilized" on the visual cortex, also becomes less distinct.

Neurones of the visual cortex in cat and monkey respond best to line-shaped targets, black or white bars, of a particular orientation and often of a certain width[4,5]. Many of them have quite large receptive fields on the retina and respond to the same sort of image anywhere within them.

There is psychophysical evidence that human visual systems also have this sort of organization, and that the neurones are central[6-10]. So presumably if we look in the middle of a pattern of black and white stripes (a grating), it is "stabilized" in our visual cortex. Within limits, wherever we move our gaze over the pattern, the same set of cortical neurones, specific for the orientation and width of the stripes, should be constantly stimulated. If our cortical cells show the same sort of attenuation of response that many sensory neurones suffer, then the activity aroused by such a pattern should gradually wane.

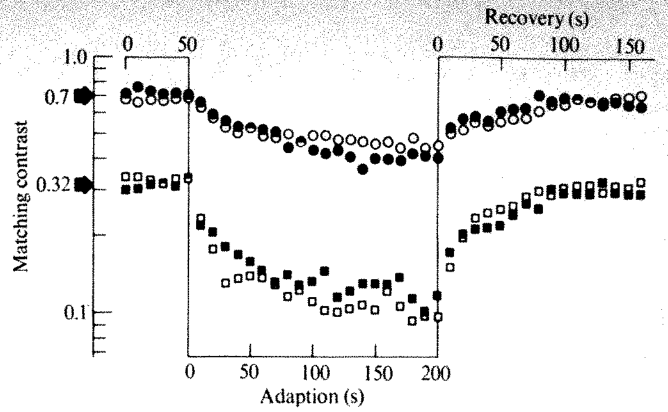


Fig. 1 Every 10 s the subject matched the contrast of the lower grating, shown on the ordinate, to that of the upper. Each symbol, open for one subject, solid for the other, is the mean of four settings made in separate experimental sessions. The average standard error of these means is only about 0.02 log units. For the circles the test grating on the upper screen had a contrast of 0.7, the same as that of the adapting pattern. For the squares the test grating had a contrast of 0.32. Contrast= (I max−I min)/(I max+I min) where I max I min are the maximum and minimum luminances in the grating. ● ■, J. M.; ○ □, C. B.

We have found that as one looks at a grating its apparent contrast declines and the pattern becomes less and less distinct. We arranged two oscilloscope screens, one above the other, each one subtending 4° in width and 3° in height. The gap between them was 24′ and in the middle of it there was a luminous horizontal fixation bar. We generated on the screens vertical dark and light stripes, with sinusoidal luminance profile[11], and the experimenter could alter the spatial frequency (number of cycles of the grating per degree of visual angle) and contrast of the two patterns independently, and turn them on and off without changing their mean luminance, which was 4.8 cd m^{-2} for both screens.

Throughout the experiment the observer held his gaze on the fixation bar and his only task was to turn a logarithmic potentiometer to match the contrast of the pattern on the lower screen to that on the upper one. He pressed a button to print out his contrast setting. Every 10 s a test pattern of 5 cycles/degree appeared and the subject adjusted the contrast of the lower grating to match the upper one as quickly as possible. In between these settings the gratings on both screens were turned off. After the sixth such setting, the lower pattern disappeared but the upper one was replaced by an adapting grating of 5 cycles/degree at a contrast of 0.7. So now the subject was adapting the upper part of his visual field to the grating, while the lower part remained merely light adapted.

Every 10 s the original test grating appeared above and a comparison pattern below. The subject matched the contrasts quickly and the adapting situation was restored for a further 10 s before the next setting. After twenty such settings, with constant adaptation between each, the upper pattern was also extinguished between readings as we followed the time course of recovery.

The open and solid symbols in Fig. 1 show the results for two subjects. The contrast setting on the lower screen is plotted on the ordinate. For the circles, the test grating was identical in contrast (0.7) to the adapting pattern; for the squares the test grating had a contrast of only 0.32. In both

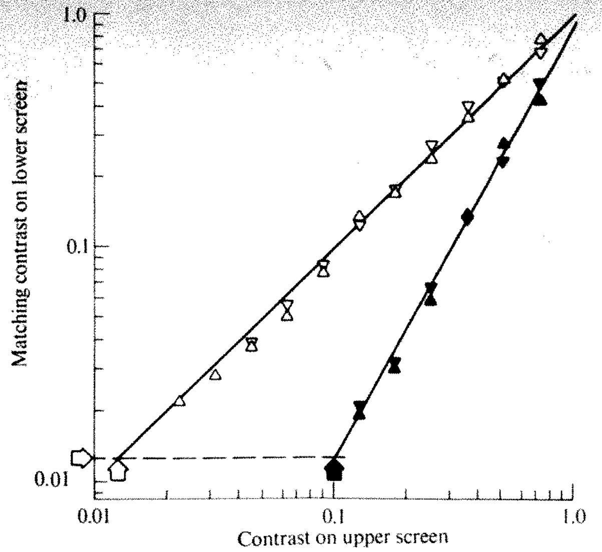


Fig. 2 The subject adapted constantly to a grating of 5 c/degree at a contrast of 0.7 on the upper screen. Every 10 s it was replaced by a test grating the contrast of which was varied from trial to trial. Each solid symbol is the mean of six settings of the contrast on the lower screen. A regression line (method of least squares) is fitted through them. The open symbols show the results for an identical series of contrast matches but with no prior adapting pattern on either screen. These points fit the expected line with a slope of +1. The open arrows indicate the threshold contrast before adapting ($N=6$; subject C. B.) and the solid arrow shows the elevated threshold on the upper screen after adaptation ($N=6$; C. B.). ▽ ▼, J. M.; △ ▲, C. B.

cases there is a clear reduction in apparent contrast, with a time constant of about 30 s, but it is much greater for the lower contrast test grating.

Having seen that adapting to a high contrast grating can cause an apparent reduction in contrast that varies with the contrast of the test pattern, we performed the following experiment. The subject made a series of contrast matches for test gratings of 5 cycles/degree at various contrast levels: in between each setting both screens went blank. In Fig. 2 these unadapted settings are plotted as open symbols for the two subjects. The abscissa and ordinate are the contrasts on the upper and lower screens respectively. Not surprisingly, the open points lie near the expected function with a slope of +1.

Now the subject adapted constantly to a 5 cycles/degree grating at a contrast of 0.7 on the upper screen for 3 min, to allow the adaptation to build up to a maximum (Fig. 1). Then, as before, a test grating appeared on the upper screen with a comparison grating on the lower one and a match was made. At least 10 s of re-adaptation to the 0.7 contrast grating on the upper screen were allowed before the next pair appeared.

The contrast of the test grating was varied from trial to trial and the results are plotted as solid symbols in Fig. 2. Obviously, adapting to a contrast of 0.7 causes a reduction in apparent contrast at all test contrasts, but the magnitude of the effect is greatest at low contrast levels. Open arrows on the abscissa and ordinate mark the threshold contrast level for the stripes to be just distinguishable, before adaptation, and the solid arrow shows the new threshold contrast on the upper screen measured after adaptation. As Blakemore and Campbell described, the threshold is grossly elevated by prior adaptation[10]. Indeed elevation of threshold can be thought of as a special case of reduction in apparent contrast.

Fig. 2 shows an interesting point. Although at every contrast level the apparent contrast is reduced during adaptation, the slope of the function is steeper than +1. So the amplification or gain of the visual system for changes in contrast is actually increased by adaptation, although the absolute signal is smaller at all contrast levels. This increase in gain seems to be the first potentially beneficial consequence of spatial adaptation that anyone has reported. If one looks at a grating, or presumably any other contour-containing pattern, one becomes more sensitive to changes in its contrast, like those effectively produced by fast eye movements, for example.

Finally, we have been able to use this new technique to measure the selectivity of human visual mechanisms for orientation and spatial frequency, at suprathreshold levels. If the adapting grating is replaced, not by a grating of identical orientation and spatial frequency, but by one that is markedly different, then the apparent contrast of the test grating is not reduced. This implies that the adapting and test gratings are handled by different neural channels. We shall be able to define the specificity of these channels and compare them with those revealed by threshold elevation[8-10].

This work was supported by a grant to C. B. from the Medical Research Council.

COLIN BLAKEMORE

The Physiological Laboratory and

JAMES P. J. MUNCEY
ROSALIND M. RIDLEY

The Psychological Laboratory,
University of Cambridge

Received March 9; revised May 5, 1971.

[1] Riggs, L. A., Ratliff, F., Cornsweet, J. C., and Cornsweet, T. N., *J. Opt. Soc. Amer.*, **43**, 495 (1953).
[2] Barlow, H. B., *Quart. J. Exp. Psychol.*, **15**, 36 (1963).
[3] Campbell, F. W., and Robson, J. G., *J. Physiol.*, **158**, 11P (1961).
[4] Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **160**, 106 (1962).
[5] Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **195**, 215 (1968).
[6] MacKay, D. M., *Nature*, **180**, 849 (1957).
[7] Andrews, D. P., *Nature*, **205**, 1218 (1965).
[8] Gilinsky, A. S., *J. Opt. Soc. Amer.* **58**, 13 (1968).
[9] Pantle, A., and Sekuler, R., *Science*, **162**, 1146 (1968).
[10] Blakemore, C., and Campbell, F. W., *J. Physiol.*, **203**, 237 (1969).
[11] Green, D. G., and Campbell, F. W., *J. Opt. Soc. Amer.*, **55**, 1154 (1965).

Iridescent Corneas in Fishes

DURING the course of underwater studies of fishes in the English Channel, Mediterranean and Indian Ocean, it was noticed that a coloured iridescent layer was often present on the inner surface of the cornea extending over the pupillary area (Fig. 1). Iridescence has been observed in a variety of animal tissues[1,2] and is caused by regular multilayer structures stacked in a medium of different refractive index. Iridescent tissues already known in fish include the tapetum in which the principal material may be guanine[1] or collagen[3] and the silvery iris and scales of teleost fish where the principal material is guanine[4].

Walls[3] noticed that in a few fishes including the gobies and particularly the Plectognaths (which include the Balistidae and Tetraodontidae) the corneal substantia propria exhibits a complex lamination with histologically peculiar intercalated layers. Iridescence is the rule among the groups Walls mentions and the iridescent layer can be seen by examination of the live fish to be situated at the inner surface of the cornea, so that it is reasonable to assume that the layers in the substantia propria are responsible for iridescence. In addition a similarly placed endothelial layer in the strongly iridescent cornea of the serranid fish *Nemanthias carberryi* possesses regular laminations that could well cause iridescence (personal communication from Locket).

Corneal iridescence has received scant, if any, attention in the scientific literature and should not be confused with the iridescent iris found in many fishes[4]. In the past corneal iridescence may have escaped notice since it is normally only

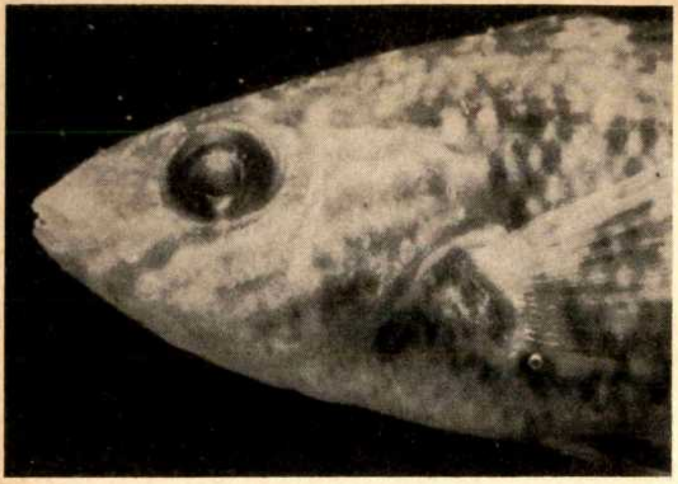


Fig. 1 *Crenilabrus melops* illuminated by electronic flash from above, showing corneal iridescence. The fish is alive, healthy and is in water. The cornea is green in colour, having a maximum reflectance in the region 520–550 nm.

visible when most of the illumination comes from above. This is the case in the natural environment[5] but is less common in aquaria.

Corneal iridescence is common in members of the Tetraodontidae (puffer fishes), Balistidae (trigger fishes), Siganidae (rabbit fish), Scaridae (parrot fish), Gobiidae (gobies) and Labridae (wrasse). It has also been observed in the Cottidae and Serranidae. The colour varies from an unsaturated red in *Epinephelus merra* through yellow–green (*Arothron citrinella*) to a saturated blue–green (*Cottus bubalis*). There is, however, no obvious correlation between the colour of the iridescence and the colour of the water in which the fish lives.

Fishes with iridescent corneas often, but not always, possess yellow filters[6] at some point in the optical pathway. Thus of the fishes with iridescent corneas mentioned here, ***Arothron citrinella*, *Crenilabrus melops*, *Cottus bubalis*** and ***Balistapus undulatus*** have yellow corneas; *Xanothon bipallidus* and *Callyodon viridifucatus* have yellow lenses. No yellow coloration has been observed in the lens or cornea of *Epinephelus merra* or *Siganus stellatus*. The aqueous or vitreous humours were clear except in the case of a yellow vitreous humour in *Siganus rostratus*. The cornea of *Nemanthias carberryi* was clear but the lens was not examined.

The data that follow were obtained from fishes collected by the use of explosives at the Royal Society's Research Station on Aldabra in the Indian Ocean. Immediately after the charge was detonated, three divers entered the water; one to collect fish, one to watch for sharks and one to study iridescence of the collected fishes.

The fish, killed by the explosion one to two minutes previously, was clamped to a plastic writing board with an inclinometer attached at the lower left hand corner. The inclinometer consisted of a glass sphere open at the bottom and marked with lines of latitude and longitude. A metal rod was fastened inside the sphere in such a way that its tip marked the geometric centre of the sphere. Underwater the sphere filled with water; a small air bubble was then introduced and this indicated the vertical. The direction of view was obtained by noting the locus on the surface that aligned with the centre of the sphere. Observations were made as near noon as possible at 15–25 m. depth. In this way the direction of maximum downwelling radiance corresponded with the vertical within the limits of experimental error.

The apparatus was held level with the eye so that the anterior/posterior axis and the axis between the eyes was horizontal. In *Callyodon*, *Xanothon* and *Balistapus* corneal iridescence was visible in this position. In *Nemanthias* the fish had to be slightly below the level of the observer's eye. The apparatus with fish attached was then rotated around the anterior/posterior axis (roll) and then around the axis joining the eyes (pitch). The angles of pitch and roll through which iridescence was visible are listed in Table 1. When iridescence is visible under natural conditions the entire corneal area is affected. When a small artificial light source is used as in Fig. 1, the sector of the cornea shaded from the light does not appear iridescent. The fishes were then viewed from different directions, keeping the anterior/posterior axis and the inter-eye axis horizontal. When viewed from below and somewhat from in front, no iridescence was visible (Fig. 2).

Table 1 The Angles of Pitch and Roll through which Corneal Iridescence is Visible

	Pitch		Roll	
(*A*) *Nemanthias carberryi*	−25°	+25°	−0°	+66°
(*B*) *Balistapus undulatus*	−61°	+54°	−49°	+59°
(*C*) *Xanothon bipallidus*	−51°	+32°	−10°	+68°
(*D*) *Callyodon viridifucatus*	−66°	+44°	−25°	+66°

The angles are measured from the vertical. Negative pitch is when the head is in the up position; negative roll is when the back of the fish is tilted away from the observer.

It is now well established that the silvery iridescent scales and iris of fish act as a camouflage device underwater[4] and it might seem natural to ascribe the same function to corneal iridescence. To the diver, however, corneal iridescence often shows up extremely brightly underwater and is rarely the same colour as the head; for instance, corneal iridescence in *Nemanthias* is blue–green whilst the head and body is golden orange. Furthermore, the cornea is not iridescent in silvery sided pelagic fishes where the black pupil may be the only visible part of a fish swimming in open water[4].

It is too early to speculate on the role of the yellow optical filters and the iridescent cornea on selective spectral absorption of light from different directions, but all the fishes so far known

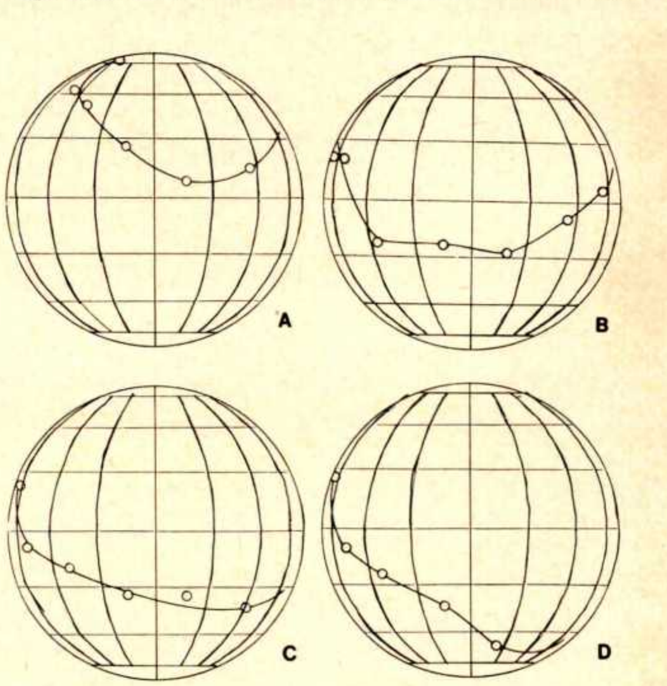


Fig. 2 Direction from which corneal iridescence is visible. The diagram represents the curved surface of a cornea oriented in the same way as in the fish in Fig. 1. The angle of view is indicated by the locus on the sphere's surface which aligns with the centre of the sphere. The line joining the circles indicates the direction of view at which the entire surface of the cornea appears iridescent. Iridescence ceases to be visible when the direction of view is from below the line. *A*, *Nemanthias carberryi; B, Balistapus undulatus* (trigger fish); *C*, *Xanothon bipallidus* (parrot fish); *D*, *Callyodon viridifucatus* (parrot fish).

to possess this iridescence live and feed near the bottom and it may be that the iridescence acts as a sophisticated sunshade designed to reduce by reflexion the bright light arriving from the upper hemisphere, whilst allowing the much less bright light entering from the direction of the sea bed to pass without significant loss.

I thank the staff of the Royal Society Research Station and my co-divers, Mr J. A. Stevenson, Dr W. R. A. Muntz and Mr A. J. Young for their support and help.

J. N. Lythgoe

MRC Vision Unit,
University of Sussex

Received June 4, 1971.

[1] Denton, E. J., and Land, M. F., *J. Physiol. Lond.*, **191**, 23P (1967).
[2] Bernard, G. D., and Miller, W. H., *Invest. Ophthalmol.*, **7**, 416 (1968).
[3] Walls, G. L., *The Vertebrate Eye* (Hafner, New York and London, 1963).
[4] Denton, E. J., and Nicol, J. A. C., *J. Marine Biol. Assoc.*, **46**, 685 (1966).
[5] Jerlov, N. G., *Optical Oceanography* (Elsevier, New York, London, Amsterdam, 1968).
[6] Moreland, J. D., and Lythgoe, J. N., *Vision Res.*, **8**, 1377 (1968).

Titration of Oral Nicotine Intake with Smoking Behaviour in Monkeys

SEVERAL studies have indicated that nicotine is an incentive in smoking. Johnston[1] found that heavy smokers reported a pleasurable sensation when given nicotine, whereas non-smokers reported an unpleasant effect. Lucchesi *et al.*[2] showed that intravenous administration of nicotine diminished the number of cigarettes smoked by heavy smokers. Jarvik *et al.*[3] found that oral nicotine administration produced a very small but significant decrease in the number of cigarettes smoked but because humans were used, they[3] were not able to use large doses of nicotine which might have produced larger decrements in smoking. Previous work in this laboratory with monkeys trained to puff cigarette smoke showed that mecamylamine, a nicotinic-blocking agent, reduced their smoking[4], and the work reported here was conducted to establish more definitely that orally administered nicotine could substitute for nicotine derived from smoking.

Four mature rhesus monkeys were trained to puff cigarette smoke. Only one (Phoebe) had served as a subject in the previous study with mecamylamine. Puffing behaviour was instilled initially by making the monkeys suck on a tube in order to drink. The mouthpiece of a cigarette smoking apparatus[5] was then substituted for the water tube. The smoking apparatus allowed a monkey to smoke lit cigarettes by automatically lighting each cigarette, spacing the cigarettes over time, and sensing changes in pressure as the monkey puffed. When the monkeys had learned to puff, they were so trained that they had to puff but were allowed to choose between smoke and air[6]. This procedure was developed to reduce the large variability from subject to subject inherent in free puffing.

A smoking apparatus was attached to a 'Plexiglas' panel covering a 36 cm square opening in the door of each monkey's home cage. Two tubes, one delivering cigarette smoke and a "dummy" tube with access only to air, were also mounted on the panel. A water solenoid was mounted between the smoke and air tubes. A puff on either the smoke or air tubes preset the solenoid and would release a small amount of water when the monkey touched the solenoid spout. A light mounted above the solenoid signalled the availability of water. A fixed ratio (FR) contingency was added, so that a monkey could be required to puff a specific number of times to obtain water.

The monkeys received all their water during a daily 4 h puffing session. Thirty cigarettes were loaded into the smoking apparatus each morning and the test was started at 1030 h. A new cigarette was automatically lit every 7.5 min. The positions of the smoke and air tubes were interchanged each morning so that side preferences would not develop. Initially, water could be won with a single puff on either the smoke or air tube. The number of puffs for a reward of water was then increased to five, ten, twenty and finally to thirty. The FR 30 schedule was then maintained. Puffs were recorded on Sodeco counters.

All four monkeys preferred smoke to air; that is, although they could get water by puffing on either the smoke or the air tubes, they reliably took more puffs on the smoke tube than on the air tube. When puffing rates on the FR 30 schedule stabilized, the nicotine experiment was begun (except for the monkey used in the previous mecamylamine experiment[4]; the present experiment was started 2 months after completion of the previous experiment).

Various quantities (50 mg to 200 mg) of nicotine tartrate were dissolved in the water which each monkey obtained through puffing. The amount of nicotine on a given day was always dissolved in 700 ml. of water although the monkeys would usually consume only 300–400 ml. during a puffing session. Increasing dosages of nicotine were self-administered in this way with at least 4 days between successive nicotine trials. Each dose was repeated two or three times before starting a higher dose.

The results obtained from such oral nicotine administration are shown in Table 1. The data on days immediately preceding oral nicotine days were used as control data in paired *t* tests. For each monkey, doses of oral nicotine were found which significantly decreased and in some cases reversed the smoke–air preference without significantly affecting the overall puffing rate.

Table 1 Changes in Smoke Preference induced by Oral Nicotine

	Phoebe		Alex		Ivan		Dot	
Dose (mg)	Total puffs	% pref.	Total puffs	% pref.	Total puffs	% pref.	Total puffs	% pref.
Control	6,103	88.7	4,844	86.4	2,786	87.2	4,322	91.1
50	6,509	87.5			2,594	87.0	3,666	88.3
75	6,466	91.0	5,191	90.0	3,465	87.0	4,437	86.0
100	6,440	92.0			3,453	75.0	4,754	81.5
125	5,784	64.1*	4,798	91.9	2,589	41.7*	4,308	14.8*
175			4,973	75.0				
225			4,623	56.7*				

* Significantly less than control at $P<0.05$.

One additional drug trial was conducted with one of the monkeys. Because previous work had shown that mecamylamine inhibited monkeys' smoking, it was of interest to determine whether mecamylamine would antagonize the inhibition of smoking by orally administered nicotine, that is, whether the combination would depress smoking behaviour less than either treatment alone. For this purpose, the monkey used in the previous mecamylamine experiment as well as the more recent oral nicotine experiment was used again. A dose of 125 mg of nicotine tartrate was administered orally as already described. In addition, a dose of 0.8 mg/kg of mecamylamine was given intramuscularly 15 min before the beginning of the same puffing session. This combination was repeated twice. Thereafter, two administrations of the mecamylamine alone were conducted. The results are shown in Table 2. All three treatments—oral nicotine alone, mecamylamine alone and the combination—produced significant inhibition of smoking behaviour. Only mecamylamine alone and the combination significantly decreased overall puffing. Most surprising, however, was the finding that the inhibition of smoking produced by the combination was significantly greater ($P<0.05$) than that produced by either drug treatment alone.

Table 2 Changes in Smoke Preference of Phoebe induced by Oral Nicotine (125 mg) and/or Mecamylamine (0.8 mg/kg)

	Total puff	% pref.
Control	6,055	88.2
Nicotine	5,784	64.1*
Mecamylamine	4,842*	65.5*
Nicotine + mecamylamine	3,358*	33.5*

* Significantly less than control at $P < 0.05$.

These results raise interesting questions about the role of nicotine in smoking. If oral nicotine depressed smoking by providing a greater than optimal level of nicotine, such that additional nicotine derived from smoking was aversive, then mecamylamine should have antagonized rather than potentiated this effect. However, different central actions of nicotine may have been responsible. Perhaps the aversiveness produced by the large oral nicotine doses was a function of nicotinic blockade rather than of activation[7]. Blockage of nicotinic activating, presumably rewarding, actions of nicotine by mecamylamine would then allow non-rewarding blocking actions to predominate. Clinical studies are being conducted in this laboratory to evaluate the therapeutic significance of this hypothesis.

This work was supported by grants from the US National Institute of Mental Health and the American Cancer Society.

S. D. Glick*
B. Zimmerberg
M. E. Jarvik

Department of Pharmacology and Psychiatry,
Albert Einstein College of Medicine,
1300 *Morris Park Avenue,*
Bronx, New York 10461

Received June 21, 1971.

* Present address: Department of Pharmacology, Mount Sinai School of Medicine, New York, NY, 10029.

[1] Johnston, L. M., *Lancet*, ii, 742 (1942).
[2] Lucchesi, B. R., Schuster, C. R., and Emley, G. S., *Clin. Pharmacol. Ther.*, **8**, 789 (1967).
[3] Jarvik, M. E., Glick, S. D., and Nakamura, R. K., *Clin. Pharmacol. Ther.*, **11**, 574 (1970).
[4] Glick, S. D., Jarvik, M. E., and Nakamura, R. K., *Nature*, **227**, 969 (1970).
[5] Pybus, R., Goldfarb, T., and Jarvik, M. E., *Exp. Anal. Behav.*, **12**, 88 (1969).
[6] Glick, S. D., Canfield, J. L., and Jarvik, M. E., *Psychol. Rep.*, **26**, 707 (1970).
[7] Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics*, 585 (Macmillan, London, 1965).

Effects of Cyprenorphine Hydrochloride on Sensory Reinforcement in the Rat

In a previous study[1] of the morphine antagonist cyprenorphine hydrochloride (M 285), we examined the possible hallucinogenic effects of this analgesic compound on the light-reinforced behaviour of rats. Control animals receiving saline injections showed typically increased response rates during test sessions in which response–contingent light (RCL) was available, whereas M 285 (0.3 mg/kg) depressed this light reinforcement (LR) effect. The drug had no effect on operant or non-reinforced responding, and so we postulated that it had its principal effect in relation to the relative significance of the stimulation to the animal. More specifically, this drug (in common with other hallucinogenic agents) may raise the arousal level of an animal that is relatively deprived visually, so that it needs very little further stimulation.

We report now a further test of the hypothesis that M 285 has a direct sensory effect. A response–contingent light offset (RCLO) situation involves sensory reinforcement, just as in the RCL onset situation, but the sensory conditions are reversed. If the visual stimulation is hallucinogenically intensified by injection of M 285, then the drug group should respond more than the saline group in order to turn the light off for a short period.

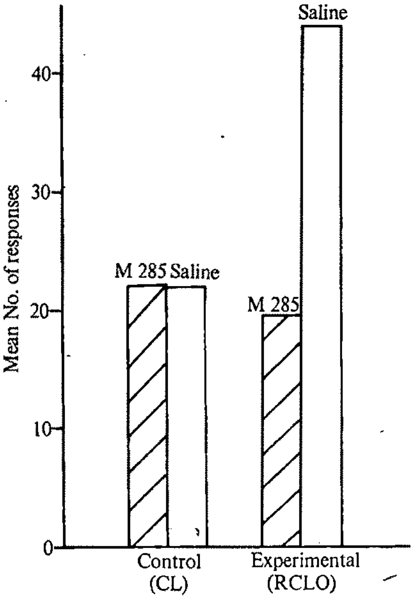


Fig. 1 Effect of M 285 (0.3 mg/kg) on reinforced (RCLO) and non-reinforced (CL) responding in rats. Response rates were recorded from a 60 min test session on day 2. All four groups ($n = 9$) were matched for mean baseline response rates in a 30 min CL session on day 1.

Thirty-six female hooded rats, 80–90 days old, were tested in the six sound-insulated, light-proof Skinner boxes described in the previous study[1]. Each rat was placed in a response box with the end ('Perspex') wall dimly illuminated constantly throughout an initial 30 min baseline session. A response (lever press) produced no change in the stimulus condition. After this "constant light" (CL) period, the thirty-six rats were matched in four groups of nine on the basis of their average operant response rate during this first CL session. On the following day, two groups received 0.3 mg/kg of M 285 intraperitoneally in 0.9% saline solution. The other two groups were injected with saline. Ten minutes after injection the rats were placed in the same response boxes with constant dim illumination. One drug group and one saline group were run in the experimental condition, in which a lever press switched off the light for as long as the lever was depressed (RCLO). The two control groups (drug and saline) were run under constant light (CL) as on day 1, and their responses produced no change in the stimulus conditions. The control (CL) and experimental (RCLO) sessions on day 2 lasted for 60 min.

Fig. 1 shows the response rates of the four groups during the test sessions on day 2. In the experimental (RCLO) condition there is a very significant difference between the drug and saline animals ($t = 4.9$; d.f. $= 16$; $P < 0.001$). The saline group shows a typical sensory reinforcement effect, since the mean response rate was twice that of the control (CL) group. Response rates of experimental animals injected with M 285, however, were no different from those of the control groups.

We have thus replicated, in a different experimental situation (RCLO), the finding that M 285 (at a dosage of 0.3 mg/kg) selectively depresses a sensory reinforcement effect without affecting non-reinforced operant responding. This result, however, contradicts the expected increase in responding of the experimental M 285 group, predicted from the hypothesis that visual stimulation is hallucinogenically intensified. A more likely explanation is that the sensory change is the critical stimulating and reinforcing factor, irrespective of whether this change involves onset or offset[2]. Thus, RC changes in sensory stimulation would be less rewarding for animals whose arousal level has been raised by M 285 than for saline animals.

We thank Reckitt and Colman for supplies of M 285, and Dr A. L. A. Boura for advice and assistance. The work was supported by a grant from the Medical Research Council.

G. Lowe
D. I. Williams

Department of Psychology,
University of Hull

Received April 5, 1971.

[1] Lowe, G., and Williams, D. I., *Nature*, **224**, 1226 (1969).
[2] Williams, D. I., and Lowe, G., *Brit. J. Psychol.*, **61**, 379 (1970).

Characterization of the Principal Steroidal Saponins of the Starfish *Marthasterias glacialis*: Structures of the Aglycones

Steroid glycosides with surface active properties have been isolated from extracts of the starfish, *Marthasterias glacialis*, by gel filtration and ion exchange chromatography[1,2]. These saponins induce avoidance reactions in the mollusc, *Buccinum undatum*, and may play a part in reducing predation by the starfish on this mollusc and other marine organisms. Mackie[3] has discussed the possible significance of this type of interaction in relation to pollution studies. Partial characterization of one of the major glycosides showed it to be a mixed conjugate of a Δ^{24}-23-ketocholestane derivative[2]. In this communication we discuss the composition of the glycoside mixture and evidence for the nuclear structure of the aglycone.

Crude starfish extracts were hydrolysed with hydrochloric acid and the water-insoluble aglycone was purified by chromatography on silica gel. Although homogeneous by thin-layer chromatography in several solvent systems, the aglycone was resolved into three components (ratio approximately 1 : 5 : 4, varying slightly with different batches of animals) by gas chromatography. The major components were then separated by preparative layer chromatography using continuous development in benzene–ether (24 : 1 v/v). The more polar of these, now called marthasterone, contained the Δ^{24}-23-ketone system previously characterized[2] (λ_{max} 240 nm; ν_{max} 1,690 cm^{-1}), while the other component exhibited no selective absorption in the ultraviolet and its carbonyl stretching frequency appeared at 1,715 cm^{-1}. This, together with its nuclear magnetic resonance (NMR) spectrum, suggested that it was the 24,25-dihydroderivative of marthasterone, and the relationship was confirmed by selective hydrogenation (Pd/C, 1 atmosphere, 1 h) of the side-chain double bond of marthasterone, which yielded the other major component. Further structural work was then carried out on this material, melting point 167°–169° C, now called dihydromarthasterone.

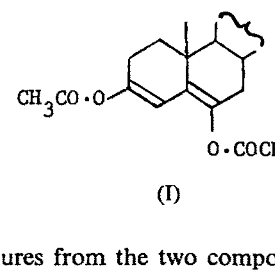
Dihydromarthasterone ($C_{27}H_{44}O_3$, M$^+$ 416.3289 (required 416.3290)) showed a mass spectral fragmentation pattern analogous to that of marthasterone, with the major cleavage (at C_{20}–C_{22}) involving loss of the six-carbon fragment containing the ketone function (*m/e* 316, 301, 299, 298, 283, 265).

Previous work indicated that the nuclear substituents of the aglycone consisted of a double bond and two hydroxyl groups[2], and spectral data established conclusively that the trisubstituted nuclear double bond was not attacked in the above conditions. Prolonged catalytic hydrogenation (50 h) was required to reduce the nuclear double bond, as shown by the disappearance of the olefinic proton signal at τ 4.68. In the resulting tetrahydromarthasterone, the C_{19} angular methyl group showed a large upfield shift (10 Hz), while the C_{18} angular methyl had moved downfield (2 Hz). Zürcher's rules[4] indicated that this was in accordance only with the presence of a $\Delta^{9(11)}$-olefinic bond in the substrate. Other trisubstituted double bonds produced markedly different effects on the positions of the angular methyl group resonance frequencies. (Hydrogenation of the side-chain double bond had no significant effect on these resonances.)

Epoxidation of the nuclear double bond, using monoperphthalic acid in ether, was accompanied by loss of the olefinic signal at τ 4.71 and the appearance of a broad one-proton doublet at τ 6.83 ($J = 6$ Hz). In addition, downfield movements of the C_{19} (14 Hz) and C_{18} (5 Hz) protons were noted. Similar changes occurred in the positions of the angular methyl proton signals following the epoxidation of a number of model $\Delta^{9(11)}$-compounds ($\Delta^{9(11)}$-tigogenin acetate, $\Delta^{9(11)}$-pregnanolone acetate, and so on). There is thus strong evidence that the nuclear double bond of marthasterone occupies the 9,11-position.

The locations of the two nuclear hydroxyl groups were next investigated. Oxidation of marthasterone with chromic acid in acetone gave a triketone (λ_{max} 239 nm, $\varepsilon = 10,120$; ν_{max} (KBr) 1,715 and 1,680 cm^{-1}). Its NMR spectrum showed downfield shifts (C_{19}, 15 Hz; C_{18}, 5 Hz) for the angular methyl groups, and the olefinic proton at C_{11} had also moved 16 Hz to lower field. This indicates that the keto-groups are relatively close to the C_{19} angular methyl group and the $\Delta^{9(11)}$-bond, but more remote from the C_{18} angular methyl group. Chemical shifts for the angular methyl protons calculated from literature values[4] were in good agreement for 3 and 6-keto groups, as were the observed values for the triketones obtained by similar oxidation of dihydro and tetrahydromarthasterone. Further evidence for this assignment was obtained by oxidation of 3β,6α-dihydroxy-5α-cholestane to 5α-cholestane-3,6-dione. This was accompanied by downfield shifts of 13 and 4 Hz, respectively, for the 19 and 18-protons. Other positions for the two nuclear oxygen functions were excluded by reference to literature compilations[4,5].

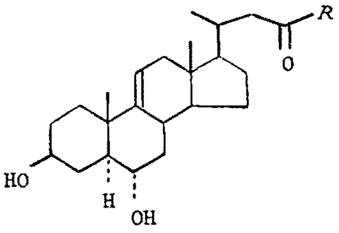
Attempts to confirm the 3,6-relationship of the new keto-groups by dehydrogenation to the conjugated Δ^4-3,6-dione system using selenium dioxide were unsuccessful. Treatment of the triketone with isopropenyl acetate, however, produced a diacetate mixture having λ_{max} 244.5 nm ($\varepsilon = 3,430$). The corresponding product mixture from 5α-cholestane-3,6-dione had λ_{max} 245 nm ($\varepsilon = 3,450$). This absorption corresponds to that of an heteroannular diene chromophore of type (I). The acetylated mixtures from the two compounds were shown by gas–liquid chromatography to consist of similar mixtures of components, resulting from the various enolization pathways open to the diketones. The major products appear to be the $\Delta^{3,5}$ and $\Delta^{2,5}$-dien-3,6-diol diacetates.



(I)

The broad nature of the C_3 and C_6 proton signals in the original aglycones and their diacetates indicate that they are both axial; hence the hydroxyl groups are equatorial, and occupy the 3β and 6α positions.

NMR spectral data on this series of compounds accord with the A/B-*trans*-ring junction, rather than the A/B-*cis*-junction, and hence the structures proposed for the major aglycones present in the *Marthasterias* saponins are 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one (marthasterone, II) and its 24,25-dihydro-derivative (III).



[II; $R=CH=C(CH_3)_2$]

[III; $R=CH_2CH(CH_3)_2$]

Two structural features of these molecules are of biogenetic interest. First, the keto-groups at position 23 are unique, oxygenation at this position in the side chain having only previously been encountered in nature in the case of 23ξ-hydroxylanosterol. This was isolated from the common fungus, *Scleroderma aurantium*, by Entwistle and Pratt[6,7]. The nuclear hydroxylation pattern occurs in the cactus sterols, peniocerol[8] and macdougallin[9,10]. Second, starfish sterols are characterized by the presence of a Δ^7-bond[11], as are those of sea cucumbers. The latter, however, also produce toxic steroidal saponins (the holothurinogenins) possessing a $\Delta^{7,9(11)}$-diene system[12,13], and $\Delta^{9(11)}$-derivatives[14].

Recent work[15,16] shows that echinoderm sterols are partly dietary in origin and partly biosynthesized by the animals. Incubation of starfish with 2-^{14}C-mevalonic acid gave heavily labelled lanosterol, although cholest-7-enol, the principal sterol component, was only weakly radioactive.

Synthetic work to confirm the structures proposed is in progress in this laboratory, as well as further work on the location of the conjugating groups in the original saponins, and characterization of the minor component.

A. B. TURNER
D. S. H. SMITH

Department of Chemistry,
University of Aberdeen,
Aberdeen AB9 2UE

A. M. MACKIE

Natural Environment Research Council,
Fisheries Biochemical Research Unit,
University of Aberdeen,
Aberdeen AB1 3RA

Received May 26, 1971.

[1] Mackie, A. M., Lasker, R., and Grant, P. T., *Comp. Biochem. Physiol.*, **26**, 415 (1968).
[2] Mackie, A. M., and Turner, A. B., *Biochem. J.*, **117**, 543 (1970).
[3] Mackie, A. M., *J. Exp. Marine Biol. Ecol.*, **5**, 63 (1970).
[4] Zürcher, R. F., *Helv. Chim. Acta*, **44**, 1380 (1961).
[5] Zürcher, R. F., *Helv. Chim. Acta*, **46**, 2054 (1963).
[6] Entwistle, N., and Pratt, A. D., *Tetrahedron*, **24**, 3949 (1968).
[7] van Lier, J. E., and Smith, L. L., *J. Org. Chem.*, **36**, 1007 (1971).
[8] Djerassi, C., Murray, R. D. H., and Villotti, R., *J. Chem. Soc.*, 1160 (1965).
[9] Djerassi, C., Knight, J. C., and Williams, D. I., *J. Amer. Chem. Soc.*, **85**, 835 (1963).
[10] Djerassi, C., Knight, J. C., and Williams, D. I., *J. Amer. Chem. Soc.*, **88**, 790 (1966).
[11] Gupta, K. C., and Scheuer, P. J., *Tetrahedron*, **24**, 5831 (1968).
[12] Tursch, B., Guimaraes, I. S. S., Gilbert, B., Aplin, R. T., and Djerassi, C., *Tetrahedron*, **23**, 761 (1967).
[13] Roller, P., Djerassi, C., Cloetens, R., and Tursch, B., *J. Amer. Chem. Soc.*, **91**, 4918 (1969).
[14] Chanley, J. D., and Rossi, C., *Tetrahedron*, **25**, 1897, 1191 (1969).
[15] Smith, A. G., and Goad, L. J., *FEBS Lett.*, **12**, 233 (1971).
[16] Goad, L. J., *Abstr. Roy. Soc.*, March 25 (1971).

Lead Accumulation by Wild Oats (*Avena fatua*) in a Contaminated Area

THE input of lead into the environment has increased during the last two decades[1-4], especially with the development of lead-containing gasolines[2]. The body-burden of lead is now much greater in man than in his modern ancestors[5] and as one source of lead is food, its acquisition, mobility and effects in plants and animals, as well as its partitioning among the air, soil, water and biotic fractions, must be understood.

The Benicia-Vallejo area, 25 miles north-east of San Francisco, is a unique site for such studies because it has been continuously contaminated with lead from a nearby smelter for approximately 70 yr[6]. The area includes both agricultural and urban uses of land. Between early 1969 and early 1970 thirteen Appaloosa horses died while grazing in a winter pasture in the area and their deaths were correlated with a high body-burden of lead. Since then we have carried out an intensive sampling and analysis of native vegetation.

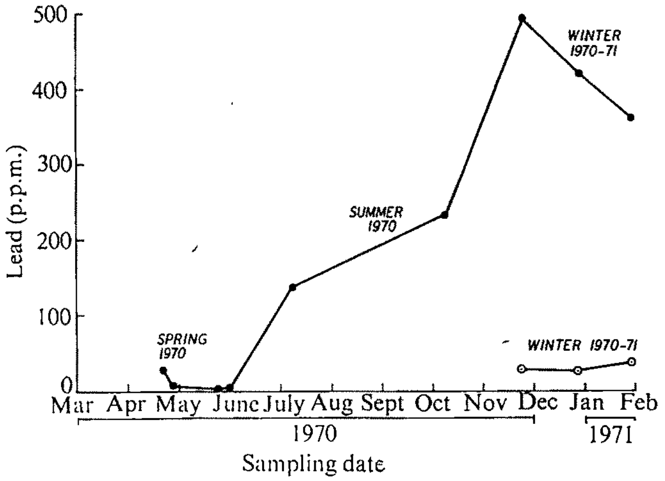


Fig. 1 The concentration of lead (p.p.m. of dry weight) in the above-ground portion of *Avena fatua* as a function of sampling date. ●, 1969–70 growing season; ○, 1970–71 growing season.

The aerial parts of various plants were analysed for lead by atomic absorption spectrophotometry[7]. Tests on two dates indicated no detectable difference in lead concentrations between washed and unwashed samples. Fig. 1 summarizes the analysis of *Avena fatua* samples. Initially, during the period of rapid growth and heading out (April–June), the lead concentrations decreased, but then increased substantially during ripening until autumn, when the plants were completely air-dry. The increase continued, accelerating after heavy rain in October and November when the new season's growth began, and reached a peak in December. Late November to late January was also a period of heavy rain. Lead contamination in the new growth was similar to that observed in the early samples of the 1970 season.

The shape of the curve in the early months of the year may reflect growth dilution. The plants are growing and are constantly absorbing lead from either the soil or the air, and a decrease in concentration might be expected. As the plants senesce and desiccate the lead concentration would increase because, although input may remain constant, the mass of the plants, is no longer increasing. Later in the season, however, when the plants and soil are dry, the transfer of lead from the soil to the shoots would presumably cease. The observed continuation of the accumulation of lead probably, therefore, derives from atmospheric inputs.

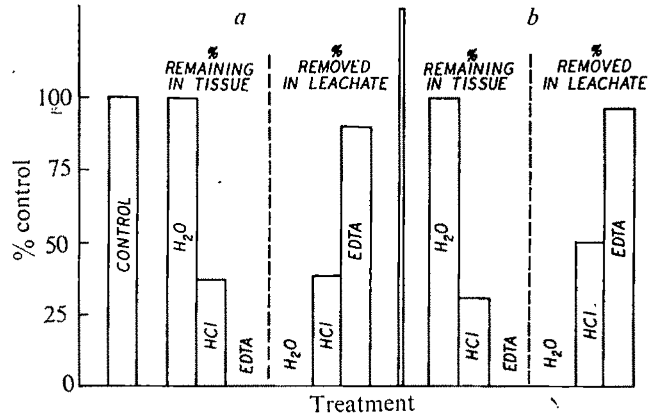


Fig. 2 Leaching for (*a*) 4 and (*b*) 24 h of ground plant material by various solutions. The results are expressed as percentage of lead in control, represented by untreated straw. Treatments consisted of H_2O, 0.01 N HCl and 0.01 M NH_4-EDTA (*p*H 6.5).

An alternative interpretation is suggested by the work of Mitchell and Reith[8] who discovered that as plants began to senesce the lead they contained was mobilized, substantially increasing in concentration in the shoot. But the pasture in which their study was conducted, unlike that reported here, was a perennial association and the plants were still viable in October when the increase occurred. Subsequent lack of growth due to the low winter temperatures, and the continued translocation of lead may have caused the late autumn and winter increase. The onset of spring growth would have then diluted the lead concentration. My own greenhouse experiments with *A. fatua*, and other reports[9,10], suggest that very little lead is translocated in representative grasses of the Benicia-Vallejo area, so that such an explanation for the increase there is unlikely.

A final alternative could be that the increase resulted from a substantial reduction in dry matter of the plant, with no loss of the associated lead. To account for the observed increase, however, a dry matter loss of at least 95% would be required but only a 10–20% dry weight loss actually occurred. This suggests that there is indeed a continuous input of lead into the system, presumably from the atmosphere, much of which is actively retained by plant material. Acting as a store in this way the plants may serve to regulate, at least partially, the cycling of lead within the system.

If the accumulation of lead results from the settling of superficial dust and atmospheric particulates on the surface of the vegetation, it might be expected that with the onset of the winter rains, significant quantities would be removed. In fact, the loss of only small amounts was detected. To investigate the effect of rain, a series of leaching experiments was conducted on dried straw collected during November.

The material was ground to pass through a 20-mesh screen, and 0.1 g samples were placed in flasks with 25 ml. of either H_2O, 0.01 N HCl, or 0.01 M NH_4-EDTA (*p*H 6.5) for periods of 4 and 24 h. The material was then filtered, washed several times and the leachates were combined. Both the residual material and the leachate were analysed for lead. The results of these experiments are presented in Fig. 2. The EDTA treatment was very efficient in removing lead, the acid less so, and the water leaching removed very little lead from the plant tissue. These data indicate the tenacity with which lead is retained by the dried plant material and to some extent confirm that rainfall can be expected to be ineffective in removing it.

The accumulation on the surface of the vegetation during the summer, therefore, is possibly followed by a binding of the lead by the dried plants. As very little rain falls in the area during the summer, only negligible amounts of surface lead would be removed, and there would be an extended period of contact during which such binding could take place. Straw consists almost entirely of cell wall remnants and heavy metals are known to be bound by such tissues in both roots and leaves[11,12]. Analyses of the surface litter reveal lead concentrations of 700–1,000 p.p.m., indicating a further concentrating effect.

The phenomenon described here has important implications for management and surveying in contaminated areas. The California Department of Public Health, in cooperation with the Air Resources Board, has carried out a comprehensive survey of lead in the Benicia-Vallejo region by sampling *A. fatua* over a large area. Because of the propensity of the grass to accumulate lead throughout the season, this may be a suitable method for monitoring the atmospheric input on a large scale. Management could be instituted to reduce the intake by stock of the previous season's contaminated plants. Because only small amounts of lead seem to be taken up from the soil[9,10], residual old growth could be ploughed in. Grazing practices might also be altered to avoid the use of contaminated pastures until most of the total feed available is represented by the current season's growth which contains only low concentrations of lead.

I thank Hector Schoo, Shirley Kunisaki and Catherine Rains for technical assistance, and Sydney Thornton and Dr R. G. Burau for their contributions.

D. W. Rains

Department of Agronomy and Range Science,
University of California,
Davis,
California 95616

Received April 13, 1971.

[1] Cannon, H. L., and Bowles, J. M., *Science*, **137**, 765 (1962).
[2] Chow, T. J., and Earl, J. L., *Science*, **169**, 577 (1970).
[3] Hammond, P. B., and Aronson, A. L., *Ann. NY Acad. Sci.*, **111**, 595 (1964).
[4] Motto, H. L., Haines, R. H., Chilko, D. M., and Motto, C. K., *Environ. Sci. Tech.*, **4**, 231 (1970).
[5] Patterson, C. C., *Arch. Environ. Health*, **2**, 344 (1965).
[6] Haring, C. M., and Meyer, K. F., *Bureau of Mines Bull.*, **98** (1915).
[7] Smith, R. G., Zajnar, J. S., and Hecker, L., *Environ. Sci. Tech.*, **4**, 333 (1970).
[8] Mitchell, R. L., and Reith, J. W. S., *J. Sci. Food Agric.*, **17**, 437 (1966).
[9] Keaton, C. M., *Soil Sci.*, **43**, 401 (1937).
[10] Marten, G. C., and Hammond, P. B., *Agron. J.*, **58**, 553 (1966).
[11] Peterson, P. J., *J. Exp. Bot.*, **20**, 863 (1969).
[12] Reilly, C., Rowel, J., and Stone, J., *New Phytol.*, **69**, 993 (1970).

Effect of an RNA-rich Extract on Acquisition of a One-way Avoidance Response in Rats

Two recent approaches to the biochemistry of memory have involved, respectively, the measurement of molecular changes after training, and attempts, using brain extracts, to transfer trained behavioural tendencies between animals. Zemp *et al.*[1] have reported differences between experimental and yoked-control mice in the synthesis of both nuclear and cytoplasmic RNA after a training session in which they learned to jump onto a platform to avoid foot shock; subsequent work[2] suggested that the change in RNA synthesis during learning is a function of the learning experience and not of stimulation, sensitization or other non-specific factors. The "memory transfer" effect was discovered in our laboratory and has now been replicated successfully in at least twenty others[3–5]. We had now attempted to interrelate these two approaches by studying the behavioural significance of substances synthesized after acquisition of the same response used by Zemp *et al.*, as measured by their effects when injected into a second animal.

RNA-rich extracts were obtained from the brains of trained animals, untrained controls, and yoked-shock controls (that is, animals receiving the same shocks as trained donors but prevented from learning the avoidance response). Animals receiving injections of extract from these three sources were compared on rate of acquisition of a jump-up avoidance task.

Forty-three 100–150 day old, male albino rats were housed in individual cages and given food and water freely throughout the experiment. An automated one-way avoidance chamber[6] was used to train animals to avoid a 0.6 mA shock by jumping onto a platform. The conditioned stimulus was the retreat of a wall that had barred access to the platform. Shock followed 10 s later. Thirty seconds after shock onset the wall returned, pushing the rat onto the grid for another trial. A correct trial was scored if the rat jumped to safety before shock onset.

Experimental donors were trained intensively to establish the learning experience firmly. They received ten trials a day for 5 days. Recipients, on the other hand, received three trials a day for 6 days. The difference in procedure reflects the fact that animals receiving ten trials a day mastered the task readily, often reaching asymptotic performance on the second day. Limiting practice to three trials a day made acquisition of the avoidance response more difficult for recipient animals, so that enhanced acquisition could more easily be measured.

The yoked-shock donors were placed in the apparatus for the same time as the experimental donors. Each yoked-shock donor received the same number, duration, and spacing of shocks as an experimental donor; but the 'Plexiglass' wall was always advanced to prevent any learning of escape or avoidance responses.

All rats were handled for about five minutes each day for 10 days before the first day of training. Fifteen of the rats served as donor animals and were assigned on a random basis to an experimental ($N=5$), control ($N=6$), or yoked-shock control ($N=3$) group. One animal was used for chemical assay of the donor material; the remainder of the animals were assigned to three corresponding recipient groups. Members of one group ($N=10$) received extract from the pooled brains of experimental animals. Members of a second group ($N=12$) received pooled extract from the brains of the control group. Members of a third group ($N=6$) received pooled extract from the yoked-shock group.

Within one hour of the last training session, donors were decapitated, and their brains, excluding cerebellum and olfactory lobes, were removed to a beaker of dry ice, where they were kept at −20° C. RNA was obtained by cold phenol extraction[7].

Twenty to twenty-six hours before its first post-injection test session, each recipient animal was injected intracranially with 0.15 ml. (0.5 brain equivalent) of extract from a trained or untrained donor[8].

The mean number of avoidances during the eighteen trials was 6.25 for animals receiving control group RNA, 5.83 for animals receiving yoked-shock control group RNA, and 10.5 for animals receiving experimental group extract. Individual rats receiving control group material ranged from four to nine avoidances, while rats receiving experimental material ranged from nine to thirteen. These individual scores were ranked and submitted to a Mann–Whitney U test. The resulting statistic was highly reliable ($U=0$, $P<0.001$). Yoked-shock recipients ranged from three to ten avoidances. When these scores were ranked against the experimental recipient scores, the resulting test statistic was again highly significant ($U=3$, $P=0.001$). When the yoked-shock and control scores were ranked against each other, however, the resulting statistic was not significant ($U=26$). Fig. 1 presents the mean number of avoidances on each of the training days. As can be seen, the group receiving "trained" extract displayed higher performance on every day after the second.

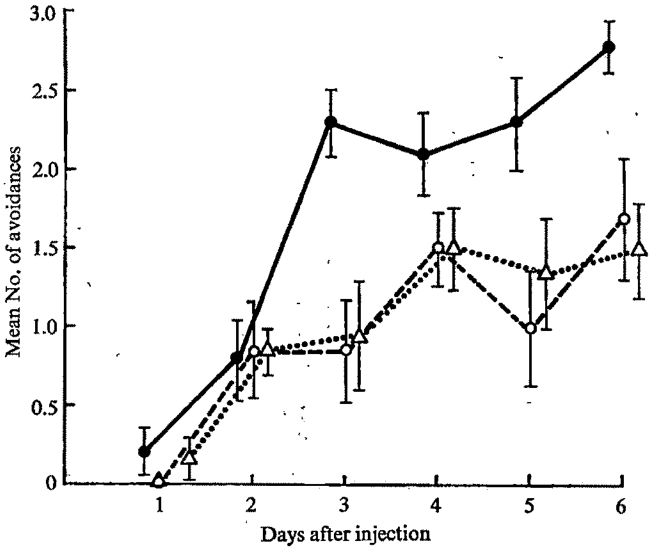


Fig. 1 Mean avoidances of experimental, control, and yoked-shock recipient groups on each of 6 days following injection of RNA-rich brain extract. (The vertical bars at each data point represent one standard error of the mean.) ●—●, Experimental; ○----○, yoked-shock; △----△, control.

The enhancement of avoidance by extracts from trained donor brains is open to several interpretations. The chemical changes in trained animals responsible for this effect might reflect learning, or they simply might reflect general stimulation such as shock trauma, violent activity, of mere exposure to the experimental apparatus. The total ineffectiveness of extracts from yoked-shock donors argues against the latter conclusion. But we did not control for all conceivable sources of stimulation, such as being pushed off the platform. It should also be noted that the cold phenol extract contained many impurities[7], in addition to RNA. Thus it is not certain that the enhancement was due entirely to RNA.

David H. Malin
Arnold M. Golub[*]
James V. McConnell

Mental Health Research Institute,
University of Michigan,
Ann Arbor, Michigan

Received July 12, 1971.

[*] Present address: Eunice Kennedy Shriver Center, 200 Trapelo Road, Waltham, Massachusetts 02154.

[1] Zemp, J. W., Wilson, J. E., Schlesinger, K., Boggan, W. O., and Glassman, E., *Proc. US Nat. Acad. Sci.*, **55**, 1423 (1966).
[2] Adair, Linda B., Wilson, J. E., and Glassman, E., *Proc. US Nat. Acad. Sci.*, **61**, 917 (1968).
[3] Jacobson, A. L., *J. Biol. Psychol.*, **9**, 52 (1967).
[4] Tunkl, Judy, *J. Biol. Psychol.*, **19**, 80 (1968).
[5] Schutjer, Marlys, *J. Biol. Psychol.*, **10**, 54 (1968).
[6] Tenen, S. S., *Psychon. Sci.*, **6**, 407 (1966).
[7] Golub, A. M., Masiarz, F. R., Villars, T., and McConnell, J. V., *Science*, **168**, 392 (1970).
[8] Lindberg, O., and Ernster, L., *J. Biochem.*, **46**, 43 (1950).

BOOK REVIEWS

Pioneers

Sex, Career and Family. By Michal P. Fogarty, Rhona Rapoport and Robert N. Rapoport. (George Allen and Unwin: London, 1971.)

THE initial research of Rapoport, a sociologist–psychoanalyst, Rapoport, a social anthropologist, and Fogarty, an economist, aimed to understand the problems of women in top jobs. However, as the work progressed it became clear to them that one could no longer look at women and work as isolated variables. Rather, as their book *Sex, Career and Family* has so convincingly demonstrated, work patterns and family relationships must be re-examined together in the light of changing social mores, aspirations, motivations and opportunities. The single-role stereotypes—the father as provider, the mother as child-raiser-homemaker—have changed. With each passing decade the number of women who work increases, with the greatest increase being among educated women. The more education a woman has, the more likely she is to be employed. For example, in the United States more than 70 per cent of women who have completed more than five years of college are in the labour force. But when we ask how successful they have been in the work they are doing, the picture darkens; this book demonstrates dishearteningly that unless society is willing to undertake major changes in the way jobs are defined, family roles assigned and attitudes challenged, we shall continue to send a double message to young women—"Aim high, but not too high". Given the same intellectual endowment and educational achievement as the men with whom she attended university, the young woman can look forward to a position somewhere in the second rank of any given profession or organization. The world abounds with women whose titles are prefaced by "Assistant".

The authors make it clear that to understand how this down-grading happens to women one must shift emphasis from women alone to men and women together. From there a short step leads to the exploration of society's legitimization of the relationships between men and women, the family and work. The authors consider family patterns and sex roles as they affect job and career potential for women and, at the same time, open up the crucial psychological issues of self-definition and interpersonal expectations. All of these are examined in the context of changing attitudes toward work and the impact of industrialization and urbanization on social and psychological processes. This matrix of interlocking variables necessarily complicates methodological structure although the authors' struggle to achieve a sensible research methodology is one of the book's important contributions.

The men and women who work out new patterns of relationship between work and family to facilitate the wife's pursuit of an effective career and still maintain a family are pioneers in several dimensions simultaneously. To break the lockstep of man breadwinner and woman homemaker which is so accepted by our social institutions, both the women and men (the dual career families) involved must also change their view of women as the weaker partners. This "creative minority", as the authors call them, are distinctly on the move, spurred by unanticipated social and technological changes.

A reason for this book's excellence, besides the vast spectrum of data drawn upon, is its awareness that one cannot blueprint how or what will be the result of a social, psychological or technological change. Events that seem unconnected emerge as forces to be integrated by these pioneers into the different family, work and personal patterns they definitely predict. As one surveys the rapidity of change in our society, it is hard to fault their insight. Who could have predicted a few years ago the almost universal militancy for equal rights of underprivileged groups which now affects the women's rights movement so directly? Or, as more cogent examples, who could anticipate how definitely one discrete technological advance could change sexual mores and another the roots of sexual identity?

It is unlikely that the puritanical Henry Ford ever thought he would be the father of America's sexual revolution. His interest was in transportation—mechanical not emotional. Yet he provided America's first bedrooms on wheels, thereby changing sexual behaviour to a greater extent than we can measure. It was a technological achievement external to individuals which began by changing social vistas and eventually reshaped psychological responses.

Now we are in a new era . . . that of the pill. Like Henry Ford, the steroid chemists (were they all men?) were attempting to solve a technological problem . . . how to provide a better method of contraception. To a large extent they have solved the technical challenge . . . for millions of women the pill is a miracle. What was unanticipated and as yet only dimly perceived, are its social–psychological side-effects. Chemists set out to develop safe, cheap, readily available contraception. Once the means to that end came into being, it turned out that the achievement of this seemingly straightforward goal necessitated broad revisions of society's attitudes toward such specific matters as contraception, conception, copulation and consent. On a more abstract level but in the long run probably more salient in determining our attitudes and behaviour will be the revision of our sexual identity.

For example, in any sexual encounter before the pill, the woman had to be protected. Concrete evidence for this assumption is provided in the name of a proto-diaphram patented as early as 1846 . . . "The wife's Protector". She could protect herself from the spectre of unwanted pregnancy, could ask the man to or could expect it of him as (to drift into the Victorian for a moment) a gentleman. Now the pill is taken along with vitamins, orange juice and morning coffee, separate from and unrelated to the act of going to bed or to the person she might be sleeping with.

The pre-pill perception of the woman was in part that she was weak, fragile and vulnerable. Can anyone imagine that this persistent image of a woman in this most basic act does not influence men and women's view of themselves and others? If the idea that she must be cared for becomes integrated as part of her sense of self, it also implies that she must be protected from the man who might be careless, that is potentially harmful. As the impregnators, men develop from the same experience similar views of themselves and women. The interaction of these intrinsic sexual identities reinforce each other and the weak–strong dichotomy is maintained. Hence, attitudes derived from the sexual act can affect the way in which children are treated from birth; they can be extrapolated to a general equation describing men and women's roles and functions. She requires protection and pays for it in status. But now she no longer needs the protection—she has it. One can see how in a society pressing towards egalitarianism, the pill would have a definitive impact on that social and psychological equation.

The above oversimplified analysis of the potential impact of the pill on sex, career and family life would probably arouse little disagreement at the nearest cocktail party or at the course, *Women in Society*, recently spawned at the local university. Does that mean we are adapting psychologically and sociologically to the coming change?

Not necessarily. The authors show how little prepared we are individually or as a social group to cope with change despite our awareness that it must come.

Their studies of highly qualified British women graduates of 1960 show that only one per cent of those now married with children definitely intend not to work and that four out of five intend to be in part or full-time work when their children are grown. These findings are similar to those in the United States, where the Labor Bureau reports a marked upswing in the percentage of young women college graduates continuing to work after the birth of their first child. Some of the technical problems associated with this are obvious. How much should be spent on "improved housing, neighbourhood service and provision of nursery schools?" Less obvious are the challenges faced by employers. If it is necessary to re-cast their recruitment and promotion practises to take into account women's different life-cycles, can the community be expected to meet some of the costs coming out of the adaptations which will pay for maternity leaves and relaunching programmes? The authors present evidence that "these programmes can be operated only if there is broad social support from the community and its formal representatives, the law and the government". Industry cannot afford and has little inclination for pioneering. This broad social acceptance and support is necessary at all levels if women are not just to be allowed in as assistants but are to reach their capacity at the top of their professions. For, although many women plan to work, the most depressing statistic presented is how few women go far or rise high whether it be in Great Britain, Eastern Europe or the United States.

Thus the change throughout must be in the potential not simply for acceptance but for status: from aspiring for auxiliary roles in work, protection in sexual encounters, or help in the house to expecting an equal opportunity to be leader, sex partner, or non-housekeeper if and when they so choose. This book offers an informed, scholarly approach to new intra-familial relationships which, while not for everyone, would offer a positive direction for families to move in—all within the definition of the family.

DOROTHY S. ZINBERG
NORMAN E. ZINBERG

Men at War

War and the Human Race. Edited by Maurice N. Walsh. (University of California, Los Angeles, Faculty Lecture Series, 1968.) Pp. 95. (Elsevier: Amsterdam, London and New York, 1971.) £2.25.

THE editor claims for this small volume that it represents an inter-disciplinary approach to the problem of war. Unfortunately it does not. The fact that the contributors are drawn from six different disciplines, and that no attempt was made to integrate their conclusions—not even in the "Summary and Conclusions"—scarcely justifies the editor's interdisciplinary claim for it. Dr Maurice Walsh, the editor, is a practising psychoanalyst with a gift for misquotation even to the misspelling of common proper names, and a style so banal that it is calculated to confer mediocrity on the most promising of subjects. The book would have benefited from the omission of his "Editorial Introduction", his lecture, "Psychic Factors in the Causation of Recurrent Mass Homicide", and his "Summary and Conclusions", all of which repeat one another, and the principal theme of which is a "new" theory of war.

The theory is that since wars take place at an average interval of 19.6 years, which is the time of the psychic stresses of late adolescence, the period without voting rights and susceptibility to the aggressive propaganda of charasmatic leaders, coupled with the intense murderous hostility of the older for the younger males (oedipus complex), the result is recurrent mass homicide (war).

As a true believer Dr Walsh has a properly idolatrous regard for the catchwords of psychoanalysis, which is not, in the end, helpful. We must, however, be grateful to him for the passionate plea he makes for more research, and for having gathered together these interesting lectures.

Dr Bernard Brodie, a political scientist, discusses "Theories on the Causes of War", illuminatingly disposing of the usual theories as to the causes of war, such as munitions makers, big business, innate aggression, arms races, neurotic leaders, and the like. Herbert Friedmann, a biologist, in "Animal Aggression and Its Implications For Human Behaviour", clarifies some of the confused issues in which the term "aggression" is used, carefully distinguishing between the "aggressive" behaviour of other animals and that of man, a very necessary distinction because it has become popular, as Dennis Chitty has recently remarked, to blame our animal ancestors for some of the distressing things we do to each other. Regrettably, when Professor Friedmann writes that man "has innate aggressive tendencies", the statement remains declarative, and no evidence is provided for it.

Dr John G. Kennedy, an anthropologist, writes on "Ritual and Intergroup Murder: Comments on War, Primitive and Modern". This is an excellent contribution and provides a sound overview of conflict and restraint among nonliterate peoples, as well as a fine analysis of their causes, functions, and meaning.

The historian, Jere Clemens King, offers a brief survey of "The Role of Warfare in History", and a journalist, Walter Wilcox, in "The Loneliness of the Long Distance Soldier", provides a first-hand examination of what makes the infantryman run, usually, in the direction in which he is ordered.

ASHLEY MONTAGU

Pesticide Journal

Pesticide Biochemistry and Physiology: An International Journal. Edited by R. D. O'Brien. Volume 1, Number 1. Pp. 122. (Academic: New York and London, March 1971.) $30.00.

THE most important question to be directed at a new scientific journal is the quality and rigour of the editorial scrutiny that will be applied to the papers proffered. No one would question the capacity of the editorial board listed in this journal: it is to be hoped that they will have the time to spare for the work.

In the first issue four of the eleven papers are from laboratories of members of the board. In general the standard attained is good. However, one paper on resistance in fungi is not related to the effects of a pesticide. The others include four papers dealing with metabolism by insect gut or microsome preparations. The mode of action of a fungicide on a fungus is described in biochemical terms and that of dieldrin on insects in neurophysiological terms. The metabolism of insecticides in resistant insects is referred to in two papers, one of which suggests that the resistant insect metabolizes one of the new "low toxicity" organophosphorus insecticides like the insensitive mammal. The binding of anticholinesterases to prevent access of meotine or decamethonium to sensitive sites is the subject of another paper. To the reader used to mammals, perhaps the greatest surprise is the explanation of why *Rhodnius* "wets its pants" when exposed to some insecticides.

If this journal can attract high quality papers describing work on the comparative effects of pesticides on target and non-target species it will provide useful background information for the eventual design of more selective but effective pesticides.

For journals whose contents cross the lines of several basic disciplines the titles of the papers are particularly important to enable the appropriate abstracting services to bring them to the attention of others. In this respect this issue is good.

It will be interesting to review this journal again in two or three years time to see whether the editorial aspirations have been maintained. By then one may probably anticipate that the price will have risen above the present £3 for 120 pages and library committees will probably still be looking critically at their budgets. J. M. BARNES

Pharmacological Methods

Methods in Pharmacology. Vol. 1. Edited by Arnold Schwartz. Pp. xiv+585. (Appleton Century Croft: New York, January 1971.) $26.50.

THE title of this book is rather misleading as are the headings of many of the chapters. For example, it is surprising to find that a chapter entitled "The Use of Microelectrode Techniques in Muscle" is concerned with microelectrodes and oxygen electrodes in cardiac muscle, not skeletal or nonstriated muscle. In fact, the book concentrates heavily on cardiovascular pharmacology, the remaining chapters being less coherent.

The sections also vary between comprehensive and useful accounts of techniques, such as those on ATPase and on the sarcoplasmic reticulum, to more restricted accounts of personal research having less general interest. The overall effect is that while much of the volume has interest for the experimental pharmacologist, it can hardly become the place to turn to for a comprehensive account of pharmacological methods. For this purpose the well known texts edited by Laurence and Bacharach and by Siegler and Moyer remain unrivalled. A. S. V. BURGEN

Phase Transitions

Phase Transitions. (Proceedings of the Fourteenth Conference on Chemistry at the University of Brussels, May 1969.) Pp. xi+256. (Wiley-Interscience: London and New York, March 1971.) £6.

IN 1952 there was an international conference in Paris on *Changements de Phases*. In 1969 the same title was chosen for the Solvay Conference in Brussels, although in the published proceedings this has been translated into English. At least ten people (including myself) took part in both meetings and to us, at least, it is instructive to see how far we have come in seventeen years.

At both meetings the emphasis was on theory. The only exact result known in 1952 was Onsager's recently obtained solution of the two dimensional Ising model in zero field. There is, alas, little more to report now. There are a few further results, notably those of E. Lieb, who reviews this topic, but these have not been so fruitful a source of inspiration as that of Onsager. On the other hand, we have now an immense amount of numerical information on both two and three dimensional lattices. This has been obtained in two principal ways.

First, we have series expansions of the partition function, usually in powers of $1/kT$, and the examinations of these series for singularities by the method of Padé approximants. The start of this work was described in 1952 by Rushbrooke; its most extensive practitioners have been Domb and his group in London, but this work was not described at Brussels.

Second, computers have arrived. These can be used to undertake calculations impossible in 1952, as, for example, the use of the matrix method for semi-infinite lattices, described here by A. Bellemans. Their greatest success, however, has been to act as a "laboratory" in which experiments on simple continuum models can be carried out by Monte Carlo or molecular dynamic methods. Virtually all we know about the melting of a system of hard spheres or about the whereabouts of the critical point of a Lennard-Jones system comes from computer simulation. L. Verlet describes this work. Our purely theoretical knowledge of continuum systems remains in a primitive state, although both J. E. Mayer and S. A. Rice sketch possible lines of attack.

The critical point is always with us. By 1952 the classical description of van der Waals was no longer accepted (at least by chemists) but there was no agreement about what should take its place. Now the nature of the singularities in the thermodynamic functions is known, if not understood, and much of the interest has passed to the less easily measured transport properties. G. B. Benedek and P. Résibois describe recent progress which has been aided by the advent of the laser, which allows us to study the spectrum as well as the intensity of the scattered light.

The remaining papers delivered at Brussels dealt with more complex systems where experiment rightly still takes precedence over theory. A. R. Ubbelohde spoke on melting and crystal structure (and was also chairman of the conference), G. Busch on liquid metals and S. Lifson on transitions in chain molecules. This welcome book contains also the discussion; some of it rather elliptic, but it may contain as many seeds of future advances as the formal papers. J. S. ROWLINSON

Band Theory

The Electron Band Theory of Solids. By G. C. Fletcher. Pp. xv+260. (North Holland: Amsterdam and London, 1971.) £6.30.

THERE are already a number of advanced texts devoted to the electron band theory of solids, but for some time there has been a need for a treatment of this particular specialization from first principles, that carries the subject to a level from where the reader can easily tackle the advanced literature, which deals with the technicalities involved in actual band theory calculations. This book adequately fulfils this need.

The basic principles of the subject are introduced, in the first few chapters, in a way that should be easily understood by the average graduate, without demanding a wide command of mathematical skills (several later chapters are devoted to mathematical techniques), yet without recourse to tedious methods nor with loss of rigour. In this section of the book the author very briefly discusses the validity of the one-electron treatment, although this is dealt with in much greater detail in the appendix. Crystal and reciprocal lattices are discussed adequately, and the appendix compliments these topics by providing a useful set of figures illustrating the Bravais lattice types and corresponding Wigner cells and Brillouin zones. Density of states and related matters are dealt with briefly, and a chapter is devoted to the problems of choice of realistic initial potentials, discussion being confined to those of "muffin-tin" type. Discussion of the exchange term occurring in these potentials is relegated to the appendix. Two chapters dealing with the free and nearly free electron approximations conclude what I would regard as the introductory section of this book. These two topics are used to provide useful illustrations of the restricted and unrestricted zone schemes, and of the formation of energy gaps.

A considerable portion of the book is devoted to an introductory review of some of the more important methods adopted for solving the one-electron equation. These methods are returned to, a few chapters later on, after ideas of symmetry and degeneracy have been discussed and it is then shown how the methods of group theory can be used to facilitate the obtaining of solutions at points of high symmetry. A chapter deals briefly with relativistic effects, too briefly I suspect, for most readers at whom the book is aimed and consultation of works listed in the reference section at the end of the chapter will probably be necessary. Other topics considered in the remaining chapters include the pseudopotential method and various semi-empirical and interpolation techniques.

An extensive appendix is provided and apart from items already mentioned, topics include the principles of numerical integration, Legendre polynomials and other mathematical functions encountered in the main text, and Lowdin's expansion. There is additional work filling in on the Green's function method and proofs connected with the theory of group representations.

In conclusion, this is a generally well-written book with its subject matter crisply presented. It should not be omitted from lists of recommended texts for postgraduate courses in solid state physics and metallurgy. It clearly fulfils the author's avowed aim to provide a primer for people wishing to carry out actual band theory calculations and to give guidance for the assessment of the reliability of such calculations.

M. GUMBRELL

Complex Permittivity

Complex Permittivity: Theory and Measurement. Compiled by B. K. P. Scaife, W. G. S. Scaife, R. G. Bennett and J. H. Calderwood. Pp. vi+170. (English University: London, 1971.) £3.45.

THIS monograph on dielectric materials consists of three separate chapters, the first, by B. K. P. Scaife, is on the theory of dispersion in polar dielectrics, the second, by W. G. S. Scaife, discusses the effects of hydrostatic pressure on the properties of dielectrics, and the final chapter, by J. H. Calderwood and R. G. Bennett, deals with experimental methods for the measurement of complex permittivity.

The first chapter gives a most readable account of the theory of dispersion. An initial section on the macroscopic approach has the fluctuation–dissipation theorem as the central theme, and a link between microscopic and macroscopic considerations follows in the next section. Details of the microscopic treatment follow, and include inertial and dipole–dipole coupling effects. A new method is described for plotting experimental results when inertial effects are important. This has the advantage that the complex plane plots are still circles, whereas the Cole–Cole ε'' against ε' plots give more complicated curves when inertia becomes important.

The next chapter, on pressure effects, briefly reviews the theory for non-dipolar fluids, and then describes the experimental techniques which have been used, with some discussion of the results obtained. Sections on crystalline solids and on polar fluids follow; in both the emphasis is on the interpretation of experimental results in the light of theory. There is a final section on dipolar relaxation processes in solids.

The final chapter on experimental techniques gives a clear and straightforward account of the principles and basic theory of a number of different experimental methods, from bridges through resonance methods to waveguides, thus covering a wide range of frequencies. Particular emphasis is placed on sources of error and practical convenience, and the influence of the computer on experimental method has not been overlooked. There are a few blemishes. No suggestion is made, for example, about sub-millimetre wave measurements, yet the first chapter contains a proposed new method for analysing experimental results in this region of the frequency spectrum, so crucial to the inertia effects which occupy so much of the book. There is no mention of resonant cavity techniques, yet the transformer ratio-arm bridge is described separately in chapters two and three.

This book will undoubtedly be useful to specialists in this field, and can be recommended. It must be understood, however, that it is a collection of three related review articles, rather than a comprehensive treatment of all aspects of dielectric materials.

A. L. CULLEN

Lasers Applied

Lasers and Their Applications. By M. J. Beesley. Pp. xii+234. (Taylor and Francis: London, July 1971.) £5.

THE author's stated aim in writing this book is "to explain the mechanism of laser action . . . and to describe some applications of the laser . . .", but it is not clear, on reading the book, to whom it is directed.

The level of the material included is highly variable — for example, it assumes that the reader will need help with the most elementary algebra but that a casual reference to free-space mode densities suffices. The initial parts of the book suffer from too extreme condensation, which has resulted in many half truths or misleading statements—for example, "for most atoms and molecules . . . the first level above (the ground state) is separated by a gap of 1.25 eV". Fortunately this does not hold for the ammonia molecule, description of which follows the above rather sweeping comment. From the text it may appear that the inversion mode is the only possible vibration mode for NH_3.

The description given of the beam focuser supplies a lot of irrelevant detail but nowhere describes why or how the system works. It is implied that the amplitude of a lightbeam reflected at a plane surface at non-normal incidence is independent of the state of polarization of the light. One can unfortunately find all too many examples of this kind of obscurity and matters are not always helped by carelessness in the exposition. (Thus the onus is on the reader to deduce whether the expression "direction of plane-polarized light" refers to propagation or electric vector.)

The author's practical experience with lasers is reflected in the brief descriptions of laboratory procedures. These parts of the book are more successful and really it looks as though the author has written the wrong book. The average scientific paper tends to contain a simple diagram showing arrays of, for example, mirrors or other optical devices. It is not uncommon for newcomers to the field to have to spend a lot of time gaining experience in this area and the ideal solution—to work under the guidance of an "old hand"—is not always possible. (This is of particular relevance in the laser field, where applications are possible in areas where no optical experience exists.) It does seem that, if the author had addressed himself specifically to this problem, a far more satisfactory result could have been achieved.

The selection of applications chosen for description is well made and gives an excellent view of the potentialities of the laser in a variety of fields. Unfortunately, as in the earlier part of the book, some of the descriptions lack the clarity required for the unfamiliar reader. Sufficient references allow further exploration of the topics described.

O. S. HEAVENS

CORRESPONDENCE

Six Years for a PhD

SIR,—The impression exists among many students of college age and earlier, and among their families, that three years is a "normal" length of post-baccalaureate time for a PhD. Many university faculty expect their graduate students to finish in four, or perhaps five, years. Those who spend six years or more in graduate study are often asked why they took so long. As we shall see here, however, six years is about the median time for a PhD at both Harvard and Caltech.

Fig. 1 shows the distribution of time between first degree and doctorate for all students receiving PhDs at Harvard University in 1969. The first degree is always used although, especially in the case of foreign students, it was not always a BA or BS. No adjustments were made for interrupted or part-time study. Obviously most of those taking over ten years were not in graduate full-time for the entire period. The curve for mid-year degrees, which at Harvard are dated "March", is shifted left by six months to provide a better fit.

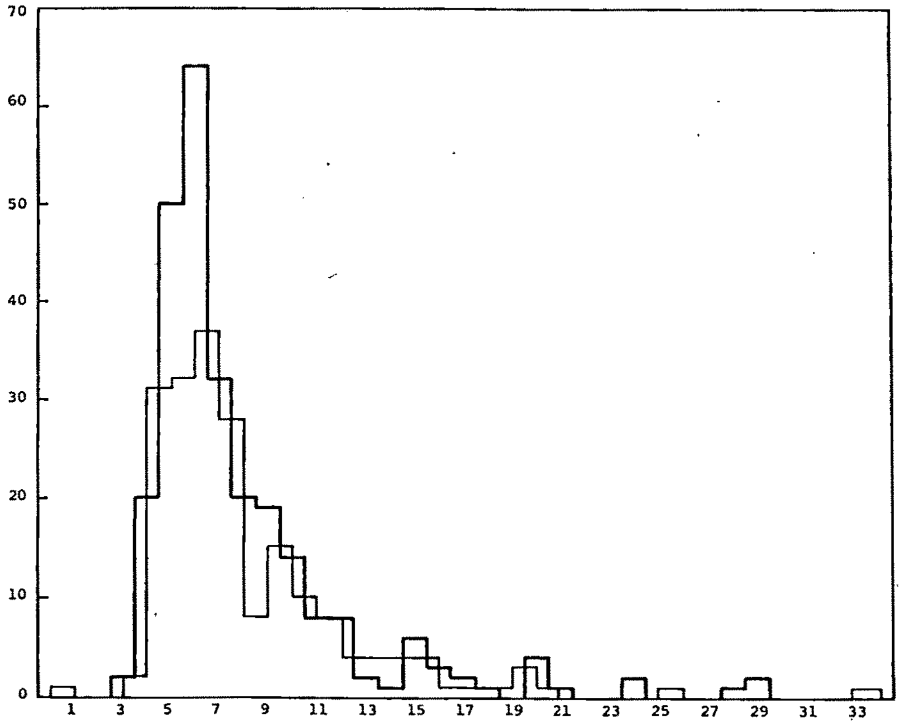


Fig. 1 Number of years between first degree and PhD for those receiving degrees at Harvard commencement in 1969. The dark line is June degrees; the light line is March (mid-year) degrees.

The curves, which are drawn to the same absolute scale, agree in the form of the distribution, which peaks at five through seven years. The average length of time spent before receiving the 262 June 1969 degrees at Harvard was 7.9 years, with a median of 6 years and a mode of 6 years. The average for the 459 total degrees was 8.9 years, with a median of 7 years. There is a long tail, gradually extending out to approximately 30 years. The number who receive PhDs in the traditional three years or less is negligible: less than one per cent.

The curves for mid-year and June degrees agree also in absolute numbers for the wings; additional recipients of June degrees are clustered at 5 and 6 years. The sharp drop thereafter probably represents the effect of the existence of deadlines, not only departmental rules but also psychological pressure felt by the individuals, both students and advisers.

The California Institute of Technology is a more specialized institution, and the 117 PhDs awarded in June 1971 were all in scientific subjects. Yet the distribution (Fig. 2) is very similar to that for all Harvard degrees, peaking at 5 and 6 years with a long tail that extends out to 16 years.

The average length of time of post first-degree enrolment before receiving a Caltech PhD is 6.4 years, with a median of 6 years and a mode of 6 years.

Clearly, the fact that so many students take more than five years for a PhD reflects more than individual delays, and must result partly from the design of

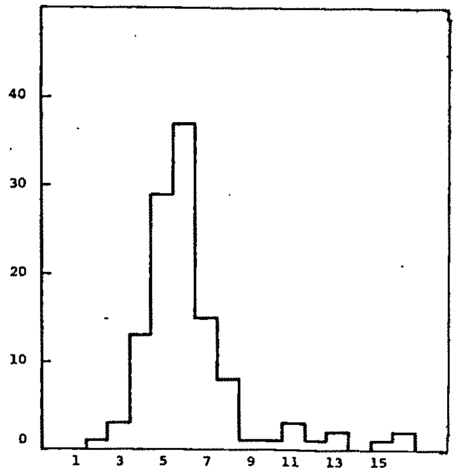


Fig. 2 Number of years between first degree and PhD for those receiving degree at California Institute of Technology commencement in 1971.

requirements laid down by the departments, including minor theses, qualifying examinations, and supplemental research projects.

Yours faithfully,
JAY M. PASACHOFF

Hale Observatories,
Carnegie Institution of Washington,
California Institute of Technology,
Pasadena, California 91109

Bird Navigation

SIR,—Two points appear to be misleadingly used to indicate that domesticateal *Columba livia* does not require sun-azimuth for route-finding when your correspondent on the homing sense in pigeons (*Nature*, **232**, 86; 1971) stated: "Recent evidence that this ("map" mind of the homing bird) can operate effectively even after many days' imprisonment away from the home loft . . ." I published elsewhere[1] examples of very long memory in pigeons including the following: a Hollywood (USA) pigeon which raced up to 500 miles, 1931–4, sold to Huntington (California) which returned on escape after kept "prisoner" 8 years; one lost racing Rennes (France) to Manchester, 1926, which returned home 1931, after presumably being stolen and kept prisoner; an Australian pigeon lost racing 550 miles Benallo–Melbourne, 1944, which returned in 1948, and another which returned 400 miles to France 2 years after sale to Newcastle, where it was kept prisoner a year. I have known pigeons lost up to a year return to their old nest-boxes, among many inside a breeding loft, and fight the new owner for occupation. As the breeding season approaches, more lost birds home than otherwise.

In all such cases which could be investigated, the birds were kept as "inside" prisoners without sky-views. Where transported birds are kept prisoner a week or more in outside, mesh-wire "aviaries", they "resettle" to the new territory. Young birds (6 months old) have thus been resettled in 1 week, exceptionally in 4 days; but older birds usually require 8 to 10 days. My operational birds in the British Army Pigeon Service in the 1939–45 war were sent forward in baskets (to be flown back to their lofts) with the instruction "it is important that birds should be so placed that it is impossible for them to see their immediate external surroundings", and that unused birds should not be kept in delivery or stock baskets for more than 4 days (in trench-baskets, 3 days), but returned for replacements. This was to avoid birds "seeing" and "remembering" the new temporary release-point.

Visual recognition of their external surroundings (e.g. sky and landscape was used to resettle birds to "home" to new loft-sites in place of old ones, using outside aviaries or baskets on top of the loft for at least an hour daily. I allowed my loftmen a minimum of a fortnight to settle pigeons to a new loft-site, but preferred 5 weeks (longer still for distances over 30–50 miles). We once settled thirty-four birds to "home" to a new loft site in 10 days. I have no evidence of birds settled to fly to a new loft-site by "inside" imprisonment, without external views, excepting the "nomad" type of training where a pigeon homed to a marked mobile loft, or basket, by recognition of the mark used also when feeding it.

While ability to home is known to be inherited (an untrained 2-months-old pigeon still squeaking homed 40 miles to one of my lofts, where it was bred) the homing territory is acquired after birth—pigeon eggs taken 100 miles to be hatched and reared "home" to the loft where they were reared, not where the eggs were laid. Pigeons returning to Scotland continued through the longer light of Scottish summer nights to arrive earlier than birds roosting in the darker English night before reaching English lofts. Pigeons liberated in fog distinct from merely ground-mist, are delayed, presumably by waiting for visibility to clear.

The more remarkable test of astral and sun navigation is not this long delayed homing of pigeons, but the annual return to the same breeding and wintering territories by trans-equatorial migrants, particularly the purely marine southern petrels and shearwaters, whose journeys cover seas with no landmarks.

Yours faithfully,
ERIC HARDY

Merseyside Naturalist's
Association,
47 Woodsorrel Road,
Liverpool 15

---

[1] Hardy, E., *Pigeon Guide: A. Complete Handbook of Pigeon Keeping* (Seeley, London, 1951).

Black Hole to the North?

SIR,—I read with extreme interest the editorial observation that, over the past ten years, "the population in the extreme North of Britain decreased, with Lancashire, Cumberland and Northumberland all returning figures lower than in 1961 . . ."[1] thus confirming the fears of those of us who dwell beyond the pale in the thirty-one counties of mainland Britain on the wrong side of Hadrian's Wall, that in fact we really do have no existence and that contracting population acting like some contracting stellar body has pulled the land mass with it leaving a "black hole" to the north of Carlisle.

Or perhaps the statement showed a trace of Anglo-Chauvinism in confusing the land of the English with the island of Great Britain?—an inauspicious error in a journal dedicated to the objective accuracy of science.

Yours faithfully,
WILLIAM F. DRYDEN

Department of Pharmacology,
Royal College,
*George Street, Glasgow C*1

---

[1] *Nature*, **232**, 512 (1971).

Unfair to Scientists

SIR,—During the period when the pay of civil service scientists has been the subject of public debate most of us concerned with the matter have felt the reports in *Nature* to be well balanced and to fairly state the case of both sides. Your conclusions have represented a fair assessment of the merits of the case. But, then, we could expect you as a serious commentator on scientific affairs to recognize what enormous damage would be done to the scientific community at large by the Civil Service Department's ludicrous pay offer to its scientific staffs. I should like, however, to emphasize the following points.

Regrettably, the arbitration tribunal has done nothing to resolve the basic problem. Anyone trying to reconcile the irreconcilable is likely to produce a nonsensical result. That was bound to be the outcome of trying to bring together "fair comparisons" and internal relativities. The Civil Service Department at least recognized the problem. They acknowledged that there were special circumstances affecting the pay of scientists but argued that they were irrelevant and, in any case, could not be measured. Thus, for them, there was no conflict. We were not prepared, and are not now, to have scientists treated as poor relations of their civil service colleagues in the technological field, nor yet of those in administration.

We will not take part in another pay research exercise and we are determined to get a joint review to establish a proper basis for future pay reviews for scientific staffs. We will press for early action on all of the structure aspects of Fulton, including the point which you rightly make about the need for scientists (and other specialists) to move across to more general administration. In part, of course, the point can be met by proper systems of accountable management but there will remain a need to move away from science for those who are going to get right to the top.

Neither this alone, nor in combination with your other point of allowing greater freedom to leave the civil service (a subject on which we are currently in discussion with CSD) will resolve the main problem. This remains the need to provide the great majority of scientific staffs—who will not become Chief Scientists or Permanent Secretaries—with a pay and career structure which matches that of those of their colleagues whose job weight, background and qualifications is unquestionably in no way superior to their own. That is not the case now; we are quite determined that it shall become so.

All this is far removed from your suggestion that we may become "entirely lost in detailed quibbles", or that we are taking an insufficiently radical view of what is needed. I should have thought that our aims are precisely those which would result in scientists assuming their rightful position in the Service. As you have recognized, it will be a sorry day for science and scientists generally if we fail. We do not intend to fail; there is too much at stake.

Yours faithfully,

CYRIL COOPER

Deputy General Secretary

The Institution of Professional Civil Servants,
Northumberland Street,
London WC2

History of "Plasmas"

SIR,—There has been speculation as to the basis for Irving Langmuir's use of the word "plasma" to describe a particular region of an electrical discharge in a gas. I was working for Langmuir when he made this innovation about 1927, and, much later, described it in a letter to a friend at the General Electric Research and Development Center, dated April 20, 1967. The pertinent extract from this letter follows.

"Meanwhile word had come down from on high that we had better do something about mercury-arc rectifiers and converters, which were the big new things in the electrical engineering world, although at that time they were all in glass bulbs. So Langmuir began to study mercury vapor discharges. He shortly invented his probe, I did the experimental work and most of the mathematics, and we soon accumulated a lot of data about ion densities and velocity distributions in the mercury arc columns, in the positive columns of Geissler tubes, and in gas-filled thermionic tubes.

"We noticed the similarity of the discharge structures they revealed. Langmuir pointed out the importance and probable wide bearing of this fact. We struggled to find a name for it. For all members of the team realized that the credit for a discovery goes not to the man who makes it, but to the man who names it. Witness the name of our continent. We tossed around names like 'uniform discharge', 'homogeneous discharge', 'equilibrum discharge'; and for the dark or light regions surrounding electrodes, names like 'auras', 'haloes', and so forth. But one day Langmuir came in triumphantly and said he had it. He pointed out that the 'equilibrium' part of the discharge acted as a sort of sub-stratum carrying particles of special kinds, like high-velocity electrons from thermionic filaments, molecules and ions of gas impurities. This reminds him of of the way blood plasma carries around red and white corpuscles and germs. So he proposed to call our 'uniform discharge' a 'plasma'. Of course we all agreed.

"But then we were in for it. For a long time we were pestered by requests from medical journals for reprints of our articles. This happens to me this day. The scientific world of physics and chemistry looked askance at this uncouth word and were slow to accept it in their vocabulary. The engineering world treated it as a GE trade name. Then all of a sudden, long after I had left the laboratory, to my pleased surprise, everybody started to talk about plasmas. This happened not long before they became thermonuclear and so government subsidized. That finally put the seal of respectability on plasmas."

Yours faithfully,

HAROLD M. MOTT-SMITH

6909 SW Freeway,
Texas 77036

British Diary

Saturday, September 18

Cell Proliferation and the Differential Response of Normal and Malignant Cells, Professor L. F. Lamerton, British Institute of Radiology, at Southampton. (Silvanus Thompson Memorial Lecture.)

Tuesday, September 21

Infra-red Techniques (three-day conference) Institution of Electrical Engineers; and the Institution of Electronic and Radio Engineers, at the University of Reading.

Synthesis of Sugar Phosphonates, Professor H. Paulsen, Carbohydrate Group (Chemical Society Subject Group), at Birkbeck College, University of London, Malet Street, London WC1.

Wednesday, September 22

Fibre Optics (7.30 p.m.) Dr M. Chown, Institution of Electrical Engineers, jointly with the Harlow Engineering Society, at Harlow Technical College, Harlow, Essex.

Nuclear and Particle Physics (three-day conference) Institute of Physics and the Physical Society, at the University of Oxford.

On-Line Computer Methods Relevant to Chemical Engineering (7.30 p.m. symposium) Institution of Chemical Engineers, jointly with the British Computer Society, at the University, Nottingham.

Process Pumps (three-day conference) Institution of Mechanical Engineers, at the University of Durham.

Thursday, September 23

MOS Integrated Circuits (7 p.m.) Mr R. G. Hibberd, Institution of Electrical Engineers, at the Medway College of Technology, Chatham, Kent.

Population and Pollution (two-day symposium) Eugenics Society, in the Meeting Rooms of the Zoological Society of London, Regent's Park, London NW1.

The Consumers' Association—Its Aims and Achievements (7 p.m.) Mrs Alma Williams, Oil and Colour Chemists' Association, at the Beech Tree Hotel, Maxwell Road, Beaconsfield.

Friday, September 24

Chlorinated Rubber Marine Paints (6.30 p.m.) Mr C. G. Reid, Oil and Colour Chemists' Association, Midlands Branch, at the Chamber of Commerce and Industry, 75 Harborne Road, Birmingham.

Some Aspects of Metal Pretreatment and Printing (7.15 p.m.) Mr P. Gollop, Oil and Colour Chemists' Association, at the Royal Hotel, Bristol.

Saturday, September 25

Science Simulation Seminar, Institute of Mathematics and Its Applications, and Chelsea College of Science and Technology, at the Centre for Science Education, Bridges Place, London SW6.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Imperial College of Science and Technology, University of London. Prospectus 1972–73. Pp. 139. (London: Imperial College, 1971.) [36

Building Research Station Digest, No. 57: Painting Walls—3. Pp. 8. (London: HMSO, 1971.) 5p. [36

Meteorites: a Concise Account. By A. A. Moss. Pp. vii+26. 20p. Bulletin of the British Museum (Natural History). Entomology. Vol. 26, No. 2: The Species of *Ardeicola* (Phthiraptera:Ischnocera) Parasitic on the Ciconiidae. By P. Kumar and B. K. Tandan. Pp. 117–158+2 plates. £1.80. Bulletin of the British Museum (Natural History). Historical. Vol. 4, No. 2: A Short History of the Libraries and List of MSS. and Original Drawings in the British Museum (Natural History). By F. C. Sawyer. Pp. 77–203. £3.40. Type-Specimens of Birds in the British Museum (Natural History). Vol. 2: Passerines. By Rachael L. M. Warren and C. J. O. Harrison. Pp. vi+628. £13. (London: British Museum (Natural History), 1971.) [36

Tin Research Institute. Annual Report 1970. Pp. 36. (Greenford, Middx.: Tin Research Institute, 1971.) [46

Rothamsted Experimental Station. Report for 1970. Part 1: Pp. 385. Part 2: Pp. 259. (Harpenden, Herts: Rothamsted Experimental Station, 1971.) £2 the two parts. [76

Job Enrichment and Employee Participation—a Study. By Margaret Butteriss. Pp. 71. (London Institute of Personnel Management, 1971.) £1. [76

Proceedings of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 323, No. 1553 (8 June 1971): A Discussion on Numerical Analysis of Partial Differential Equations. Organised by J. H. Wilkinson, FRS, Pp. 151–304. £1.75; $4.55. Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 261, No. 837 (27 May 1971): A Discussion on New Developments in Electron Microscopy with special emphasis on their Application in Biology. Organised by H. E. Huxley, FRS, and A. Klug, FRS. Pp. 1–230+plates 1–44. £9.50; $24.50. Vol. 261, No. 838 (27 May 1971): On the Upper Triassic Mammals. By F. R. Parrington, FRS. Pp. 231–272+plates 45 and 46. £1.25; $3.25. (London: Royal Society of London, 1971.) [86

At Your Service 1971/1972: An Outline of Advisory and other Services, Grants and Subsidies available to Farmers and Horticulturists. Pp. 40. (Belfast: Ministry of Agriculture, 1971.) [96

Other Countries

Institute of Applied Science of Victoria. Report of Activities 1968/1969. Pp. 22. (Melbourne: Institute of Applied Science of Victoria, 1971.) [245

Unesco. Science Policy Studies and Documents No. 25: Science Policy and the European States. Pp. 208. (Paris: Unesco; London: HMSO, 1971.) 12 francs; 90p; $3. [245

The American Phytopathological Society. Monograph No. 6: Fusarium Wilt of Tomato. By J. C. Walker. Pp. 56. (St. Paul, Minnesota: The American Phytopathological Society, 1971.) $3. [245

New Zealand: Ministry of Transport. Meteorological Observations for 1967—Stations in New Zealand and Outlying Islands, including the Cook Group. Pp. 68. (Wellington: Government Printer, 1970.) [245

Brands, Generics, Prices and Quality: The Prescribing Date After a Decade. Pp. vii+118. (Washington, DC: Pharmaceutical Manufacturers Association, 1971.) *gratis.* [255

Standards in Canada. By Robert F. Legget. (A study prepared for the Economic Council of Canada and the Science Council of Canada.) Pp. 248. (Ottawa: Information Canada, 1971.) $3.25. [255

US Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. The 1965 Salt-Water Angling Survey. By David G. Deuel and John R. Clark. Pp. 51. (Washington, DC: Government Printing Office, 1968.) $0.40. [255

Ecology and Land Use of the Supply Ponds Natural Area, Branford, Connecticut. By Diana Starr Cooper and William Lee Hotaling. Pp. x+56. (New Haven, Conn.: Yale University, School of Forestry, 1970.) $2. [255

US Department of the Interior: Geological Survey. Water-Supply Paper 1929: Surface Water Supply of the United States 1961–65. Part 2: Pacific Slope Basins in California. Vol. 2: Basins from Arroyo Grande to Oregon State Line, except Central Valley. Pp. ix+673+1 plate. (Washington, DC: Government Printing Office, 1970.) $3.25. [255

Canada: Department of Energy Mines, and Resources. Geological Survey of Canada. Memoir 360: Paleozoic Geology of the Bruce Peninsula Area, Ontario. By B. A. Liberty and T. E. Bolton. Pp. 163. $4.50. Paper 70–35: Paleozoic Geology of Wilfe Island, Bath, Sydenham and Gananoqie Map-Areas, Ontario. By B. A. Liverty. Pp. 12+4 maps. $3.50. (Ottawa: Information Canada, 1971.) [255

Malaysia. Ministry of Lands and Mines. Annual Report of the Geological Survey Malaysia, 1968. Director: S. K. Ching. Pp. viii+199+20 plates. Sandakan Peninsula, Eastern Sabah, East Malaysia: Explanation of Sheets 6/337/16, 6/118/13, 5/117/4, and 5/118/1. By D. T. C. Lee. (Report No. 6.) Pp. xiii+75+36 plates. M$12. (Kuching, Sarawak, Malaysia: Principal Geologist, Geological Survey, 1970.) [255

World Health Organization. Technical Report Series No. 466: Methodology for Family Studies of Genetic Factors—Report of a WHO Scientific Group. Pp. 37. (Geneva: World Health Organization; London. HMSO, 1971.) 3 Sw. francs; 30p; $1. [16

Nova Scotia Research Foundation. Selected Bibliography on Algae, No. 11. Pp. 169. (Dartmouth, Nova Scotia: Nova Scotia Research Foundation, 1971.) [16

Ministerio de Agricultura y Pesqueria, Direccion General de Investigaciones Agropecuarias. Boletin Técnico. No. 72: El Cultivo de los Citricos en el Peru. Pp. 60. No. 73: El Cultivo del Palto en el Peru. Pp. 40. No. 74: El Cultivo del Mango en el Peru. Pp. 39. No. 75: El Cultivo de la Pina en el Peru. Pp. 32. (Lima, Peru: Ministerio de Agricultura y Pesqueria, 1969 and 1970.) [16

Smithsonian Contributions to the Earth Sciences. No. 6: Distribution of *Echinarachnius parma* (Lamarck) and Associated Fauna on Sable Island Bank, Southeast Canada. By Daniel J. Stanley and Noel P. James. Pp. 24. $0.45. Smithsonian Contributions to Zoology. No. 72: Revision of the Fish Genus *Ecsenius* (Belnniidae, Blenniinae Salariini). By Victor G. Springer. Pp. 74. $1.25. No. 73: Synopsis of the Tribe Salariini, with Description of Five New Genera and Three New Species (Pisces: Blenniidae). By William F. Smith-Vaniz and Victor G. Springer. Pp. 72. $1.25. No. 82: Pontoniinid Shrimps from the Ninth Cruise of R/V *Anton Bruun*, 110E, 1964: I. *Palaemonella* Dana and *Periclimenes* Costa. By A. J. Bruce. Pp. 13. $0.25. No. 83: The Distribution and Patterns of the Major Arteries of the Iguanids and Comments on the Intergeneric Relationships of Iguanids (Reptilia:Lacertilia). By George R. Zug. Pp. 23. $0.40. (Washington, DC; Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [16

US Department of the Interior: Geological Survey. Bulletin 1350: Lexicon of Geologic Names of the United States for 1961/1967. By Grace C. Keroher. Pp. iv+848. $3.50. Professional Paper 629-A: Upper Paleozoic Rocks in the Oquirrh Mountains and Bingham Mining District, Utah. By E. W. Tooker and Ralph J. Roberts, *et al.* Pp. iv+76. $1. Professional Paper 661: Mississippian Stratigraphy of the Diamond Peak Area, Eureka County, Nevada. By David A. Brew. Pp. iv+84+1 plate. Professional Paper 674: Stratigraphy of the Outcropping Post-Magothy Upper Cretaceous Formations on Southern New Jersey and Northern Delmarva Peninsula, Delaware and Maryland. By James P. Owens, James P. Munard, Norman F. Sohl and James F. Mello. Pp. iv+60. $0.65. Water-Supply Paper 1899-C: Sediment Transport by Streams in the Palouse River Basin, Washington and Idaho, July 1961/June 1965. By P. R. Boucher. Pp. iv+37+plate 1. $0.70. (Washington, DC: Government Printing Office, 1970 and 1971.) [16

Publikationer fra det Danske Meteorologiske Institut, Isforholdene i de Grønlandske Farvande, (The Ice-Conditions in the Greenland Waters), 1964. Pp. 25+32 Ice-Charts. (Charlottenlund, Danmark: Det Danske Meteorologiske Institut, 1971.) [16

US Department of the Interior: Geological Survey. Water-Supply Paper 1932: Surface Water Supply of the United States 1961/65. Part 12: Pacific Slope Basins in Washington. Vol. 1: Pacific Slope Basins in Washington except Columbia River Basin. Pp. ix+679+1 plate. (Washington, DC: Government Printing Office, 1971.) [26

Smithsonian Contributions to Zoology. No. 78: A Manual for the Study of Meiofauna. By Neil C. Hulings and John S. Gray. Pp. xi+83. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) $2.75. [26

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 139: Der Gelbrost, Piccinia Striifromis West. II. Befallsbild, Morphologie und Biologie der Sporen. Infektion und Weitere Entwicklung. Wirkingen auf die Wirtspflanze. Von Professor Kurt Hassebrauk. Pp. 111. 32 M. Heft 140: Beitrag zur Kenntnis der *Phytophthora*—Arten in Iran und ihrer Phytopathologischen Bedeutung. Von Dr. Djafar Ershad. Pp. 84. 33.95 M. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1970 und 1971.) [26

Annals of the New York Academy of Sciences. Vol. 171, Article 2: Down's Syndrome (Mongolism). By V. Apgar *et al.* Pp. 303–688. (New York: New York Academy of Sciences, 1970.) $24.50. [26

Annual Summary of Information on Natural Disasters, No. 4, 1969. Pp. 73. (Paris: Unesco; London: HMSO, 1971.) 10 francs; 75p; $2.50. [26

Recueil de Travaix du Bureau International de Poids et Mesures, Volume 2, 1968–1970, 29 reprints. (Sèvres, France: Bureau International des Poids et Mesures, 1971.) [26

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wheat Research Unit 1969/1970. Pp. 26. (Ryde, NSW: CSIRO, 1971.) [46

US Department of the Interior: Geological Survey. Water-Supply Paper 1899-J: Ground Water for Irrigation Near Lake Emily, Pope County, West-Central Minnesota. By Wayne A. Van Voast. Pp. iii+28. (Washington, DC: Government Printing Office, 1971.) $0.25. [46

Smithsonian Contributions to Zoology. No. 68: A Systematic and Ecological Study of Nearctic Hydrellia (Diptera:Ephydridae). By D. L. Deonier. Pp. 147. $1.75. No. 79: Western Atlantic Shrimps of the Genus *Metapenaeopsis* (Crustacea, Decapoda, Penaeidae), with Descriptions of Three New Species. By Isabel Pérez Farfante. Pp. 37. $0.45. No. 94: Type Specimens in the Hans Eggers Collection of Scolytid Beetles (Coleoptera). By William H. Anderson and Donald M. Anderson. Pp. 38. $0.45. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [46

World Meteorological Organization. WMO Sea-Ice Nomenclature: Terminology Codes and Illustrated Glossary. Pp. 147. (Geneva: World Meteorological Organization, 1970.) [46

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:
12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Airfreight Canada
Nature (Friday)	**£14**	**$48**	**$52**
Nature+ Nature Physical Science	**£24**	**$83**	**$90**
Nature+ Nature New Biology	**£24**	**$83**	**$90**
All three editions	**£29.50**	**$108**	**$116**
Annual Index	**£1**	**$3**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431

American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004

All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone: 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, September 17 1971

NATURE Volume 233 SEPTEMBER 24 1971

Why Not to Test Cannikin

THE United States Administration seems bent on making everybody wait until the last minute before knowing whether there will actually be an underground explosion of a five megaton weapon in the Aleutians in October. Both the Department of Defense and the Atomic Energy Commission have said that they see no reason why the Cannikin test should not take place, but President Nixon is wisely determined to make the final decision himself. After all, it is he and not the designers of the warheads for the Sprint ABM rocket who will have to travel to Alaska two weeks from now to spend an afternoon with the Emperor of Japan. He or the Department of State, and not the technical people, will also be the first and principal recipients of any trouble which may come from the way in which the Cannikin test is likely to offend the Soviet Union and disturb the SALT negotiations in Helsinki. And finally, of course, with the Atomic Energy Commission and the Department of Defense in constant trouble on issues as various as the proper siting of nuclear power stations and the continuing war in Vietnam, it is understandable that President Nixon should find it necessary either to fortify a decision to go ahead with Cannikin by the prestige of his own office or—perhaps—to abandon the project at the highest level.

In all the circumstances, the arguments against the Cannikin test need to be appreciated for what they are—chiefly political and diplomatic but also technical. After all, the Cannikin explosion will amount to five megatons. In previous underground explosions, the AEC has moved gingerly towards one megaton. The most powerful underground explosion so far under American auspices, the Milrow test carried out in the Aleutians in 1969, was, to be sure, a fairly reasonable sign that Cannikin will do very little damage. It is improbable that the event will trigger off tsunamis. It is also improbable that the worst fears of the protesters will be realised and that earthquakes will follow the explosion. But there is a greater risk of a leakage of radioactive material from the site. No doubt the cautious fellows at the AEC have done all they can to ensure safety, but it is only as recently as December 1970 that a miscalculation led to a leakage of radioactivity from an underground explosion in Nevada. That was only a small test, but even so it stopped the weapons testers in their tracks for close on half a year. Can they now be sure that the much larger undertaking in the Aleutians planned for October will be safe? And are they in a position to predict what diplomatic fall-out there will be if radioactive material should find its way either to the Soviet Union or to Canada?

Another and more serious issue is the extent to which such an obvious test of a warhead for an ABM may upset the negotiations at Helsinki. It is true, of course, that the issue is by no means simple. In the past few weeks it has begun to seem as if an agreement on the mutual limitation of anti-ballistic missiles would be the chief component of an agreement between the Soviet Union and the United States on the limitation of strategic weapons. The fact that a comparatively small item in the package, the agreement that there should be an improved method of instant communication between Washington and Moscow based on a communications satellite, was made public prematurely last week is a sign that there are serious difficulties in reaching a more comprehensive treaty. In circumstances like these, to be sure, it may be entirely appropriate that one potential signatory or the other should be seen to be pushing vigorously ahead with preparations for defence—if the Soviet Union earnestly desires an agreement on ABMs, testing by the United States may help to encourage an agreement. The difficulty, unfortunately, is that the Russians have been talked into believing that ABMs are bad. A show of obduracy may therefore be more a provocation than a pressure in the right direction.

It is possible, of course, that President Nixon has made an exactly similar calculation and that he intends, at the last minute, to call a halt to the Cannikin test. In that case no lasting harm will have been done. But if the explosion goes ahead as at present planned, it will be a great misfortune if the managers of the AEC are able to persuade themselves and the Administration, as they have done before (see page 230), that opposition to this event is entirely unfounded.

Creating a Professional Labour Market

THERE is in Britain a remarkable coincidence of view emerging about the steps which need to be taken to bring the output of the universities and other institutions of higher education more accurately into line with the demand. In the past few months (see page 225), pure mathematicians have been told by the Science Research Council that they must modify postgraduate courses in such a way as to produce graduates who are at once more broadly based than in the past, by which the Science Research Council implies a greater awareness of the fields of study in which pure mathematics can be profitably applied. The fact that some mathematicians are up in arms about this advice, and are pleading that such instructions entail an infringement of academic freedom, should be no surprise. This, after all, is what physical scientists said when the Science Research Council first embarked on its policy of increasing the proportions of students on short advanced courses in the entire population of postgraduates. To some extent, the mathematicians who protest will no doubt learn a lot to their discomfort in the report, issued this week, of the Working Group on Biological Manpower (see page 224), which comes out with an endorsement of the Science Research Council's old policy which is in turn endorsed by no less a person than Sir Frederick Dainton. On the face of things, at least, the Science Research Council will have no difficulty in winning its case. That, however, is only half the battle. It is also important to understand just why these policies are now in vogue.

The first thing to be said is that universities are by no means in scorn of academic ideals if, for some reason or another, they seek to educate students in ways which are considered a preparation for life in the outside world. It is, however, important that such a concern does not imply that higher education should consist of what is more commonly known as vocational training. Indeed, it should by now be apparent to all concerned that many of the difficulties which science graduates are experiencing in finding jobs are simple consequences of the narrow base of their education, however academic its character. Indeed, the university curriculum in science has over the years become a strange amalgam of education in the broadest sense, and training in techniques which are essential preparations for careers in professional science. The result, quite understandably, is that a great many scientists are hampered by their training from moving easily from one branch of science to another. The implications of this state of affairs for the labour market in professional science, clearly defined by the Working Group on biological manpower, are profound.

Jobs in professional science, in teaching, research or development, are bound in a relatively stable society to increase at a measured pace. The numbers of people employed in research, for example, are unlikely to grow much faster than the Gross National Product although, of course, there will be variations from one industry to another. To be sure, the pace of expansion in fields such as medicine may grow with great speed as a result of policy decisions to invest more social capital in health, but these are exceptional circumstances. And it is beyond dispute that all societies are likely in the next few years to want to increase their commitment to higher education as quickly as their commitment to any other form of expenditure. The consequence, of course, is either that courses of higher education which are vocational or partly vocational in character will grow less quickly than the universities as a whole, or that they they will keep pace with the general rate of expansion, but turn out graduates unable to find jobs. The figures for British higher education are striking enough. The output of graduates of all kinds was in the late sixties growing at 23 per cent a year at a time when the conventional demand for biologists, one of the most buoyant parts of the labour market in science, was growing at only 10 per cent a year. It has been conventional wisdom for several years to say that the growth of secondary education entails changes in the school curriculum, particularly in the sixth form. So far as undergraduate education is concerned, the only novelty in the present situation is that the same effect has been translated into higher education.

By good luck, or perhaps even by good judgment, there have in the past few years been some important experiments in the design of broader university courses of a kind likely to turn out graduates who are likely to be comparatively flexible in their choice of a career, either within science as a whole or in such a way that a scientific background is considered an important part of a general education. Without in any sense decrying the value of the boldest experiments of all, the attempts to introduce into universities thoroughly liberal courses of scientific study, much must be said in favour of more arbitrary marriages between courses of different kinds—physics and economics, for example. To be sure, in these respects British universities and other institutions of higher education are still much more narrow than their counterparts elsewhere, especially in the United States, but there is at least a chance that some of the new courses being tried out may have the advantages of breadth, and of the discipline that goes, or is supposed to go, with depth. Although in the past, universities (especially in Britain) have been inclined to think that new courses or combinations of courses can be launched after a sufficiently long period of committee meetings within the faculty, the truth is that much remains to be done before there is an objective appreciation of the advantages of different methods of educating science undergraduates more broadly. All those who pronounce on the subject, the Science Research Council and the Working Group on biological manpower included, have an obligation to say as much and, when they have the power, to spend money on this cause.

The issue of postgraduate education is more complicated. For four years, the declared policy of the Science Research Council has been to maintain in postgraduate courses a constant proportion of those taking first degrees in science and engineering, but to ensure that increasing proportions of the postgraduate students will follow courses of advanced study lasting one or two years. In other words, although the numbers of PhD graduates will continue to increase, the proportion of first degree graduates completing PhD courses will at the same time decline. It is of course important that this policy has been linked with the deliberate attempt by the research councils to divert science students towards practical studies and with the policy of selection and concentration by means of which some departments in certain universities have been encouraged to develop in directions in which they excel. Those pure mathematicians who are now protesting might equally well have cried scandal at any time in the past five years, for the intentions of the Science Research Council have been declared policy all along. Moreover, the question asked about them is not whether they entail an infringement of academic freedom whatever that may mean, but whether they are in the best interests of students and of society at large.

There is at least some reason to believe that the attitudes towards PhD courses which are now becoming conventional are too draconian. To be sure it is easy enough to argue that if undergraduate courses in British universities have a dangerously strong vocational flavour, then PhD courses, which prepare people for research and virtually nothing else, are even more restrictive. This is one of the reasons why the Science Research Council has come down strongly in favour of postgraduate courses which last for only one or two years and, with such a pattern, it is of course easy to see how the selection of PhD students might be carried out in such a way as to provide cohorts of young men and women even more dedicated to research and the ivory tower than they are at present. The flaw in the argument is of course the assumption that a long postgraduate course consisting to a large extent of original research is exclusively a preparation for a career in research or academic life. To be sure, it is remarkable that in the past few decades, even such a narrow preparation has made it possible for growing proportions of the PhD population to work at other tasks. Moreover, if a scientific education may be a good intellectual preparation for a general career, may not a properly organized PhD course be still better? This is one question that should be answered objectively before too much is done to concentrate PhD courses still more narrowly on the needs of research and the universities, for that will only create a set of circumstances in which the assumptions underlying the new policy become self-justifying. And as at the undergraduate level, so in the graduate schools, nobody knows what kinds of curricula would exploit these opportunities most efficiently. In short, those committees which are inclined to follow the Science Research Council and the biological manpower committee in asking for smaller proportions of PhD students should first of all apply themselves to learning more about the functions of postgraduate education.

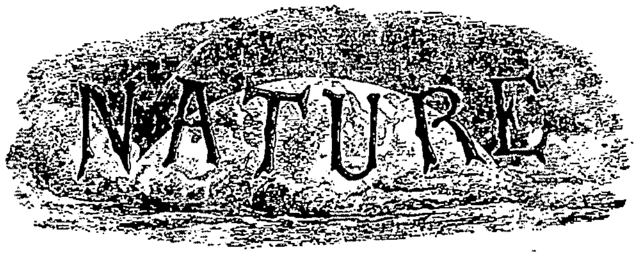
Pugwash Joins the Establishment

As the years go by—there have been fifteen of them so far—the Pugwash conferences have become respected and even respectful. The twenty-first conference, held at Sinaia in Romania at the end of August, seems to have been one of the most sober and constructive so far. Certainly the document now published by the Pugwash Continuing Committee is much more closely argued than many of its predecessors. For what the declaration of a private group of people is worth, the document also serves to bridge many gaps which have on previous occasions been contentious issues in Pugwash and elsewhere. On this occasion, for example, it seems to have been widely agreed that anti-ballistic missiles should be abandoned by the nuclear powers and that indeed the SALT agreement should not merely limit the possession of ABMs by the United States and the Soviet Union, but should actually abolish them. The conference appears to have taken a cheerful view of the decision to ban biological weapons, and the throng of signatories to the Geneva Convention in the past few months, but in pleading that governments should not therefore be diverted from the search for an agreement on chemical weapons, the delegates to the Pugwash meeting appear to have been divided—as indeed their governments are—on the need for on-site inspection. Sensibly enough, the meeting has reaffirmed its belief in a comprehensive test-ban treaty as well as its scepticism about the supposed economic benefits of nuclear explosions for peaceful purposes.

The surprising familiarity of these views does not of course imply that Pugwash has ceased to be a valuable institution. Over the years, it has demonstrated that it can at times of stress serve as a valuable channel of communication between East and West. It is a fact, even if now a comparatively old fact, that Pugwash helped enormously in the negotiation of the partial test-ban treaty in 1963 and 1964. As it has developed, the organization is no doubt regarded by all governments as a valuable listening post, to say nothing more of it. In the circumstances, however, it may not be surprising that Pugwash has fallen into one or two familiar traps, at least as far as can be told from its latest declaration. In Romania this year, there seems to have been a general feeling that political circumstances now favour an international conference on European security along the lines of the proposal being canvassed by the government of Finland. The notion is that there should be to begin with a mass renunciation of the use of force and of tendencies to interfere in other people's internal affairs, and the recognition of boundaries, renegotiated as necessary. The latest statement includes a string of detailed proposals about the regulation of tactical nuclear weapons in Europe that might be a sensible basis on which to construct a more permanent agreement. Outside Europe, on the other hand, the statement of the Continuing Committee consists largely of hand-wringing over the war in Indo-China and the renewed threat of war in Korea. But is this not too Western a view of what needs to be done to make the world a safer place in the decades ahead? Pugwash is wise not to chase some of the hares which used to distract its attention, but is there not a strong case for keeping permanently on its agenda the search for the issues which may replace the threat of a European war in the seventies and eighties? Should not, for example, Pugwash be working hard at the design of international institutions likely to help with such things as the regulation of fish or telecommunications, two obviously contentious issues? Another trap into which there is a danger that Pugwash might fall—the latest statement from Romania skirts carefully around the edges—is the tendency to repeat clichés about the environment.

---

100 Years Ago



When ocean cables were first submerged, various apprehensions of probable injury were entertained, some of which have proved to be well founded, and others less so. It was supposed that worms or mollusks would burrow in the substance of the envelope, and ultimately penetrate to the centre of the wires; or, again, that the attachment of barnacles, mollusks, or other marine animals on the exterior would invite the attacks of the sharks, rays, and other fish of powerful jaws, and induce them to subject the bunch of matter to such a mastication as should produce serious harm to the cable. To what extent any accidents have happened from this source it is perhaps difficult to say; but we now learn from *Harper's Weekly* that the Florida cable between Punta Rosa and Key West has been injured in numerous places, as supposed by sea-turtles biting through or crushing it in their teeth, to such an extent as to destroy its continuity. It is, perhaps, a question whether the turtle be chargeable with these operations; and we think it is quite as probable that, under the circumstances, some ray or other fish has attacked it, and for the reasons already suggested.

From Nature, 4, 436, *September* 28, 1871

OLD WORLD

ENVIRONMENT

Global Watch

from a Correspondent

Canberra, September

A FRUITFUL meeting in Canberra on September 1–3 has ensured that several ambitious and promising proposals for putting global environment monitoring on a firm scientific footing will be put to the Stockholm Conference on the Human Environment in June 1972. The proposals, prepared by working groups of the Special Committee on Problems of the Environment (SCOPE) of the International Council of Scientific Unions (ICSU), were adopted at SCOPE's first general assembly here. The chief proposal is for a world-wide monitoring system aimed at helping to detect significant and potentially harmful changes in the environment early enough to take preventive action, and eventually to predict harmful trends before they occur. To back this up, a SCOPE committee is examining methods of analysis for major pollutants in air, water and biological tissue and hopes to make recommendations on the best methods for measuring each pollutant by the end of 1972.

The proposals were prepared at the request of Mr Maurice Strong, the Secretary-General of the Stockholm Conference, who was an invited guest at the meeting. They call for the regular collection of data from a global network of a hundred or more selected areas exposed to minimal, medium and intense human impact. Every nation is being urged to set up a national monitoring office to integrate its own monitoring effort and to contribute to international coordination. Also existing monitoring activities would be fed into the system.

The working group listed three sets of problems which deserve the highest priority for global monitoring: potentially adverse climatic changes resulting from human activities; potentially adverse changes in biota and man from contamination by toxic substances; and potentially adverse changes in biological productivity caused by improper land use. Highest priority is given to atmospheric measurements of turbidity, carbon dioxide, surface solar radiation and standard meteorological variables; monitoring mercury, lead cadmium, DDT and polychlorinated biphenyls in air, water, soils and biota; keeping check on a variety of data reflecting the state of human health; and implementing studies of vanished or endangered ecosystems and vertebrates and of the population size and distribution of birds. The documents say that research activities and pilot studies are essential for defining and evolving detailed monitoring programmes where necessary, and recommend the establishment of internationally funded and staffed laboratories in at least three monitoring areas subject to minimal human impact.

Mr Strong told the assembly there had been a gratifying response to requests for contributions to the conference from governments, United Nations agencies and scientific organizations. He predicted that the main constraints on necessary environmental projects after Stockholm would be political unwillingness on national levels, not a lack of sufficient ingredients for action. Mr Strong also said that another conference, similar to that at Stockholm, might be needed within three or four years, and argued a case for continuing and consolidating the present organization for Stockholm—in effect advocating a new UN agency for the environment. This proposal runs counter to the views of the existing UN agencies with fingers in the environmental pie (for example, UNESCO, FAO, WHO) and the scientific unions, who see it as, on the one hand, a threat to their existing responsibilities and, on the other, as an unnecessary proliferation of international bureaucracies. In pressing his case, Mr Strong said "I don't think we are at a sufficient state of readiness in terms of knowledge and particularly political willingness, to do all the kinds of things that are going to be necessary".

BIOLOGICAL MANPOWER

Broadening the Base

PROFESSIONAL biologists are the latest to be told that their education should be broader and that they must be more ready to change disciplines in mid-career. The *Report of the Working Group on Biological Manpower* (HMSO Cmnd Paper 4737, £0.85), published this week, also echoes previous declarations by, for example, the Swann committee (of which it is a descendant) that qualified scientists must broaden their concept of what constitutes a professional job as the output of the universities and other institutions of higher education grows so as to outstrip what is at present considered to be the demand. The working group, the chairman of which is Dr Richard D. Keynes, director of the ARC Institute of Animal Physiology at Cambridge, is, like other bodies, anxious that university departments and grant-giving bodies should in future discriminate more carefully between graduate students who can make proper use of a PhD course and those who have less aptitude for original research.

Sir Frederick Dainton, chairman of the Council for Scientific Policy, is one who plainly finds the group's recommendations to be acceptable. In his introduction to the report, he says that "much of the report could be applied to science in general". Taking his cue from his own report on the subject, now five years old, he goes on to say that "it will have to be recognized increasingly that on the one hand a specialist scientific education will not guarantee employment at a professional level in the subject of a graduate's specialization" and that graduates in science have valuable jobs to do outside the confines of their profession.

The members of the group are as follows: Dr H. O. J. Collier, Mr D. J. B. Copp, Dr F. A. E. Galley, Professor J. M. Mitchison, Professor S. J. Pirt, Professor M. M. Swann and Professor C. P. Whittingham. Apart from statistics which exist already, the working group had access to the results of employment surveys carried out within the research councils and a questionnaire survey within industrial organizations.

By 1968, the 10,918 biologists employed in Britain accounted for more than ten per cent of all scientists, but their numbers were growing more quickly, at an annual rate of 5.5 per cent, compared with 4.4 per cent for scientists as a whole. Understandably, most biologists appear to be teachers at some level, 8,100 of them in schools, universities and further education. These calculations, of course, omit those who work in medicine, dentistry and health, more than 95,000 in 1966.

The essence of the employment problem identified by the working group is that the annual growth of the production of first degrees in biology is accelerating and amounted to 19.9 per cent per year in the late sixties, while the annual percentage growth of biologists with higher degrees amounted to 21.7 per cent per year. These rates of growth are greater than the corresponding rates in other branches of science, but not markedly so. On the other hand, the number of extra jobs likely to arise each year by natural causes is estimated to be something between rather more than 1,000 and rather less than 1,200 qualified people, or roughly ten per cent of the working population of qualified biologists. Either the remaining ten per cent have to be unemployed or have to find jobs elsewhere.

On the broadening of the curriculum, the working group says that this is the best way of minimizing "the undesirable effects of an imbalance between the supply of biological graduates and the demand for their services in specifically biological fields". It recognizes that one consequence must be more mathematics and physics in the training of biologists at all levels. The working group is a little heartened to see that only 33 out of 44 universities now offer separate courses in botany and zoology. At the postgraduate level, the working group is a little taken aback to find that employers seem to prefer what it calls "conventional" postgraduate courses but unabashed, and suspecting that the predilections of government research establishments have had an undue influence in previous inquiries, the working group goes on to say that the PhD course should be improved. It fears that too many specialist PhDs might mean "individual frustration and unhappiness" and asks for bold experiments, for more stringent selection for PhD courses and for graduate courses aimed both at providing professional people with up to date information and with matching the education of biologists to the non-professional jobs which they will increasingly be required to take.

MATHEMATICS

Pressure of the Purse

MATHEMATICIANS in Britain are concerned that academic freedom may be infringed by an apparent change of policy by the Science Research Council Mathematics Committee. A protest on the subject from Professor W. B. Bonnor of Queen Elizabeth College, University of London, appeared in *The Times* last week. He complained that the SRC is instituting standards other than those of academic excellence in allocating research studentships.

The issue has been brewing since March this year, when heads of mathematics departments at British universities were told in a letter from the secretary of the SRC Mathematics Committee of anxiety about career prospects for MSc and PhD graduates in pure mathematics, many of them pensioners of the SRC. The committee said that in the next few years, research in pure mathematics is likely to offer fewer opportunities for jobs than the number of students being trained and that in the circumstances it would be prudent for mathematics departments to broaden the training given to graduate students by means of courses in applied mathematics, theoretical physics, engineering subjects, computer science or business economics. In March, mathematics departments were asked to let the Science Research Council's committee know how they were responding.

Fuel was added to the flames of protest at the end of June by a second letter asking that within twelve months the committee should be told what had been done and saying that it would then "hope to feel well-enough informed to be able to reflect departments' efforts in next year's quotas of studentships".

Professor Bonnor sees this as an attempt to dictate how mathematics should be taught at postgraduate level. He said last week that "although a case can be made for what the SRC is trying to do, there is no justification for making mathematics departments carry it out by threatening their financial support." He also said that the SRC's suggestions were ill-conceived. First, a course of twenty or so lectures on any of the suggested topics would not help students to find jobs. Second, such courses of lectures would lower the standard of the PhD degree. Third, courses would not make students more valuable to employers. Professor Bonnor is only a little more cheerful about intensive courses after completion of the PhD course. He emphasizes that the only criterion that should determine the support for a university department is the excellence of its research.

A written explanation from Sir Brian Flowers, chairman of the Science Research Council, to Professor Bonnor says that "the SRC would not dream of dictating to mathematics departments how they should do their research or teach their postgraduate students and I cannot see that anything that has been written implies this; however, we do hope to encourage mathematics departments to think about the design of their postgraduate activities in the light of the fact that many of the students almost certainly will eventually be seeking employment outside the purely academic field".

Heads of other mathematics departments share Professor Bonnor's views but less strongly. Professor G. Higman, University of Oxford, said last week that his department had answered the letters but that unemployment of Oxford PhD mathematics students is not a problem—general courses for mathematicians are, however, already offered at Oxford. Professor D. J. Bartholomew, University of Kent, is concerned about the implications of the letters and feels that as a matter of principle the SRC should not dictate to departments on matters of curriculum. Professor H. B. Griffiths, University of Southampton, said that the SRC request does not necessarily infringe academic freedom but that the mathematical postgraduate teaching at Southampton had already been "broadened".

The Association of University Teachers is particularly interested in Professor Bonnor's claim and it is on the agenda for discussion at the AUT's meeting in Bath today. Dr Sheila Brenner, senior vice-president of the AUT and chairman of the AUT's education committee, said earlier this week that "although the AUT expects graduates at both first and higher degree level to tackle a variety of problems in industry and other occupations, it is not the university's function to give them job training." She went on to say "Our general opinion is that external direction to educate students in particular ways may well prove counter productive in the short run and in the long term will be damaging to the national interest".

Dr Brenner also said that "many university departments are under considerable financial pressure at the moment and while we would welcome additional funds for those departments which have plans for experiments to improve the education they offer their students, we shall watch carefully to see that this is not done at the expense of departments which do not consider a particular pattern of education appropriate."

RESEARCH AND DEVELOPMENT

No Wolf at the Door?

DR BASIL BARD, managing director of the National Research Development Corporation since July, seems unperturbed at the Docksey Inquiry for the Department of Trade and Industry into the NRDC's arrangements for the exploitation of inventions. The inquiry could mean that the NRDC's days are numbered, but Dr Bard, speaking after the publication of the corporation's Twenty-second Annual Report (HMSO, £0.45), said that until the results of the inquiry became known towards the end of the year, the NRDC was carrying on as usual and that he had "no reason to believe that we won't carry on substantially as usual" after the results become known, with possibly some changes of direction and emphasis. In the report Dr Bard welcomes the inquiry, and says "it seems to us timely that the corporation and its work be reviewed in the light of the present-day and future requirements of the country", adding that "the salient figures for the past four years offer in our view a reasonably encouraging picture" of the corporation's work.

The report shows that the corporation's income exceeded £6 million for the first time and that its revenue account showed a small surplus (£68,960) for the third successive year.

The activities of the corporation expanded during the year with 96 new development projects being undertaken and with 404 projects receiving support compared with 377 in the previous year. Of these projects 186 are joint ventures with industry. The corporation's investment in development projects rose from £5.8 million in 1969–70 to £5.9 million last year, and recoveries from the projects amounted to £2.4 million last year compared with £1.3 million the year before.

The corporation has a borrowing limit from the Department for Trade and Industry of £50 million with which to finance projects, and £24.8 million of this was in use to September 1971, £1.3 million more than at the same time last year. The actual annual borrowing for the year fell from £4.88 million in

1969–70 to £2.85 million. Licence income from patents, the corporation's biggest source of income amounted to £3.7 million, of which £2.7 million were foreign currency earnings. The drug cephalosporin C was again the largest revenue earner.

Submissions of new projects for support rose from 2,194 to 2,535, but the numbers presented by the universities and industry both fell, the increase coming from submissions by private inventors. The corporation felt that the fall in applications from industry was "a possible reflexion of the cutting back of research and development by firms encountering cash flow difficulties". Commenting on this Dr Bard said that he was convinced this was "only a hiccup" in the growth of research and that it would be "the greatest mistake at the present time to cut down on research and development".

RESEARCH AND DEVELOPMENT

Small Firms Feel Pinch

A REPORT on *Scientific and Engineering Manpower and Research in Small Firms* (HMSO, 40p), published last week, concludes that the use of scientific and technical personnel in small companies may be linked to the capital intensiveness of the industry concerned.

The report, one of the research studies commissioned by the Bolton Committee of Inquiry on Small Firms, sets out to examine the degree to which small companies—those with between eleven and 200 employees—are in a position to benefit from, and contribute to, the technological advance of British industry.

The report shows that small companies employ about a third (2.5 million) of all employees in manufacturing industries, but only 15.5 per cent of the equivalent of graduate qualified engineers, technologists and scientists (QSEs). (Figures are for 1968.) This is a proportion of QSEs to other employees of 0.7 per cent, only half that of larger companies. The distribution within small companies is not even, but small electronics companies alone have a higher proportion of QSEs than their larger competitors. The proportion of QSEs in small electronics companies is higher than in other small companies because research in electronics is a rapidly advancing technology at a highly theoretical as well as a purely practical level, and because "materials are relatively inexpensive and large capital facilities are unnecessary". Innovation in fields such as mechanical engineering requires a bigger "technological step" and large expenditure on the trial and testing of new products.

This led to the conclusion that capital-intensiveness may be the key to QSE employment in small companies, and the observation that many small companies rely on contracts with outside laboratories for their research work as this limits the extent of their expenditure and removes the necessity of maintaining their own full-time research facilities.

The expected growth rate for small companies from 1968–71 was 27.1 per cent compared with 20.9 per cent for all companies. If the report's suspicions about a "technological step" are right, then this "implies a barrier to greater employment of QSEs and further technological advance".

MUSEUMS

Timbers to Greenwich

from a Correspondent

THE National Maritime Museum (NMM) at Greenwich is advertising for an archaeologist to be appointed at the relatively elevated rank of Assistant Keeper. This is certainly novel, and may be thought bizarre, but it is the direct result of the archaeological "rescue" of the Graveney boat unearthed in Kent salt marshes last October (*Nature*, **228**, 402; 1970) and since then carbon-dated by the British Museum as late ninth century.

The new director of the museum, Mr Basil Greenhill, has a number of sweeping plans for a new look for the NMM and one of them is to extend the exhibition of boats on display. Apart from the *Cutty Sark* nearby, the museum has relied on its superb collection of contemporary models and numerous fine marine paintings to demonstrate the art of shipbuilding. At present its largest gallery, the Victorian Neptune Hall, is being converted to accommodate boats of different periods, not all of them small, such as the 1900 paddle-wheel tug *Reliant* whose fore-end including the bridge and engine room will form the centrepiece of the display when the hall is reopened next year. The opportunity of adding a pre-Conquest boat to the group was too good to miss although, by any estimate, the Graveney boat is no beauty.

Very considerable expertise is required to derive the full information retained in the lines and timbers of an antique vessel especially when it is sodden with salt water. The expectation is that the successful candidate, whether his or her training is in archaeology, anthropology or naval architecture, will have to acquire a number of other special skills. There is nowhere to train in the field of nautical archaeology, so the unit's archaeologist will have to resort to self-improvement. Besides looking to the eventual display of the original Graveney boat in Neptune Hall, there is a plan to build a replica and row it across the North Sea as the Anglo-Saxons did when they journeyed between Kent and the Continent. Altogether some £50,000 has been committed to this unit.

Once the unit is in existence it will obviously do more than simply process the Graveney boat; a committee of experts will screen other candidate timbers or craft for treatment and preservation. This will provide a unique facility for Britain. There are few other places in the world where waterlogged wood up to 50 foot long can be handled. Will there be a rush of old timbers to Greenwich? One thing is clear—the line is being drawn at boats, and specifically boats less than 50 foot long. There is no possibility that the eighteenth century East Indiaman, *Amsterdam*, buried at Hastings, or Henry V's *Grace Dieu*, still in the mud of the Hamble river, will be imported to the Greenwich tanks.

EDUCATION

Computing in Schools

THE view that teacher training is the most urgent need in the expansion of computer science teaching in secondary schools is put forward in a report of an international seminar held in Sèvres during March, 1970 (*Computer Sciences in Secondary Education*, OECD, Paris; 1971 ; £1.90).

The report, like the papers presented at the six-day meeting, recognizes the need for several types of school computing course, which could be taught by teachers with quite different educational backgrounds. These include appreciation courses which require almost no computer time and could, in principle, be taught by any numerate teacher, and other courses which contain more instruction in programming and in the working of computers; these would have to be taught by mathematicians or scientists. Ultimately, says the report, the aim should be to include at least a computer appreciation course in all secondary school teacher training.

Much of the seminar was taken up with discussions of the significance and objectives of the teaching of computer science in schools. There was agreement that one of the important functions of computer education is the encouragement of skills in organizing and handling information, which are useful in the study of most subjects and which help to convince pupils that information is a means to an end but not an end in itself.

Even the simplest appreciation course requires a certain amount of access to a computer, if the subject is not to appear to be too abstract, and the report suggests that agreements should be encouraged which allow pupils' programs to be run on computers belonging to local authorities, universities and companies. Twenty of the 160 local authorities in England and Wales, for example, encourage the use of their management service computers for educational purposes and about 30 per cent of schools which run computing courses make use of these facilities.

AIRPORTS

Up, Up and Away

For the first time, each of the four airports of the British Airports Authority has shown a net profit over a financial year—that ending March 31, 1971. Taken together, Heathrow, Gatwick, Stansted and Prestwick have been profitable since 1962, but only because the large profits at Heathrow have more than compensated for losses elsewhere. Although the total profit in the past financial year is seven per cent less than the record of £9.5 million in 1969–70, there is obviously merit in the even spread of profit. Edinburgh airport, handed over to the authority on April 1, 1971, is also budgeted to show a small profit in the current year.

Apart from economic considerations, recent months have been marked by the controversy over the siting of the third airport for London, and the final decision, made on environmental grounds, in favour of Foulness. This decision is discussed in the recently published annual report and accounts of the British Airports Authority (HMSO). The authority's view is that none of the four sites on the Roskill Commission's short list is really suitable for a major new airport and that of the four, Foulness is, on economic grounds alone, the least suitable.

Other environmental issues also appear less disheartening than when the 1969–70 report of the authority was published. The noise problem remains, but it has been shown that jet engines are much cleaner than those of surface traffic, so that in the past year—the first in which jets accounted for more than half of the world's airline fleet—airports had less concentrations of pollution than ordinary urban districts. Concentrations of smoke and sulphur dioxide recorded at Heathrow in the summer of 1970 demonstrate this dramatically (see Table).

Table 1 Comparative Average Concentrations of Smoke and Sulphur Dioxide in the Atmosphere at Heathrow, Summer, 1970

In Westerly Winds	From the West (outside the airport)	From the Airport
Smoke	16	15
SO$_2$	152	59
In Easterly Winds	From the East (outside the airport)	From the Airport
Smoke	29	26
SO$_2$	208	91

What of the further growth of air traffic, and the difficulties of coping with this without damaging the environment? Between 1960 and 1970, the number of flights to and from British airports grew from 402,000 to 607,000, while the average number of passengers per air transport movement increased from 25 to 52 as the size of average loads increased. The authority expects that the average load per flight will continue to increase by six per cent a year throughout the 1970s, and that by 1985 the airports of the UK will be handling, on a "middle" forecast, 130 million passengers, 5 million tons of cargo and 1.1 million flights a year. In 1970, the equivalent figures were 32 million passengers, 0.5 million tons of cargo and 600,000 flights. It will, indeed, be a "substantial task" to meet these demands, even if the Foulness development lives up to its proponents' greatest expectations.

Miscellaneous Intelligence

Britain's problems of what to do with some of its graduates and school-leavers do not seem to be shared by the Soviet Union. Mr Boris Kostin, head of the Moscow Employment Board claimed in a recent interview in the Soviet press that no school-leaver in the Soviet Union has any difficulty in getting a job. Those without any professional skills are trained on leaving school for several months at half pay, and a wide choice of jobs awaits them upon completion of training. Moscow in particular has an "insatiable" demand for workers in the service spheres, and for clerical and municipal workers. Moscow's largest construction company alone is building more than 100,000 flats a year.

Between 1960 and 1970 the number of visitors to those of Britain's principal monuments in the care of the Department of the Environment almost doubled from 6,800,000 to 12,500,000 and total fees received quadrupled to almost £1.1 million, a sizable part of the department's 1970 budget for ancient monuments of £2 million.

These figures were quoted by Dr A. J. Taylor, Chief Inspector of Ancient Monuments and Historic Buildings, in Hamburg, where one of the department's two touring exhibitions of "Historic Monuments in Britain" is showing this week. The two exhibitions have toured Britain and the mainland of Europe (the second exhibition is now at the Banqueting House, Whitehall) and the exhibition now at Hamburg will go next to Tokyo.

Scientists from the Soviet Arctic and Antarctic Institute have been studying the movement of glaciers in the Antarctic by means of laser beams. The experiments were carried out in the Molodezhnaya station area on the coast of the Sea of Cosmonauts and were implemented by installing a 20 cm diameter mirror on a glacier. The glacier was then tracked by a reflected laser beam and it was concluded that its movement was random—movement occurred sometimes within seconds and sometime within hours.

The fossils of fifteen dinosaur eggs found at Ologoi Ulan Tsab by the 1968–69 Soviet-Mongolian Geological Expedition bring the number of types of dinosaur eggs found in the Ghobi desert to eight. All are ornithoid in structure, the Ologoi Ulan Tsab eggs having a diameter of 14–15 cm with an estimated fluid content of 1.5 l. The eighteen amino-acids identified by chromatographic analysis suggest that the shell membrane was basically keratin, as in modern reptiles. A comparison of these eggs with the testudoid dinosaur eggs found at Aix in France should provide data on the evolution of the different orders of dinosaurs and their relationship to other fossilised and modern vertebrates.

Research in the USSR has shown that the migratory isabelline wheater (*oenanthe isabellina*) is a carrier of bubonic plague. The bird nests in rat holes and it has been found that its fleas feed as readily on the blood of rats, notorious plague carriers, as on their original hosts which they inflict upon returning to them. It was found during studies that birds so infected lived on for more than ten days, which presents a serious problem in controlling the spread of plague as the migratory birds would travel some distance in that time. The discovery helps account for the resurgence of the plague in areas of Russia where anti-rodent programmes in the early fifties had successfully banished the disease for some years.

NEW WORLD

Yet Another Prescription for Cancer

by our Washington Correspondent

THE National Institutes of Health may yet be rescued from dismemberment by the setting up of an independent cancer research authority. The proposals, which have so far achieved the dubious distinction of almost uniting the Senate in support and the biomedical community in opposition, last week entered a critical stage in their passage through Congress when they became the subject of hearings before the House Subcommittee on Public Health and Environment. But just as it seemed that members of the House of Representatives, like their colleagues in the Senate, would be placed in the unfortunate position of either voting for a measure likely to break up the National Institutes of Health or of recording their dissent and being accused of refusing to support cancer research, Mr Paul G. Rogers, chairman of the Health Subcommittee, offered a solution that may preserve both the structure of NIH and the integrity of Congressmen.

With the support of five members of his subcommittee, Mr Rogers last week introduced a bill designed to give the National Cancer Institute authority for coordinating all the activities of the NIH related to cancer research, and a budget of $400 million for 1972, rising to $600 million in 1974. The bill, which was written with the help of cancer researchers, also keeps the National Cancer Institute firmly within the NIH.

Before Rogers had introduced his bill, it seemed likely that the House would follow in the footsteps of the Senate by voting through a bill which in effect would take the National Cancer Institute outside the NIH umbrella and place it in an agency operating under the unfortunate and misleading name of the Cancer Cure Program. The Senate gave its blessing to the creation of the agency by a vote of seventy-nine to one, and since the bill also bore the sponsorship of the Administration, it looked set fair to sail through the House and into law with the opposing voices of the biomedical community drowned in the political rhetoric.

As passed by the Senate, the bill would set up the Cancer Cure Agency as an "independent agency within the National Institutes of Health" and would endow it with "such sums as may be necessary". The agency would have its own budget, a director at the same executive level as the director of the National Institutes of Health and its own bureaucracy To call it an integral part of the NIH on the basis of its geographical location in Bethesda would therefore clearly be a misnomer, and any doubts about the subsequent bad health of the national institutes if the bill were to become law should have been swiftly cast aside when the National Heart and Lung Institute asked for similar treatment. There is little doubt in Bethesda that the Senate version of the bill would signal the break-up of NIH if it were passed unchanged by the House.

In contrast, the bill introduced last week by Congressman Rogers and his colleagues is designed to represent as closely as possible the views of the biomedical community, but at the same time it makes a few obvious concessions to the thinking that lay behind the Senate's adoption of the Cancer Cure Program. If adopted, the bill would give the National Cancer Institute more independence and considerably more dollars than at present, and make it better known, but its standing in the National Institutes of Health's bureaucracy would not be greatly different from that of other institutes.

The key strategem in Rogers's defence of the NIH is to seek to buy off other proposals with the promise of $400 million to the National Cancer Institute for the present year—more than $60 million above the amount appropriated by Congress last month, which itself included an extra $100 million requested by President Nixon in May. The Rogers bill would therefore distribute Congressional largesse to the National Cancer Institute at almost double the rate for 1971, and by 1974, the NCI would have nearly three times as much money as it is now accustomed to spend each year. Even this bid may, however, be too low to succeed in tempting the supporters of a separate agency to abandon their ideas, for the original proponents of a cancer cure agency were asking for funding to reach $1,000 million by 1976.

As for the bureaucratic arrangements for cancer research within the National Institutes of Health, the Rogers bill would promote the director of the NCI to the level of Associate Director of NIH. And before other agencies have a chance to complain that they would be relegated to the status of poor relations, the bill similarly promotes the directors of the National Heart and Lung Institute and of the National Institute of Neurological Diseases and Stroke and makes all three positions political appointments directly from the White House. The grouping together of these three institutes is suggested on the basis that, between them, cardiovascular diseases, stroke and cancer account for about 75 per cent of deaths in the United States, and it firmly underlines Rogers's belief that the political dust kicked up during the debate about cancer research should not be allowed to obscure the legitimate claims of other areas of biomedical research. Ironically, however, previous suggestions that the director of the NCI should be a political appointment have come under fire for placing unnecessary political ties on biomedical research, and the move to extend this provision to other institutes will do little to head off those criticisms.

Perhaps the most important provision in the Rogers bill is that the budget of the National Cancer Institute should be submitted directly to the President for review and referral to Congress. Although the Director of NIH, the Secretary for Health, Education and Welfare, and the National Cancer Advisory Council would have a chance to look at and make comments on the NCI budget, they would be constitutionally powerless to alter it. This proposal in effect may help to turn the cancer budget into a political football, to be debated separately in Congress each year, but it will also keep public attention focused on the efforts of Congressmen to help find a cure for cancer—a sure way of ensuring that funding for cancer research is maintained at a high level.

This proposed budget mechanism, although generally regarded as a lesser evil in comparison with setting up an entirely separate agency, will present a few difficulties for the National Institutes of Health. For one thing, the flexibility built into the present system, whereby budgets for the separate institutes are channelled through the director of the NIH, would be lost if the Rogers bill were put into effect. At present, revisions are made in each institute's budget right up to the time that the Administration's budget goes to press. But if the NCI's budget is to be reviewed by the Director of NIH, the Secretary of HEW and the National Cancer Advisory Council, not only must it be drawn up a good deal earlier but it would also not be open to last minute change One official of the

NIH described this arrangement last week as a "formula for chaos", but Rogers's supporters defend it on the basis that it will assist the NCI in its planning if it can be sure that its budget request is transmitted to the President without arbitrary alterations designed to make the NIH's budget request fit the global sum allowed by the Office of Management and Budget.

Another provision in the Rogers proposals that has been billed as a means of speeding up grant applications, and therefore, by implication, the development of a cure for cancer, is the suggestion that all the directors of the research institutes should be able to distribute grants of less than $20,000 without first seeking approval from the National Cancer Advisory Council. In the past, the advisory council has met only three times a year, and this has led to accusations of unnecessary delays in the processing of grant applications. But the force of the new proposal is probably more rhetorical than actual, for it will only speed up grant applications by at the most one or two months in a process that now takes between six and eight months, and there are no suggestions that researchers and equipment are standing idle during the application process.

The proposed arrangements are, nevertheless, being welcomed for two reasons. First, they will take some of the burden of reviewing straightforward and minor grant applications from the shoulders of the National Cancer Advisory Council, and make it less of a rubber stamping body. For another, if the proposal is accepted, it would do little damage to the established review processes of the NIH. NIH officials are naturally suspicious of any change in the grant review process, and they are particularly concerned to preserve the scientific peer review, but they accept that the force for change has probably gained irresistible momentum. The Rogers bill is regarded as doing the least damage since it applies to about 26 per cent of the NCI's grants, and in any case to the grants which usually generate the least controversy.

What chance has the new bill of succeeding where previous attempts to inject some rationality into the debate about cancer research have failed? One of the chief obstacles in the path of the bill is clearly the fact that an independent NASA-style agency dedicated to finding a cure for cancer makes an ideal subject for political hyperbole—the Senate debate on the proposal provides a good example—while a proposal in effect to give more money to existing institutions lacks the same lustre. Another difficulty facing Rogers and his supporters is the fact that the Senate has already passed the Administration's bill designed to give the National Cancer Institute independent status, and whatever the House passes must be amalgamated with the Senate bill in a conference committee. Although members of the biomedical community can find many acceptable elements in the new bill, it is already undoubtedly a compromise with the Senate position, and may become badly emasculated in the conference committee.

Whatever compromise is finally worked out by the committee and by the House, the new bill has placed the Administration in an embarrassing position, for when the Administration's bill was first introduced, it advocated elevating the National Cancer Institute to a bureau, but keeping it firmly within the bureaucracy of the NIH. The Administration's original proposal was designed to head off attempts to create a separate cancer agency that were embodied in a bill introduced into the Senate by Edward M. Kennedy. The Administration found it politically expedient, however, to compromise with Kennedy's supporters by agreeing to a compromise bill that retained the essence of Kennedy's proposals, but which bore the Administration's name. The introduction of Rogers's bill has therefore put the Administration in the situation of defending a position which it had previously attacked, against proposals which it once supported.

Guide to the Bill

THE bill introduced into the House last week by Congressman Paul G. Rogers and five members of his Subcommittee on Public Health included the following major provisions:

- The National Cancer Institute would be preserved as an integral part of the National Institutes of Health, and it would be charged with the task of coordinating all the cancer research in NIH.
- The National Cancer Institute's budget would be transmitted separately to the President, but the Director of NIH, the Secretary of Health, Education and Welfare and the National Cancer Advisory Council would have the chance to comment on, but not alter it. The NCI would be authorized to spend $400 million in 1972, rising to $600 million in 1974.
- Directors of the Heart and Lung Institute, the Institute of Neurological Diseases and Stroke and the National Cancer Institute would all be promoted to the level of Associate Director of the NIH, and all would be direct appointments by the White House.
- All the institutes would be able to approve grants of less than $20,000 without having to seek approval from the advisory councils, although scientific peer review for all grants must be maintained. It is hoped that this would help prevent the National Cancer Advisory Council from being bogged down with minor decisions.
- Finally, so that Congress can keep a check on the way the provisions are working, the director of the National Cancer Institute is required to present an annual report to the President for transmittal to Congress.

DETERGENTS

Algae Better than Burns

by our Washington Correspondent

THOSE who take a delight in watching bureaucratic somersaults will have been particularly amused last week to witness three government agencies vigorously backtracking on their policies for controlling phosphates in detergents. Not so amused, however, are detergent manufacturers, who have spent millions of dollars seeking alternatives to phosphates only to be told that the cure is worse than the disease; housewives, who have switched in droves to the low phosphate detergents in their desire to do something for the environment; and congressmen who have submitted bills designed to force detergent manufacturers to remove phosphates entirely from their products. Placed in an even more invidious position are those counties and states that have banned the use of detergents containing phosphates, only to be told last week to reconsider their policies.

What caused the Environmental Protection Agency, the President's Council on Environmental Quality, the Food and Drug Administration and the Surgeon-General to declare last week that although phosphate detergents are the largest single cause of eutrophication of waterways, they should be used in preference to other detergent types? The answer is simply that substitutes for phosphates are undesirably toxic. The problem is that legitimate concern over deterioration of the environment has reached such a pitch that environmental groups are blessed with the political force to influence legislators and with enough consumer backing to pack a painful punch against industries that can be shown to abuse the environment, and this irresistible force is apt to produce overhasty reactions. As far as detergents are concerned, the environmentalists' punch forced manufacturers into producing a product that provides less of a hazard to the environment, but more of a hazard to people.

The scenario leading up to the abrupt policy reversal of federal agencies last week is an object lesson in how the lobby system can overreact to produce a result that is acceptable neither to the harassed manufacturer nor to the concerned consumer. As evidence began to accumulate about the contribution of phosphate detergents to the eutrophication of lakes and rivers (the stimulation of plant life at the expense of the oxygen supply to other aquatic organisms), they became an ideal object on which environmentalists could vent their anger. A congressional committee in April last year recommended that detergent manufacturers should be made to remove phosphates from their products by 1972, the Canadian government banned phosphate detergents from that date, several states and counties, including Indiana and New York, outlawed the sale of phosphate detergents, and housewives began to switch to detergents labelled as phosphate-free. In addition, countless other state legislatures have bills pending before them, enjoining manufacturers either to remove or severely restrict the amount of phosphate in their detergents.

Flailed by the public and Congress, detergent manufacturers set to work developing alternatives to phosphates in detergents—the chief role of phosphates is to soften the water. The most promising substitute seemed to be nitrilotriacetic acid (NTA), and chemical manufacturers were hard pushed to meet orders from the detergent manufacturers. But doubts about the carcinogenicity and teratogenicity of NTA, first voiced by Dr Samuel Epstein, then a pathologist at Harvard, led eventually to a voluntary ban on the substance by detergent manufacturers in December last year. That ban was upheld last week by the federal agencies charged with protecting public health, chiefly on the basis of a study carried out at the National Cancer Institute, which produced no direct evidence of carcinogenicity at low dose levels, but which nevertheless failed to remove the doubts.

After that setback, manufacturers began to substitute caustic materials—such as sodium hydroxide—in place of phosphates. This led to action by the FDA, forcing some products to be labelled as toxic. Detergent manufacturers protest that they cannot produce a non-toxic substitute for phosphates, and legislators were continuing to force them to produce phosphate-free detergents. Finally, the Environmental Protection Agency, the Council on Environmental Quality, and the Surgeon-General decided last week to advise the housewife to choose detergents containing phosphates.

AEC

How To Sell Cannikin

by our Washington Correspondent

It has been esimated that Washington boasts more public relations personnel per square mile than any other city in the world. While most of these are hired to impress their clients' views on the Federal Government, the government itself has a small army of information officers whose role is to keep the public informed of departmental and agency projects and policies. The difference between the two groups is that the lobbyists are expected to deal in propaganda, while the government public relations officers are expected to deal in facts.

That at least is the theory, but few people accept it as the fact. A particularly fine example of how one particular government agency, the Atomic Energy Commission, set about selling a potentially distasteful project, the explosion of a nuclear weapon under Amchitka Island in 1969, and how it intends to use the public relations experience gained in that exercise to sell the Cannikin project, was uncovered last week by Senator Mike Gravel of Alaska. Gravel, a vociferous opponent of the Cannikin nuclear test scheduled to take place under Amchitka Island in October, published in the *Congressional Record* an internal AEC report describing how the AEC "is running a well-oiled, well-financed, well-coordinated machine to tell Alaskans and other Americans about the need for Cannikin".

Gravel's chief complaint is that the AEC report provides ample evidence that the commission dismissed out of hand the views of its opponents by suggesting that their opposition to the test was motivated by a desire for headlines, disillusionment with the political system or by opposition to the ABM. The AEC, he complains, "discounts all legitimate criticism of its programs" and uses tax dollars to support an illegitimate propaganda campaign. "I call this situation to the attention of the Senate, not to beat the ghosts of past errors out because the issue is very much alive today", Gravel declared. "Cannikin is upon us and the selling job is all too familiar."

The report itself describes how the AEC's public relations effort for the Milrow test in 1969—the nuclear explosion that was designed as a proving shot for the five megaton Cannikin test—was planned in three stages. The first, preliminary stage started in December 1966, and gradually picked up momentum during the two and a half years to July 1969. The chief object of this phase of operations was to inform the public that the AEC was conducting a feasibility study of Amchitka to explore the possibility of using the island for nuclear testing. "Throughout this period", the report points out, "policy did not permit confirming that a nuclear test would be detonated on Amchitka. Public statements were framed in such language as 'determining the feasibility of Amchitka for possible use in underground nuclear testing' ". The policy, however, became less than credible when it had become widely known that the AEC had let a $12 million drilling contract, but the public relations team nevertheless stuck to its original story.

From July 18, 1969, when the AEC finally let it be known that an explosion was planned to take place under Amchitka, to October 2, when the Milrow test took place, the report describes an intensive build up in its public relations effort. Until September 20, the chief effort of the AEC's propaganda was directed towards building up public support and good will. An office was set up in a hotel in Anchorage, films documenting the AEC's efforts to save sea otters were made and distributed, and thousands of publications outlining why the AEC tests weapons, how it ensures the safety of its testing, and other background information were distributed. The report also explains how it enlisted the support of a newspaper editor by associating him with the sea otter film and keeping in touch with him by letter and telephone, so that in the end he would accept and print AEC statements that the Alaskan public would not be endangered. The AEC also took space in two of the state's largest fairs and showed exhibits of archaeology on Amchitka and a pictorial history of the development of the site on Amchitka Island.

The report finally gives the details of a campaign conducted during the two weeks before the test took place and designed to offset public opposition in the press and on the radio and television. The most disturbing aspect of this phase in the activities, Gravel maintains, is the AEC's unwillingness to accept that its opponents were motivated by anything other than desire for publicity. For one thing, the report consistently terms the demonstrators in Canada as "hysterical", and castigates the press for giving extensive coverage to opponents of the AEC. Seemingly, anyone who wanted to get his name in the newspapers or on radio—regardless of expertise or qualifications—could do so by making a statement against the test, the AEC complains. The commission also resented its opponents bringing in seemingly irrelevant material, but nevertheless carried on distributing all kinds of information on other activities of the AEC to the state schools.

NEWS AND VIEWS

Does Molecular Shape Determine Odour Quality?

TRAINED odour judges are said to be able to distinguish as many as 15,000 different odours. One of the puzzling aspects of the sense of smell, however, is that there is little relation between chemical constitution and odour. Theories of olfaction are thus legion; all attempt to answer the central question as to what properties of the odourant molecules are responsible for the wide variety of odour quality.

M. M. Mozell holds the theory that the olfactory epithelium behaves towards odourants in a manner analogous to a gas chromatograph and that it is the temporal as well as the spatial aspects of the resulting neuronal response which gives rise to differences in odour quality (*J. Gen. Physiol.*, **56**, 46; 1970). J. T. Davies has the theory that the initial event in the olfactory stimulus is the puncture of a lipid membrane by the adsorption of an odourant molecule onto it (*J. Theoret. Biol.*, **8**, 1; 1965). He finds that when the molecular cross-sectional area of odourant molecules is plotted against their free energy of desorption from a lipid water interface, compounds of similar odour quality are found together in certain areas of the plot. These areas merge into each other indicating that there is a continuous spectrum of odour quality. Neither of these two theories requires the existence of specific receptor sites which respond to only certain types of odourant molecules.

Of the theories which start from the assumption that there are several different types of receptor sites, those of J. E. Amoore and of R. H. Wright at present excite the most interest. Wright starts with the idea, gleaned from Hainer's "information theory of olfaction", that there are about twenty-five different types of receptor. He considers that only the wide range of low energy molecular vibrations possible for different molecules could give sufficient variety to satisfy so many different receptors. The evidence for most of these vibrational modes is to be found in the far infrared spectra of the molecules and hence Wright has attempted to correlate odour quality with the frequencies of the peaks found in this spectral region. This correlation is not at all apparent on looking at the spectra and, even after Wright has picked out what he calls "osmically active bands", statistical tests are required to bring out a correlation.

From a study of the comparison between molecular shape and odour quality, Amoore came to the conclusion that there are seven primary odours, five of which are characterized by a particular molecular shape and each fits a corresponding receptor site with an appropriately shaped cavity. Other odours, supposedly more complex, are associated with molecules that fit into more than one of these hypothetical sites. This theory has met with some success in the prediction of the odour quality of new compounds. Amoore has since modified his ideas as to the number and types of primary odours and their corresponding sites, but still maintains that molecular shape is of major importance in determining odour quality.

On page 270 of this issue of *Nature*, Amoore makes an attempt to get a direct comparison between his theory and that of Wright. To do this he uses twenty-five compounds, the spectra of which have been measured by Wright and which have odours similar to bitter almonds or cumin. In order to get a measure of odour quality he uses a panel of observers, selected for their olfactory acuity, to compare the odours with that of benzaldehyde. The numbers which he gives for odour similarity are essentially a rank order scaling. The procedure of comparison is analogous to asking persons of good aural discrimination to place a series of notes in their correct relationship to each other by comparing them with a standard note by ear only. This would probably result in the notes being placed in the correct sequence but the numbers assigned to the notes would show no more than a crude correspondence to their frequencies.

In the absence of any known odour property analogous to sound frequency, the odour similarity values are the only available parameters which can be used in testing theories of odour quality. Amoore finds a good correlation between his values and a computer generated index of similarity of molecular shape to that of benzaldehyde. He challenges Wright to show as good a correlation between these same odour similarity values and some measure of spectral similarity. This places Wright in the unenviable position that whereas failure to show a correlation will tend to discredit his theory, a correlation as good or better than Amoore's is unlikely to convert anybody not already committed to it.

Limits on Seyfert Galaxies

DECIDING whether the infrared flux from extragalactic objects such as galaxies and quasars varies significantly over short time scales has recently occupied much of the time of infrared astronomers. Significant flux variations on time scales of months or days would place quite severe constraints on the size of the infrared source in these objects But infrared astronomy is a young science, and published evidence for time variations has so far been habitually followed—after a decent interval—by expressions of doubt that the evidence is convincing enough to be regarded as real.

Whether the same sequence of events will overtake the work reported by F. J. Low and G. H. Rieke of the University of Arizona on page 256 of this issue of *Nature* remains to be seen, but Low and Rieke find their evidence for a short-term variation in the infrared flux from NGC 1068 "compelling". Yet the fact that the change in the flux occurred at a time when there was a change in the instrumentation that was being used to monitor NGC 1068 is bound to raise a few eyebrows. But to be fair, Low and Rieke have done a convincing job in demonstrating that the low fluxes that have been recorded from NGC 1068 this year are likely to reflect an intrinsic change in the source, and are not an artefact introduced by the alterations to the equipment.

NGC 1068 is one of the Seyfert galaxies, which are characterized by small, bright nuclei and highly broadened emission lines setting them apart from other spiral galaxies. On average the Seyfert galaxies are no mean emitters of infrared radiation, but NGC 1068

Pure Cl [illegible]
[illegible] COLLEGE

surpasses any of them at these wavelengths—at the infrared wavelength of 22 μm its flux density is thirty times that of the otherwise comparable Seyferts NGC 4151 and NGC 1275. But the placing of constraints on the model for the emission is of more general interest than its applicability to Seyfert; for the infrared emission from Seyfert galaxies is likely to arise by a similar mechanism to the unexplained infrared emission from quasars.

The infrared emission from extragalactic objects such as quasars and galaxies has previously been interpreted either in terms of synchrotron emissions from electrons, or by thermal emission from a shell of dust grains surrounding a compact source of energy. Neither view is entirely satisfactory, the synchrotron mechanism because an unusual spectrum of emission would be required which, although not totally impossible to obtain, would place difficult constraints on the behaviour of electrons in extragalactic objects which would be hard to fulfil. On the other hand, the dust model is usually regarded suspiciously because of the fear that time variations of the infrared emission would one day show it to be untenable, even though the observational evidence has not been convincing.

In the dust model, a central source in the nucleus of the Seyfert galaxies is taken to emit energy in the form of ultraviolet radiation, or possibly as particle radiation, which is thermalized by the shell of dust grains around the central source. The grains reemit the energy, chiefly in the infrared. But as Low and Rieke point out in their article, and as others have, of course, done before them, short-term variability of the emission will place constraints on the size of the dust shell, the constraints becoming more severe as the time scales are reduced from months to days.

According to Low and Rieke, the observations reported this week are consistent with an optically thick shell of dust having a diameter of the order of 3 parsec (about ten light years) containing dust at 400 K, or an optically thin shell having a diameter less than about 0.3 parsec at a temperature greater than 1,200 K. So, in addition to verifying the variability by continuing the observations on NGC 1068 and other Seyferts, astronomers will want to discuss whether these size and temperature constraints can be met.

DEVELOPMENTAL BIOLOGY

Embryologists Meet in Glasgow

from a Correspondent

THE tenth international conference on embryology, which took place in Glasgow on August 30 to September 3, was well run, with only three principal speakers in each session. Each speaker therefore had enough time to develop an argument and could speak to a relatively fresh and attentive audience. The sessions included oogenesis, genetic interactions in development, the control of protein synthesis, the development of pattern and neuroembryology. There was also a film session, several meetings of discussion groups and about ninety demonstrations.

Dr. F. H. Ruddle (Yale University) showed that the assignment of linkage groups to particular human chromosomes was proceeding rapidly. In his work he uses the technique of intraspecific somatic cell hybridization and half-selection to produce hybrids in which the expression of particular differentiated traits can be examined. For example, in a hybrid derived from a mouse renal adenocarcinoma (RAG) and a human fibrocyte (Wi 38), cells were either deficient in esterase-2 activity, which is characteristic of the human parent, or possessed it, which is characteristic of the mouse parent. On prolonged cloning, the $ES\text{–}2^-$ strain occasionally reverted to $ES\text{–}2^+$, but $ES\text{–}2^+$ never reverted. Ruddle concluded that it was possible that the expression of the mouse phenotype could be controlled by the presence or absence of a human chromosome, either C8 or C9.

Dr E. Sidebottom (University of Oxford) uses hybrids between chicken erythrocytes, which do not normally show nuclear activity, and HeLa cells in combination with antisera to human nucleoli, nucleoplasm and cytoplasm. He was able to show a good correlation between the renewal of DNA and RNA synthesis by the reactivated chick nucleus and the appearance of chick antigens on the cell surface shortly after the reappearance of chick nucleoli. He found that although human nuclear material occasionally appeared in chick nuclei, human cytoplasmic material never did. Similarly, in mouse–human hybrids he demonstrated the synthesis of a human enzyme under the control of mouse nuclear material.

Dr P. J. Bryant (University of California, Irvine) discussed the properties of *Drosophila* imaginal disks—the segmented larval tissues from which adult structure develops. Each disk is a more or less tubular structure which is folded and attached by a stalk to the epidermis. After the last moult the disks evert, unfold and differentiate to produce a segmental structure such as an eye or a limb. He used genetic mosaics, made by irradiating flies heterozygous for "yellow" and "singe" (pale cuticle and bent bristles). This leads to somatic crossing-over in cells of the imaginal disks, some of them becoming homozygous by recombination so that the characters are expressed. Adults therefore have patches of pale cuticle and

Selection in Quasar Redshift Measurements

IN the flurry of publications concerning the observed clumping of quasar emission line redshifts at certain values, very little attention has been given to the problem of selection effects. Nobody would argue against the suggestion that the observations favour some values of redshift (z). But it is a different matter when the question of periodicities in the favoured values of z are considered, and this is an opportune time for R. C. Roeder's analysis in next Monday's *Nature Physical Science*.

The most well known and least questionable region of favoured redshifts lies around $z=1.95$. Various authors have claimed that other peaks in the histogram of quasar redshifts can be related to this peak and one another, with a "periodicity" in z of 0.06. Other workers have argued with equal conviction that the statistics are inadequate to support such a claim, and the possibility of selection effects has also been raised, although not usually in any explicit form. Roeder's work now suggests that in some objects there are simply more spectral lines to measure—in a few cases at the other extreme redshifts have been claimed on the basis of the "identification" of only one line in the spectrum of a quasar.

The correlations observed by Roeder are statistically significant, and of course if there are more lines to measure at some redshifts then it is easy to understand why more quasars have had their redshifts measured at these values. It seems that the "ease of measurement effect" may be related to the movement of spectral features across the optical window as z increases. Most notably, the deficiency of redshifts measured at $z=1.25$ could be attributed to the shift of Mg II out of the window, and the rise at $z=1.8$, peaking around $z=1.95$, could be attributed to the shifting of Lyman α into the window.

epidermis bearing "singe" bristles on a normal background. Each pattern presumably derives from a single clone, because its outline is entire and cells are never individually determined.

From experiments in which the legs were transected, Dr Bryant showed that there was good evidence for an axial developmental gradient which could partially regulate. The upper part of the limb tends to develop normally, the lower half to duplicate with mirror symmetry. This is as if a single gradient is halved: the low end of the top half relaxes towards zero, presumably because of diffusion of the substance in the gradient, the high end of the bottom half doing the same. Some positional values for specifying the limb structure thus only occur in the original top portion of the gradient, whereas those in the relaxed lower portion are duplicated. (Schubiger has reported similar results and it is interesting that there is a lethal mutant with giant disks in which structures tend to duplicate, possibly because the disk becomes too large to be covered by a single gradient.)

The most significant result of the conference was the rejection, by Dr R. M. Gaze (National Institute for Medical Research, Mill Hill) and others, of the ideas of neuronal specificity and modulation. By neuronal specificity is meant the possession of specific chemical labels by neurones and their processes. Correct recognition of such labels is supposed to be instrumental in the formation of ordered connexions between, for example, the optic nerve and the rectum in amphibians. Remodulation is the relabelling of central neurones as a function of their peripheral connexions. Neuronal specification came to be discussed because in those situations for which it has been theoretically required it now seems that there is relatively little specificity involved.

Furthermore, connexion patterns can regulate after damage in such a way that axons become inputs to neurones that they would not originally have contacted. For example, if half the optic tectum is removed and the optic nerve regenerates, the system regulates to preserve a retinotopic projection. As Gaze has shown, normal retinal growth is by division of cells in a ring at the ciliary margin. It is likely that each cell divides to leave a central daughter which will differentiate and a peripheral stem cell which will divide again. The axes whereby the position of retinal ganglion cells are specified are determined when the retina is only twenty cells in diameter, so that the specificity involved might not be very great—connexions subsequent to those of the first disk of cells could be made in the order in which ganglion cells mature and send out their axons. The tectum grows from a single line of dividing cells, however, so that the actual geometry of connexion formation is still difficult to envisage.

Most experiments in which remodulation is supposed to occur can apparently be reinterpreted as if random neuromuscular connexions are made after a motor nerve is cut. The patterns of movement of a reinnervated limb are in fact so limited that they cannot afford evidence for either specific reconnexion in detail, or for remodulation. Indeed, Dr L. Marotti (Monash University) showed that in the goldfish the inferior and superior oblique eye muscles can both be reinnervated by the nerve supplying the inferior oblique. Apparently, however, even though "incorrect" synapses are formed they are not functional under physiological conditions, though she has not yet checked to see whether they are electrically excitable.

RNA SYNTHESIS

Messenger Mathematics

from our Cell Biology Correspondent

THERE are obviously two potential ways in which a bacterial cell might increase or cut back its synthesis of RNA and protein in response to changes in its environment which either increase or decrease the growth rate. The rate of elongation of RNA and polypeptide chains could be altered, or the number of new chains initiated could be regulated while the rate of elongation of chains once initiated remained constant. During the past several years at least half a dozen groups have grappled with this question and the majority opinion is that the rates of elongation remain more or less constant in cells which are growing at rates differing by up to sixfold. The implication of these results is, of course, that regulation occurs at the initiation step. But one or two dissenters have reported evidence which suggests that rates of elongation may vary and this evidence, together with the fact that in most previous experiments the range of growth rates of the cells was restricted, prompted Coffman, Norris and Koch (*J. Mol. Biol.*, **60**, 1; 1971) to measure the kinetics of appearance of β-galactosidase after the addition of inducer to cultures of *Escherichia coli* with doubling times ranging from 50 minutes to 24 hours.

Exploiting the sophisticated techniques now available for measuring the early kinetics of enzyme induction and a sensitive fluorometric assay for β-galactosidase, Coffman *et al.* found that the delay time—the interval between the addition of inducer and first appearance of detectable β-galactosidase activity—was 95 ± 5 seconds in cells with doubling times ranging from 50 minutes to 12.5 hours. And even in cultures doubling extremely slowly (doubling time 24 hours), the delay time was a mere 150 seconds and in this extreme case the binding and penetration of inducer may be retarded.

In short these experiments confirm that over a thirteen-fold range of growth rate the elongation of RNA and polypeptide chains in *E. coli* is invariant. Furthermore, the average lag between adding inducer and the achievement of a steady-state of β-galactosidase activity does not vary significantly with changes in the growth rate; this finding presumably means that messengers survive for about as long in cells doubling every 50 minutes as in cells doubling every 12.5 hours.

In an appendix Koch presents an algebraic analysis of the induction curves for β-galactosidase, obtained when cells are either pulsed with inducer or allowed to grow in the continued presence of inducer, in an attempt to decide between the various models proposed to explain the decay of a messenger RNA. He claims that the results of experiments in which inducer is added and not removed from the medium exclude models which envisage that each messenger is read for a fixed number of times before it is degraded. The data are, however, compatible with a model which envisages that messenger decay is random with respect to time and place, or a model which envisages that there is a constant probability that a "killer" ribosome will initiate translation and inactivate the messenger as a result.

While Coffman and his colleagues have been measuring the kinetics of expression of the *lac* operon—and the objection that their data are relevant only to inducible operons cannot as yet be ruled out—Eron, Morse, Reznikoff and Beckwith (*ibid.*, **60**, 203; 1971) have been looking at the transcription of the *lac* genes in a strain of *E. coli* which because of a deletion has the structural genes of the *lac* operon fused to the *tryp* operon. In this strain the expression of the *lac* genes is regulated by the controlling elements of the *tryp* operon, and Eron *et al.* have tested to see if a *tryp*/*lac* fused messenger RNA is ever made. They find that some 4–10 per cent of the messengers made from these two fused operons are indeed covalently linked. They conclude that an RNA polymerase can initiate at the *tryp* promoter and read through the *tryp* and into the *lac* operon. But why is the amount of fused messenger so small? Either read through is rare, or it is the rule, but for one reason or another the fused messenger is broken during isolation or its 5′ end is rapidly degraded *in vivo*.

REEFS

Old and Modern

from a Correspondent

UNDER the auspices of the Palaeontological Society, a well attended meeting on the palaeoecology of reefs was held at the University of Edinburgh on September 9, being one of the events held to celebrate the centenary of the Department of Geology.

Drs D. J. Bellamy (University of Durham) and E. Drew (University of St Andrews) described their work on the zonation of hermatypic corals off the reef front at Aldabra including reference to a major system of channels which are explicable only on the basis that they represent the effect of erosion during aerial exposure of a preglacial formation. Dr P. Garrett (University of Leeds) transferred attention to the reefs of Bermuda and emphasized the complexity of a reef mass formed primarily by coral skeletons, the overlapping growth of which produces internal cavities encrusted with algal and animal growths.

Dr J. Taylor (British Museum (Natural History)) drew attention to the high diversity of shallow water macro-invertebrates in coral reef areas. Reefs present a unique diversity of habitats—a consequence of the resistance their formation presents to the forces of the sea—ranging from exposed reef faces to sheltered sandy and mangrove areas. Computers are now used to obtain objective comparisons of habitats of molluscan species.

Dr C. J. R. Braithwaite (University of Dundee) was concerned with the need for criteria for the recognition of fossil reefs and drew attention to those features of modern reefs which are particularly significant, notably the platform with its superimposed organisms. The fact that recent reefs have grown upon Pleistocene formations presents a major difficulty when comparing these with fossil reefs. Dr B. R. Rosen (University of Newcastle upon Tyne) considered ecological stratification in coralline environments in terms of hard and soft non-living substrates for life on which corals are very differently adapted.

Dr T. P. Scoffin (University of Edinburgh) described conditions of growth in the Wenlock reefs where the most vigorous growth occurs on what was the seaward fringe; colonies are preserved in the condition of growth indicating calm conditions. Dr C. T. Scrutton (University of Newcastle upon Tyne) spoke about the effect of the environment on fossil colonies and indicated that rugose corals were generally less well adapted for life in rough. "high energy", environments than were the probably poriferan stromatoporoids. Drs F. M. Broadhurst and I. M. Simpson followed by Dr J. Miller (all University of Manchester) were concerned with descriptions of limestones of coral reef origin. In the Castleton area of Derbyshire the limestones show marked faunal changes marginally, and the limestones present in knolls around the Clitheroe area, described by Dr Muller, are associated with dense crinoid thickets. Dr D. B. Smith (Institute of Geological Sciences, Leeds) dealt with the well known Upper Permian barrier reefs of the Guadalupe Mounts and compared these with smaller but similar reefs in Durham.

The meeting concluded with a lively discussion on many of the matters raised by the various speakers; a satisfactory definition of the term "reef" proved hard to find. Finally, Sir Maurice Yonge drew attention to some recent work on living corals which is providing some interpretation of the factors controlling the distribution and ecology of reef-building (hermatypic) corals.

FLUID MECHANICS

Pneumatic Conveying

from a Correspondent

THE behaviour of gas-suspended solid particles flowing in pipes is of much interest in modern powder processing industries. For many years this topic of research has also been a satisfying bone for many academic fluid dynamicists to chew on. For the first time a conference has been devoted entirely to pneumatic conveying. The meeting was held at Churchill College, Cambridge, from September 6 to 8, and was sponsored by the British Hydromechanics Research Association in conjunction with the City University of London.

On the applications side, systems for conveying some rather troublesome materials were described; asbestos slurry was the theme of a contribution by Dr D. C.-H. Cheng *et al.* (Warren Spring Laboratory, Stevenage); on-site con-

---

Messenger Transport *in vitro*

MESSENGER RNAs transcribed in the nuclei of eukaryotic cells have to be transported to the cytoplasm to be translated and there is increasing circumstantial evidence that this transport process is an important regulatory step in the expression of genes. The process is, however, extremely difficult to analyse not least because of the heterogeneity of nuclear RNAs and the biochemical complexity of cell cytoplasm and these factors prompted Raskas, as he describes in next Wednesday's *Nature New Biology*, to attempt to set up an *in vitro* system for the analysis of RNA transport across the nuclear envelope. He elected to use cultivated human KB cells infected with adenovirus type 2 as a starting material because some 18 hours after infection most RNA being made in the cell nucleus is adenovirus specific, and he now claims that, in the presence of an ATP generating system, nuclei isolated from such cells release RNA by a mechanism similar to that occurring *in vivo*.

The release of RNA by isolated nuclei is strictly dependent upon a continuous supply of ATP, without which less than 10 per cent of the RNA leaves the nuclei, and once released the RNA remains precipitable by TCA and presumably, therefore, undegraded. Analysis of the released RNA by centrifugation in sucrose gradients reveals two broad peaks, one sedimenting at about 35S and the other at about 10S. When the salt concentration is increased, the 10S RNA behaves as before but the 35S peak disappears because, Raskas suggests, it consists of ribonucleoprotein complexes which are dissociated at high salt concentrations. The buoyant density of this material is consistent with this suggestion.

Both 10S and 35S components contain viral RNA which will hybridize with adenovirus DNA, and an analysis of the total RNA released by the isolated nuclei in 20 min on polyacrylamide gels reveals RNA molecules long enough to be adenovirus messengers, in addition to low molecular weight RNAs, and the 6S RNA synthesized at late times during adenovirus 2 infections. Raskas suggests that the release of these RNAs *in vitro* is relevant to transport *in vivo* for several reasons. First, the release is specific; significant amounts of nuclear protein and DNA are not released. Second, the size of the released RNAs suggests that a maturation by cleavage goes on inside the isolated nuclei as it does *in vivo*. Third, the release *in vitro* cannot, of course, depend on the synthesis of new ribosomes and studies of intact infected cells indicate that movement of adenovirus RNA into the cytoplasm does not depend on ribosome synthesis. Finally, much of the adenovirus RNA released *in vitro* is in the form of a ribonucleoprotein and the same seems to be true of transport *in vivo*.

There is therefore every reason to believe that further analysis of this *in vitro* system will throw light on the process of RNA transport and, as Raskas comments, one of the first things to do is to analyse the protein components of the 35S ribonucleoprotein material to see if only a few, specific "transport" proteins are present.

crete distribution was described by Dr R. H. Jones (University of Nottingham); even proposals for a domestic refuse-disposal system were outlined by Dr J. A. Hayden (Hayden, Harding and Buchanan Inc., USA) and Dr A. W. Breidenbach (Environmental Protection Agency, USA).

On more fundamental issues the meeting was rather disappointing in that few contributions added much to present understanding of basic flow mechanics. The influence of dense, locally-concentrated particle clouds on basic fluid mechanics was scarcely mentioned. Furthermore, agglomeration and other multi-particle collision effects received rather scant attention, as was pointed out by Dr C. J. Stairmand (ICI Ltd, Teeside) in the discussion.

More definite advances were reported in the contributions describing experimental work. In particular, a more interesting statistical approach to particle continuum mechanics was evident in the work of Dr M. S. Beck *et al.* (University of Bradford) and Drs R. G. Boothroyd and P. R. Arundel (University of Birmingham). Drs J. Jokati and Y. Tomita (Kyushu Institute of Technology, Japan) described another example of more useful presentation of experimental data: they gave probability distributions of particle velocities at different points in a pipe. This break from earlier research, in which the interest has tended to remain in time-averaged fluid-dynamic parameters, is a most welcome and necessary development. Professor S. L. Soo (University of Illinois) twice expressed the opinion that the facility with which experimenters can examine the motion of individual particles should not be an excuse for ignoring the continuum viewpoint of the solid phase. To illustrate his point he reminded his audience that the motion of molecules is rather unimportant in one-phase fluid dynamics.

HISTORY OF SCIENCE

Kepler Honoured

from a Correspondent

THE thirteenth international congress of the history of science, held at Moscow State University from August 18 to 24, and followed by an international symposium at the Leningrad Planetarium from August 26–28, held to celebrate the four hundredth century of Kepler's birth, provided delegates from many countries with some chance of meeting Soviet scholars and seeing the fine collections in the host country.

Academician B. M. Kedrov opened the congress with an address on the history of science and principles of its research, in which he emphasized the importance of linking together past, present and future in the development of science. Colloquia topics (for which full simultaneous translation facilities were available) included the Rutherford centenary and seventy-five years of radioactivity studies (see *Nature*, **233**, 167; 1971); the evolution of classical mechanics; Mediaeval science: East and West relationships; ways of Development of Functional Analysis; and new technology in the developing countries from the eighteenth to twentieth centuries.

The general standard of contributions was high, but discussion time was, as so often is the case, somewhat restricted. The colloquium on the teaching of history of science was rather disappointing and uncoordinated; the Royal Society *Report on the Teaching of the History of Science, Medicine and Technology in Universities and Technical Colleges in the United Kingdom 1971* was presented, but was felt by many participants to be an uneven document.

The sections were less formally organized and did not entirely avoid language problems. It was felt by some participants that some of the "questions" asked were more of the nature

More about Interstellar Grains

IN spite of increasing efforts to identify the composition of interstellar grains, conclusive results remain elusive. Observations in the past were restricted to visible wavelengths where the shape of the interstellar extinction curve is fairly uniform throughout the galaxy and almost featureless, apart from a slope discontinuity at 4300 Å and several diffuse bands. Now that ultraviolet and infrared astronomy have widened the scope of investigation, the accumulating evidence points to large differences in the general trends of extinction from region to region. There are also several observed spectral features to be explained and the discovery of more can be anticipated. The conspicuous 2200 Å peak has been frequently attributed to graphite, although theoretical calculations tend to predict too broad a feature. Silicates and hydrocarbons have recently been proposed as alternatives and no doubt others exist.

In the infrared, much of the activity is concerned with emission from circumstellar shells (for example, Gillett *et al.*, *Astrophys. J.*, **164**, 83; 1971). The characteristic 10 μm excess in luminous M stars is suggestive of silicates and a limited amount of 20 μm photometry has supported this. Spectral features observed in absorption may, however, be more representative of intrastellar dust, because circumstellar grains are supposed to have been produced locally in the stellar atmosphere. Several unsuccessful searches for the 3 μm ice band indicate that most interstellar grains contain little ice, at least in the proposed form of H_2O ice (Knacke *et al.*, *Astrophys. J.*, **158**, 151; 1969); the 3 μm absorption in NML Cygnus, however, remains unexplained. Medium bandwidth observations of the bright infrared continuum of the galactic centre (20 to 30 magnitudes of visual extinction) combined with other observations have been interpreted by Hackwell *et al.* (*Nature*, **227**, 822; 1970) as showing absorption near 10 μm and 20 μm due to metallic silicates. These results must be regarded as tentative because of the considerable difficulty in establishing the true continuum against which absorption or emission is to be distinguished. Observations of three B supergiants were inconclusive because of the much lower amount of extinction, and an earlier measurement of 10 μm absorption in the M2 Ib star 119 Tau was not confirmed.

Following up these observations W. A. Stein and F. C. Gillett report in next Monday's *Nature Physical Science* a search for 11 μm absorption in the highly reddened B8 Ia star IV Cyg No. 12. Although most of the ten magnitudes of visual extinction probably arise from dust localized in the OB association, possibly even in the immediate vicinity of the star (Reddish *et al.*, *Pub. Roy. Obs. Edinburgh*, **5**, 111; 1967), evidence such as the shape of the extinction curve, the amount of polarization and the strength of the diffuse 4430 Å band suggest a similarity to interstellar grains. The continuum beyond 5 μm seems well established as the Rayleigh-Jeans portion of the stellar spectrum; there is no circumstellar emission out to 11 μm although there could be at longer wavelengths. More important, no evidence for absorption was found, the upper limit to the 11 μm optical depth being τ=0.1. This limit is at least a factor of 2 to 3 lower than predicted by scaling the observations of Hackwell *et al.* ($\tau \simeq 0.7$) but abundance variations of this size might be expected.

Stein and Gillett also estimate that less than a third of the mass of the grains can be metallic silicates. With the improved infrared optical constants reported by Nandy and Kelly (XVII Liège Symposium, June 1971) this estimate could be refined and extended to several other minerals. Clearly, many more observations with as high resolution as possible and at as many wavelengths as possible will be required before a definitive identification of the interstellar grain materials is finally achieved.

of doctrinal filibusters than requests for clarification or information. Questions such as methodology, the organization of research and the development of technologies, are obviously more open to controversial social and political interpretations than is, say, mediaeval mathematics; the time range of sectional topics from ancient science, medicine and technology to the development of transport, electronics, constructional engineering and aircraft, rockets and space science, provided most speakers with a forum.

The Kepler symposium at Leningrad was a much smaller and more homogeneous affair. The location was very appropriate because most of Kepler's surviving manuscripts and related papers are in Leningrad, and many of these were included in a small and interesting exhibition of works on and by Kepler. There was also an opportunity to visit the nearby Pulkova observatory, rebuilt following its wartime destruction, and see and discuss many aspects of modern astronomy. Academician Mikhailov opened the proceedings, and was followed by Dr W. Gerlach (Munich) who spoke on Kepler's life and work. Subsequent contributions (not all presented in person) dealt with Kepler's contributions to celestial mechanics, optics, the development of mathematics, gravitation theory, comets, the supernova of 1604, structural crystallography, the origin and derivation of the laws of planetary motion and his neopythagoreanism. There were also contributions on Kepler's connexions with Galileo, his time in Prague, his astronomical instruments, his theory of hypotheses, the Kepler manuscripts in the Soviet Union, as well as his relationship to the interest of modern physicists. Although no particularly outstanding new information or insight into Kepler's work was presented, it was clear that his scientific and methodological achievements continue to be of interest and even inspiration to many historians of science, quite apart, of course, from the historical problems involved.

VIRAL INFECTIONS

Green Monkey Disease

from our Medical Virology Correspondent

In August and September 1967, an outbreak of a severe haemorrhagic fever occurred among monkey handlers and laboratory workers in Marburg, Frankfurt and Belgrade. Thirty-one cases of severe illness with seven deaths were traced to direct contact with the tissues or tissue culture of a batch of African green monkeys (*Cercopithicus aethiops*), which had been trapped in Uganda. Several secondary cases occurred in hospital personnel by contact with the blood of patients and one further case was apparently transmitted by sexual intercourse. Many of the features of the illness, including liver and renal damage, a haemorrhagic diathesis and the association with monkeys originating from Uganda, suggested initially that the infection was caused by yellow fever virus or some other arbovirus. It soon became apparent, however, that this was a previously unrecognized disease caused by an infectious agent probably new to medical science. Today, four years after the infection, the origin of the disease remains obscure.

Studies in several laboratories established that guinea pigs, Rhesus (*Macca mulatta*) and vervet monkeys could be infected and the agent was subsequently isolated in tissue culture. The structure of the agent, which was named the Marburg virus, is that of an elongated sinuous organism 800–1,000 nm in length but generally curled in the form of a figure 6 or a horseshoe. Single organisms longer than 2 μ were occasionally found in the blood. A periodic cross-striation of 5 nm along the surface and an inner structure of about 65 nm in diameter with a central axis was also described. It was suggested that this agent was related morphologically to the Rhabdovirus family.

The possible origin of the infection has been investigated intensively since the outbreak. The monkeys were sent from Uganda at the time when there was considerable disruption of air traffic during the Six Days war. The monkeys were held overnight in London in a room containing many other animal species including birds and reptiles. A disturbing report from the United States based on serological study of complement-fixing antibodies to the Marburg agent revealed a high prevalence of antibody in many African monkeys. Strickland-Cholmley and Malherbe (*Lancet*, i, 476; 1970) pointed out, however, that the antigen used in the United States was not fully specific and that it probably contained a second non-specific component for the Marburg agent. W. Slencza and his associates (*Amer. J. Epidemiol.*, **93**, 496; 1971) were also unable, after extensive studies, to find evidence of infection with the Marburg agent (based on antibody results) in free-living monkeys.

The origin of the infection is thus still unknown and it must therefore be assumed that infection of man may recur. Furthermore, infection may come next time from other species of animals because it is now clear that vervet monkeys are not the maintenance hosts of this bizarre agent and it cannot be predicted where it may next occur.

Protein Kinase in Tumour Viruses

In *Nature New Biology* next week, Strand and August add a protein kinase to the list of enzymes, which includes reverse transcriptase, ligase and nucleases, that have been detected in RNA tumour viruses. The kinase, which is present in mouse leukaemia virus, avian myeloblastosis virus and the non-oncogenic vesicular stomatitis virus, catalyses the phosphorylation of several virion proteins. Its presence in these particles suggests that modification of proteins by phosphorylation may play a part in the interaction between an infecting enveloped virus and its host cell.

The kinase was detected by simply incubating mouse leukaemia virus particles with $(\gamma\text{-}^{32}\text{P})$ ATP and assaying for the incorporation of ^{32}P into virion proteins. Further experiments established that the kinase seems to be an integral part of the virion and is not adventitious; the phosphorylation reaction is stimulated by added protamine, depends on the presence of metal ions, is inhibited by caffeine and is enhanced by first disrupting the virions with detergent. Cyclic AMP, which stimulates by some two to three-fold the activity of other cellular kinase, and by this route exerts at least some of its regulatory effects, stimulates the phosphorylation of leukaemia virus proteins by only 25 to 50 per cent in the absence of protamine and in its presence stimulation is even less marked. As Strand and August remark, this lack of response of the virion kinase to cyclic AMP may mean either that the enzyme already has bound to it this nucleotide or it may lack the regulatory subunit of kinases which binds cyclic AMP.

The virion kinase will phosphorylate exogenous substrates including lysine and arginine rich histones, and a protein of *Escherichia coli* which is one of the two host factors required for the replication *in vitro* of QB RNA and which closely resembles histones. In the absence of added substrates the kinase phosphorylates at least ten different proteins or polypeptides in leukaemia virus particles including the internally located interspecies specific antigen (gs 3) and the two group or species specific antigenic determinants. Chemical tests indicate that serine and threonine residues are phosphorylated. Strand and August have not, however, been able to determine whether or not the glycoproteins in the envelopes of the virions, including the sub-group antigens, are phosphorylated.

NUCLEIC ACIDS

Collapsible DNA

from our Molecular Biology Correspondent

WITH a metre or so length of DNA in each of one's cell, it is fitting that one should from time to time ponder the question of how this relates to the well-known hydrodynamic rigidity of the thread of life, once this is out of the living cell and in the ultracentrifuge cell, where in the view of many people it properly belongs. Its properties, one might hazard, are modified by the basic proteins in which, in higher organisms, it is packaged. In other surroundings, such as phage heads, such a facile evasion will not do, and this is the starting point of Lerman's efforts (*Proc. US Nat. Acad. Sci.*, **68**, 1886; 1971) to find conditions in which native DNA will pack into a compact form, as it does in the phage.

In dilute salt solutions DNA is a rather stiff "worm-like coil". In the presence of high concentrations of neutral, unreactive polymers, Lerman reasoned, an effective phase separation, such as occurs macroscopically in mixtures of mutually repelling polymer species in solution, could be achieved by a collapse of this extended structure into a compact domain. Moreover, if the intermolecular forces in such a system are indeed predominantly repulsive, one would not expect that this would have to be accompanied by denaturation of the double helix.

The argument was evidently sound, for in the presence of the neutral polymer, polyethylene oxide ('Carbowax'), and at a sufficient salt concentration, presumably to minimize internal repulsions, a dramatic collapse of the DNA structure occurs. T4 phage DNA, which normally sediments at about 80S, now forms a broad band in a sucrose-D_2O gradient, centred at about 300S, and extending to 500–600S. With T7 DNA the results are qualitatively similar: from a normal sedimentation rate of 62S, it changes to give a peak at 200–250S, trailing out to 400S. The transition to the compact state occurs within a critical range of neutral polymer concentration and of sodium chloride (between 0.2 and 0.3 M). The polyethylene oxide itself undergoes no detectable change, as judged by its viscosity, in this ionic strength range, and moreover another neutral polymer, polyvinylpyrrolidone, will serve just as well. A striking demonstration of the existence of a threshold polymer concentration was achieved by sedimenting the DNA through a sucrodensity gradient containing an inverse concentration gradient of polyethylene oxide. On encountering a low polymer concentration the DNA reverts to the slowly sedimenting form, so that the back of the sedimenting zone catches up the front, and practically stops.

Examination of the buoyant density of the compact DNA after sedimentation excludes any question of denaturation. The sedimentation patterns are independent of DNA concentration, so that one may take it that the rapidly sedimenting form is not aggregated material. The high partial specific volume of the neutral polymer means that the formation of a complex with DNA, even with a large excess of polymer, would make little impression on the sedimentation coefficient of the DNA. Moreover, a similar effect is exerted by polyacrylic acid, which it would be hard to envisage forming a complex with DNA. It thus seems justified to infer that a true compact form is indeed generated. One may calculate sedimentation coefficients of the DNA molecule in various forms, and one may with reasonable certainty exclude supercoiling, which would change the sedimentation rate far too little, and a range of other possibilities. A compact sphere would sediment too rapidly, but an ellipsoid of axial ratio about 7:1 would give a result of the right order, and so would a platelet made up of double-helical stretches of DNA some 160 Å long folded concertinawise on each other.

So far as theory for phase relations can be applied, it is by no means inconsistent with a microscopic phase separation in the DNA-polymer system. Lerman suggests that the intracellular environment, pullulating as it is with other macromolecules, may be not unlike his model system. The implications of the ready transition from a stiff to a highly folded form in relation to the structure of the double helix will have to be carefully considered.

Reverse Transcriptase Initiates on RNA Primer

IN next Wednesday's *Nature New Biology*, Baltimore and four of his colleagues report that reverse transcriptase requires an RNA primer to initiate the synthesis of a DNA chain, which is covalently bound to the primer RNA. They describe a series of experiments which prove beyond doubt that the enzyme initiates DNA synthesis by adding a deoxynucleotide to the 3′ hydroxyl group of the primer. In short, the mechanism of DNA synthesis by reverse transcriptase seems to be essentially identical to that of the Kornberg DNA polymerase I of *Escherichia coli*, except the reverse transcriptase can use an RNA primer and an RNA template. The similarities between the two enzymes are indeed so great that Verma *et al.* even suggest that an RNA molecule might serve as the initiator or primer for DNA synthesis in *E. coli* by enzymes such as DNA polymerase I.

The first real clue that reverse transcriptase might require a primer RNA came from experiments with exogenous templates. Baltimore and Smoler found that although the enzyme often uses exogenous templates more efficiently than the endogenous tumour virus genome, it does so only when a primer, which can be as short as a tetranucleotide, is present; the product made comprises the new DNA chain physically attached to the primer. Having made these observations Baltimore's group asked the obvious and important question—does the enzyme when it transcribes the endogenous tumour virus genome require a primer and if so what is the primer?

They first noticed that the 20 minute product of the endogenous polymerase reaction with murine leukaemia virus particles was a single stranded DNA with a density slightly greater than expected. By treating this DNA with ribonuclease or alkali its density was reduced to that of single stranded DNA. Turning to avian myeloblastosis virus particles, which can be had in large amounts, they analysed the product DNA made after 2 to 30 minutes by the endogenous reaction. As the reaction proceeds the density of the DNA product shifts from that of a 1:1 RNA.DNA duplex to close to that of single stranded DNA.

After a series of treatments which hydrolyse RNA all the reaction products assumed the density of single stranded DNA. Using purified avian myeloblastosis virus enzyme and isolated avian myeloblastosis virus RNA instead of the virus particles, they obtained essentially identical results. And experiments with the inhibitor of reverse transcriptase, dideoxythymidine triphosphate, which have yet to be published by Baltimore's group apparently confirm that DNA synthesis is initiated by the attachment of the first deoxynucleotide to the 3′ hydroxyl group of the primer.

But what acts as primer in the endogenous reaction? It could of course be that the 3′ end of the 60–70S viral RNA genome forms a hair pin bend; alternatively small RNA molecules might associate with the 60–70S RNA. Small RNAs, including transfer RNAs, are present in tumour virus particles and Baltimore's group favour the idea that these act as primers, not least because many DNA molecules are made per viral genome.

ACOUSTICS

Hungarian Meeting

from a Correspondent

THE seventh international congress on acoustics, held at Budapest from August 18 to 26 under the presidency of Professor Tarnóczy of Hungary, was the first occasion on which the meeting had been held in a country of the Eastern block and it is noteworthy that just over 40 per cent of the 1,200 delegates came from these countries. There were also 300 guest members and the size of the gathering emphasizes the growing importance of the subject.

The revived interest in models was evident in the appearance of a few contributions on the subject such as those on the use of a model to evaluate various possible changes to a studio by Dr A. N. Burd (BBC Research Laboratory), and on diffusion and absorption of sound in a model reverberation room by the fathers of modern acoustics, Drs V. O. Knudsen and L. P. Delsasso (University of California, Los Angeles). It is sad to report that Delsasso died suddenly when travelling to the congress.

A balanced selection of topics over the wide field of acoustics had been chosen for the invited fourteen plenary one-hour lecturers. Unfortunately for many listeners nearly half of these were in French or German. Dr J. L. Flanagan (Bell Laboratories, Murray Hill) gave an excellent lecture with demonstrations of advances in selected areas of speech communication. The advances in research in this field are largely due to the expanding and the in-depth understanding of the theory of sampled-data systems and also to the wide adaptability of the moderately sized, fast digital computers. The transmission of speech in digital form, by contrast with analogue transmission, can be made effectively independent of distance as regards quality of reception, and a variety of signals—for example, voice, telegraph and the like—may be interleaved without special consideration. Digital encoding methods for speech are very varied and source coding leading to lower bit rates has resulted in an increased study of speech synthesis. Synthetic speech provides storage economies and vocabulary flexibility which promotes different possibilities in new automatic information services, and every push-button telephone becomes a potential computed terminal. For use in computer answer-back the synthesis of formant data or of printed text both seem attractive. With printed text, the machine stores a pronouncing dictionary which includes a phonemic transcription of each word, word stress and rudimentary syntax information. It generates connected speech solely from a knowledge of the linguistic rules of the language and the dynamic acoustic properties of the human vocal apparatus.

The frequency range of acoustical investigations has been extended considerably upwards in recent years and, as was mentioned by Dr E. G. S. Paige (Royal Radar Establishment, Malvern), mechanical surface waves can now be generated up to 10 GHz. Mechanical waves of these frequencies (up to 100 GHz) are often termed hypersonic and are being used in physical investigations of the condensed state of matter involving their interaction with electromagnetic, spin and acoustic waves (Dr J. P. Joffrin, University of Paris). At the other extremity of the frequency range (Dr R. W. B. Stephens, Imperial and Chelsea Colleges, London), the wavelength of the sound radiation becomes comparable with the natural frequency of various parts of the human body, and unpleasant, if not dangerous, effects can arise in consequence. There is a real need for more planned investigations in this frequency region.

The growing field of ultrasonic applications to medicine was made evident by the appearance of several contributions from workers in various countries. Dr J. C. Somer (TNO, Utrecht) described his development of an array of electroacoustic transducers which comprises twenty-one elements, spaced a half wavelength apart and suitably phased. This technique has been adopted for electronic sector scanning, and provides two-dimensional scanning with a substantially stationary probe. It has particular application to diagnosis of the brain where mechanical scanning has not proved very successful chiefly because of the high absorption of the skull.

Bacterial Purple

NEXT Wednesday's *Nature New Biology* contains a study of the purple membrane found in a species of halophilic bacterium, *Halobacterium halobium*, which shows it to have remarkable resemblance to retinal rods. The cells flourish only at extremely high concentrations of sodium chloride, and towards the end of the logarithmic growth phase there is a rapid accumulation of the purple membrane. Oesterhelt and Stoeckenius have found that when the cells are caused to disintegrate by removal of salt, the fragments of the purple membrane are easily separated by centrifugation and washing from other parts of the membrane.

The absorption spectrum of the purple membrane is essentially that of a visual pigment, the maximum in the visible being nearer to that of the cone pigment, iodopsin, than rhodopsin. Denaturation with acid causes bleaching, the purple colour giving way to yellow. The bleached chromophore can be extracted with solvents, and is identifiable as retinal.

In SDS-gel electrophoresis, the dissociated membrane displays only one protein band, with an estimated molecular weight of 26,000. This is also the equivalent of protein found by nitrogen analysis to be present in the membrane for each equivalent of the chromophore. Thus the protein may be supposed to have a molecular weight of 26,000 (supported also by minimum numbers of the sparsely represented amino-acids, for example, one histidine residue corresponding 26,000 molecular weight), which is at the low end of the range of molecular weights at various times offered for rhodopsin. The analogy extends also to the attachment of the retinal to the protein: when reduced after bleaching with borohydride it can no longer be extracted with solvents, and is evidently therefore attached in the native state by way of a Schiff base linkage to a lysine side chain. Oesterhelt and Stoeckenius make the reasonable inference that the purple membrane may be a photoreceptor.

An X-ray diffraction study of the isolated purple membrane fragments by Blaurock and Stoeckenius accompanies this characterization. The characteristic spacings for a membrane bilayer, corresponding to two dense layers 40 Å apart, are observed. Furthermore, there are reflexions that show the presence of a large hexagonal lattice, which must therefore be the form of surface packing of the protein molecules. Freeze-fractured specimens of whole cells show in the electron microscope evidence of a regular lattice on patches of the membrane (it being assumed that the cleavage plane is in the bilayer). Because these increase in number towards the end of logarithmic growth, it seems that they correspond to the purple areas. Light-diffraction patterns are also consistent with a hexagonal array.

From measurements of partial specific volume, Blaurock and Stoeckenius have calculated that the unit cell in the purple membrane preparations contains some forty molecules of lipid and three or four molecules of protein. Because hexagonal packing in a plane demands that the unit cell contain three units, Blaurock and Stoeckenius suggest that the protein is disposed in two layers rather than one. The visible absorption band of the chromophore shows strong dichroism, the long axis of the retinene lying in the plane of the membrane, exactly as found in retinal rods. This seems to be the first example of the existence of specialized functional areas in cell membranes.

Combustion Temperatures: The Future?

F. J. WEINBERG
Imperial College, London SW7

An examination of the relative merits of burning at high and low temperatures leads to new proposals for designing future combustion systems for particular purposes so as to minimize pollution and optimize intensity and efficiency.

A 100 h.p. horse would be inconveniently large. In every other way, biological converters oxidize their fuel most efficiently, give rise to very little pollution and, indeed, dispose of those effluents which are caused by the low quality of their fuel in a convenient and elegant way. To go to the other extreme, a stabilized hydrogen–oxygen detonation could achieve the throughput equivalent to 100 h.p. in a disk of less than 1/4 cm diameter and of very small thickness. The main reason for this vast difference in volume, and hence in combustion intensity, lies in the steep dependence of the rate on temperature (see Fig. 1), the horse being some 3,000° C cooler than the detonation.

Even if we omit biological systems and confine ourselves to combustion reactions, the history of mankind has been intimately associated with this steep temperature dependence of the rate law. The observation that fuels and oxidants will not react at any appreciable speed when left alone at normal temperatures, but will do so at an alarming rate once they are raised to a high temperature, must have been one of mankind's first scientific discoveries. Since then the trend, very broadly, has been to ever higher temperatures and combustion intensities—that is conversion rates averaged over the volume of the combustion chamber—and under more controllable conditions. In view of the diminishing resources of fossil fuels (and their potential usefulness for producing more interesting chemicals than carbon dioxide and water), of the falling costs of nuclear power generation and of the public awakening to the effects of pollution, it seems opportune to reassess the principles available for controlling the temperature in combustion reactions and to give serious consideration to any suggestion which may emerge for modifying practical systems.

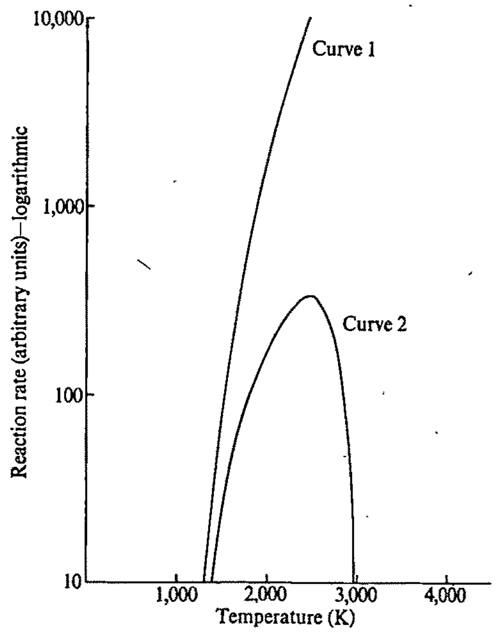


Fig. 1 Curve 1 shows the variation of reaction rate with temperature at constant pressure taken as $T^{3/2}\exp(-44\times10^3/RT)$. Curve 2 as curve 1 but with depletion and dilution of reactants, that is the temperature rise is due to reaction and/or mixing with hot products. It is curve 1 multiplied by $[1-(T-300)/(3{,}000-300)]^2$.

The potential advantages of high combustion temperatures are: high reaction rates, high thermodynamic efficiency in the case of conversion to power, high heat transfer rates to surfaces on which dissociated species can recombine, higher ionization (which may be useful in particular applications such as magnetohydrodynamic control or power generation) and the opportunity of producing endothermic products by sudden quenching. These advantages are obviously over-riding for some purposes. For others, however, they are vitiated by the production of certain pollutants at high temperatures—particularly NO—and, chiefly, by the difficulty of handling such hot gases. These are so much above the melting points of the devices used to contain (for example, combustion chambers) and exploit (for example, turbine blades) them that one cannot realize the potential advantages in practice. In the case of heat transfer rates to surfaces there are applications—such as cutting, melting, welding, spheroidizing, and so on—in which the heat sink is not intended to remain intact. In most others, however, the melting of the confining walls would be disastrous. In this way, the chief advantages such as high combustion intensity or thermodynamic efficiency may be wasted if the high temperature cannot be maintained over the entire volume of the combustion chamber or converter. Yet the high temperature may nevertheless be necessary, in conventional combustors, in order to keep the combustion zone stable at the desired throughput. Thus we may be forced to accept the disadvantages of high temperatures—for example, excessive heat losses and formation of NO—yet at the same time have to cool the gases before they become acceptable, for example, to turbine blades.

The combustion temperature range is limited, at its lower end, by the least temperature at which a stable flame can be maintained—not much below 1,000° C under optimum conditions. This is determined by the interplay between the above mentioned temperature dependence of heat release rate with that of heat loss from the reaction zone. The upper limit is set, in practice, by the difficulty of containment within a solid container—or at best a liquid melt held against the cylindrical walls of its parent solid by rotation-induced centrifugal force. Ionization does not become adequate for magnetohydrodynamic confinement until enthalpies attain values which dwarf the contribution from the heat of combustion. There is also some much higher temperature limit beyond which the heat of reaction is not retrievable from the dissociated species until they are allowed to cool down. Both these limits depend on the application envisaged and do not apply in cases where maximum heat transfer—for example, for cutting or welding—is the desired objective.

In thinking about pre-mixed flames we tend to associate flame temperature absolutely with the initial reactant composition—that is, the fuel to air ratio. The adiabatic final flame temperature is given, for a given mass of mixture, by

$$Q_c = \int_{T_{\text{unburnt}}}^{T_{\text{burnt}}} C_p \mathrm{d}T = [H_b - H_0]$$

where Q_c is the heat of combustion and C_p the heat capacity of the products at constant pressure. It is convenient to work in terms of enthalpy, H, so as not to have to take into account the effect of dissociation. For a simple flame (Fig. 2*a*), the reaction occurs over a range of temperatures close to T_b. If a converter is operated at this temperature its efficiency generally falls far short of the Carnot cycle value.

To maintain flexibility, however, it is better if we allow ourselves the variable of adding, subtracting, or recirculating some of the energy. Addition of energy to the reactants is perhaps the most obvious further degree of freedom and method of control. In its simplest form, we can preheat the reactants, though doing this by means of a solid preheater is limited to relatively low temperatures, both by the onset of preignition and by melting of the containing walls. Beyond this, we can use radiation, adiabatic compression, or electrical heating. As regards radiation, the difficulty is that in a gas (unlike in a dust cloud, for example) there is virtually nothing to absorb it. To succeed, the radiation flux has to be high enough to create a sufficient concentration of free electrons to absorb the energy. This can be achieved transiently at the focus of a Q-switched laser[1] but cannot, as yet, be done continuously. Adiabatic compression in continuous flow systems has been applied in the form of a stabilized detonation or, at least, shock initiated combustion[2,3]. The burner here looks more like a supersonic wind tunnel, the gases emerging under-expanded from a nozzle and being rapidly heated across the standing shock wave. Experimentally, it is easier and cheaper to add the energy electrically by means of some kind of discharge and various devices based on a.c., d.c., magnetically rotated, arcs and radio frequency discharges have been discussed in the literature[4].

In all these cases, if Q_a is the energy added, the final temperature, T_f, is obtained from

$$\int_{T_0}^{T_f} C_p \mathrm{d}T = Q_c + Q_a = H_f - H_0$$

and the idealized thermodynamic efficiency corresponds to the higher temperature level. Again, this cannot be approached in practice, the additional problems here being associated with the containment and utilization of the much higher temperatures involved. Now there are some special purposes[4] for which such augmented flames promise to be valuable, because of the large heat transfer from their dissociated products, their ability to stabilize very large reactant throughputs by generating specific radicals when they are used as pilot burners in high intensity combustion systems[7], or for producing endothermic compounds by quenching their products. As the chemical energy from fuels tends as yet to be much cheaper than that from other supplies mentioned above, it is economically unacceptable for general purposes to heat these systems from an external source. The above mentioned increase in efficiency is small compensation for having to supply Q_a as electrical power, particularly when losses in a compressor, or to arc electrodes, have to be taken into account.

Of greater economic viability is the concept of recirculating some of the combustion energy. Perhaps the most frequently used method of flame holding which operates on this principle is the recirculation of some of the hot combustion products. Widely used, particularly in propulsion devices, in its simplest form this requires only some baffle or change in contour of combustion chamber which will induce a vortex in the appropriate region. This kind of recirculation is, however, always accompanied by dilution of reactants with products and is useful only because of the particular form of the rate law which implies that the effect of increasing temperature is very considerably greater (see Fig. 1, curve 2) than the corresponding consumption of reactants and dilution with products, over a large part (at least the first 75%) of the conversion scale. The normal flame temperature (and hence the maximum efficiency of any converter) remains unaltered. Only the maximum throughput of the combustion chamber is increased because the volume giving a high combustion intensity is greatly enlarged by increased mixing over that in a laminar flame in which it is confined to a very narrow zone by the small scale of molecular transfer processes. Further improvements in mixing eventually lead to homogeneity and the idealization of the perfectly mixed reactor[5] in which conditions are deemed to be uniform. The dilution effect, however, places an absolute maximum on the reaction rate attainable in this way. This is reached when the conditions of the apex of the reaction rate curve (2, Fig. 1) apply to the entire volume of a perfectly stirred chamber.

I shall now consider a simple alternative to which the above limitation does not apply—recirculating heat alone. In its simplest form, consider a burner whose premixed reactants are being preheated by a single encounter with the flame products (Fig. 2*b*). The enthalpy chart is included as a corrective to the impression that this device is suitable for producing infinite temperatures in the steady state. Indeed, once the steady state has set in, the exit temperature is the normal T_{burnt} value, calculated as above, since the outgoing enthalpy must be equal to the energy input. At this point it does not differ from the scheme based on recirculating hot products. In between the initial and the ultimate final stages, however, we have achieved a higher temperature on borrowed heat and this will produce a higher reaction rate (following a curve such as 1 in Fig. 1) or any of the other desirable features mentioned above. On taking the inlet tube round once more and postulating two perfect single stage heat exchangers (Fig. 2*c*) the maximum would correspond to double the borrowed enthalpy and so forth. To extend the concept, let us introduce a counter flow heat

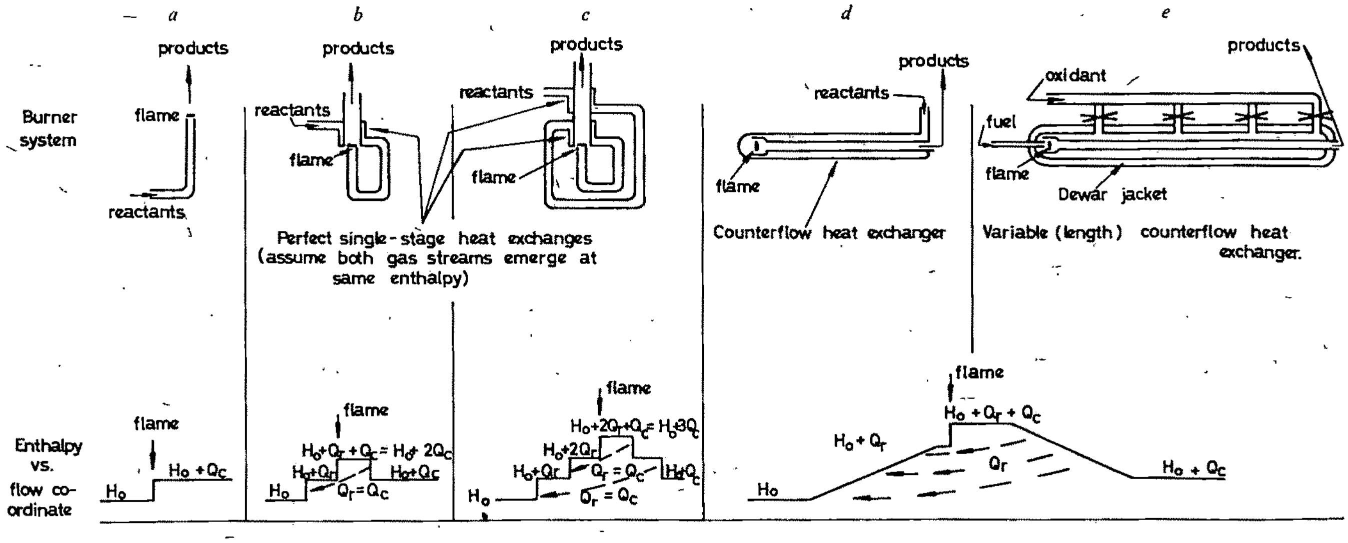


Fig. 2 Relation between enthalpy and flow coordinate for various burner systems.

exchanger (Fig. 2*d*) which we can make as long as we wish. In Fig. 2*e* some further refinements have been added: a vacuum flask surround to minimize heat losses from the device; a separate fuel and oxidant inlet, to prevent premature ignition; and a means of varying the heat transfer between outlet and inlet during operating conditions—all of which are perfectly feasible in practice. As regards the latter, there are, of course, more elegant methods of varying heat transfer than by the variation of effective length by selecting the appropriate inlet—the drawing is purely schematic. These devices dissociate the flame temperature from the initial composition. The enthalpy immediately beyond the reaction zone, H_c, is now given by

$$H_c - H_0 = Q_c + Q_r$$

being determined as much by the heat recirculated—Q_r—as by the input energy, Q_c. Moreover, the efficiency of a converter now operates on $(H_0 + Q_c + Q_r)$ instead of $(H_0 + Q_c)$. The output is therefore correspondingly higher and the overall efficiency can be increased by increasing Q_c, providing only that a small temperature differential is left to drive the heat transfer. In the case of a suitable converter it is possible to reject the thermodynamically unusable heat to the incoming gas—making the power cycle part of the heat exchanger. (For example, a thermoelectric generator could be operated between the combustion chamber and the inlet tube.) At first sight it seems surprising that such increases in overall efficiency should be possible without violating the laws of thermodynamics. In fact, we are merely "building a dam in the heat flow" and using the additional potential of releasing the chemical energy at its highest point.

Of course, in the case of mixtures of the now usual heats of reaction the heat exchangers in Fig. 2 would melt before more than 75% of the enthalpy could be recirculated. If, however, we can increase temperature arbitrarily by recirculating heat, we are no longer confined to such flames. At this point it will therefore be convenient to divide the entire combustion field into a high and a low temperature branch, the dividing line being drawn at the highest available melting point of any material of which a heat exchanger could be constructed. For the high temperature branch, if it is desired to raise the reaction temperature further, the principle of Fig. 2 must be interpreted schematically. Instead of the heat exchanger we might use a generator of electric power downstream which would supply an arc heater ahead of the flame, or we could make use of a detonation, in fact. In a steady state detonation, the degradation of momentum of the exit gases feeds the shock wave in front, which preheats the gas and is thus responsible for the high rate of conversion. Burners in which detonation is constrained to rotate in a torroid while the reactants are fed in, and products removed through slots in its side, have been proposed[6].

For many applications, however, it may well be that the low temperature branch will appear more promising. Since the melting point of the container may not be exceeded even immediately downstream of the combustion chamber, this makes possible the use of extremely lean mixtures, or of very low grade fuels. In this way it would be possible, for example, to burn the ventilation air of gassy mines; it has been shown that a 1% mixture of methane and air will produce a selfsustaining flame, when it is preheated to less than 1,000° C, even though its own heat release contributes only another 250° C or so. This is, of course, an extreme case and, by varying the fraction of heat recirculated, the burner could be adapted to any mixture, so long as the normal adiabatic final flame temperature itself does not exceed the melting point. At the same time the scheme retains all its advantages of producing no high temperature pollutants, providing gases at temperatures acceptable to other mechanical components without an intermediate cooling stage and allowing some of the energy to be drawn at a higher temperature, and therefore efficiency, level, using a suitable thermoelectric converter or a Stirling motor, perhaps. An experimental quartz burner of this type (Fig. 3) instrumented with thermocouples to monitor gas temperatures in various parts of the stream is now being operated in very lean mixtures of methane and air.

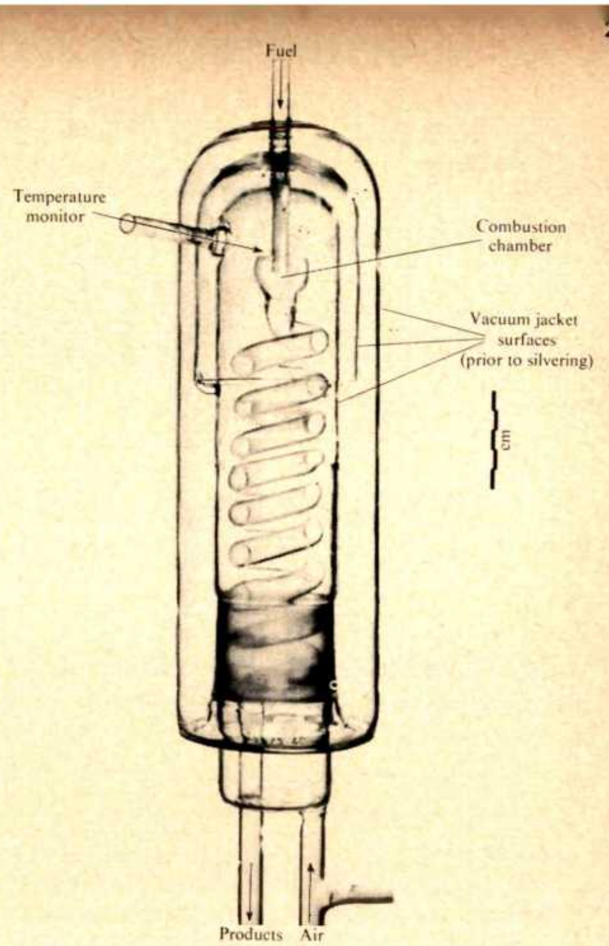


Fig. 3 Heat recirculating burner for lean fuel to air ratios.

With the advent of coal dust and other fluidized bed reactors, the subject has been drifting in this direction during the past few years. A fluidized bed does, of course, act as a very efficient heat exchanger. In the more remote future it may well be, when further improvements in the combustion intensity of low grade fuel mixtures are required, that they will be achieved by hybridizing the high and low temperature branches. It has become apparent already that an augmented flame pilot producing radicals at high temperatures would greatly help to stabilize a large throughput of mixtures of low heats of combustion[7], particularly if these have already been preheated by the recirculation scheme.

Thus, once we can control the reaction temperature independently of the heat of combustion of the mixture by one of the methods mentioned, it becomes feasible and desirable to design combustion chambers for specific purposes. In this way we can increase combustion intensity and converter efficiency, minimize pollution, burn low grade fuels efficiently and generate product gases at a temperature acceptable to machinery downstream without either subsequent cooling or having to reduce reaction temperature and hence throughput.

I thank Drs A. R. Jones, D. R. Hardesty and J. B. Cox for helpful discussions.

[1] Schwar, M. J. R., and Weinberg, F. J., *Combustion and Flame*, **13**, 335 (1969).
[2] Gross, R. A., *Amer. Rocket Soc. J.*, **29**, 63 (1959).
[3] Nicholls, J. A., and Dabora, E. K., in *Eighth Intern. Symp. Combustion*, 644 (Williams, and Wilkins, Baltimore, 1962).
[4] Lawton, J., and Weinberg, F. J., *Electrical Aspects of Combustion* (Oxford University Press, 1969).
[5] Longwell, J. P., and Weiss, M. A., *Ind. Eng. Chem.* (Ind. Intern. ed.), **47**, 1634 (1955).
[6] Voitsekhovskii, B. V., *Sov. Fiz. Dokl.*, **4** (trans. from *Dokl. Akad. Nauk SSSR*, **129**, 1254 (1959).
[7] Harrison, A. J., and Weinberg, F. J., *Proc. Roy. Soc.*, A, **321**, 95 (1971).

Obsidian Source Identification by Fission Track Analysis

S. A. DURRANI, H. A. KHAN & M. TAJ*

Department of Physics, University of Birmingham, Birmingham B15 2TT

COLIN RENFREW

Department of Ancient History, University of Sheffield, Sheffield S10 2TN

Fission track analysis has been used to determine the age and uranium content of obsidians from sources in south-east Europe and Anatolia, and from archaeological deposits in mesolithic levels at the Franchthi Cave in southern Greece. It is confirmed that the Franchthi obsidian came from the Aegean island of Melos. This is the earliest positive indication available for maritime travel, and carries the history of seafaring back a thousand years.

OBSIDIAN, or volcanic glass, was widely traded in prehistoric times, and the investigation of the trading pattern has long been a preoccupation of archaeologists and prehistorians. To characterize the obsidian, of which an artefact is made, physical or chemical properties of the material must be recognized which distinguish it as deriving from a specific natural source. Optical spectroscopy, X-ray fluorescence, and neutron activation are among the analytical techniques which have been used[1] for this purpose.

Trace element analysis usually works well: when concentrations of appropriate elements (for example, barium against zirconium) are plotted, obsidians from single sources form distinct clusters, well separated from one another. Difficulties, however, arise when obsidians from several different sources show convergent compositions. This has been observed[2] in Greece, where obsidians from the sources on the Cycladic island of Melos in the central Aegean Sea resemble in composition those from the Dodecanesian island of Giali in the eastern Aegean, lying 240 km east of Melos. As a further complication, both resemble, in their trace element composition, obsidian from the Tokaj region of Hungary, 1,300 km to the north-west, and from the central Anatolian source at Acigöl near Kayseri, 1,000 km to the east of Melos. These are the sources nearest to the Aegean, and it has been suggested that Hungarian or Anatolian obsidian reached Greece by trade in prehistoric times.

When obsidian artefacts are found in very early archaeological contexts in mainland Greece, an Aegean source for the material (in Melos or Giali) seems the most likely. But clearly the possibility of trade with Anatolia or with Hungary cannot be ruled out, especially because recent work[3] has confirmed that there was already an extensive early trade in seashells between the Aegean and the Balkans, in neolithic times. The possibility also remains, although it is remote, that obsidian was also obtained from some Aegean source at present unknown.

To overcome these problems it seemed useful to consider other variables which might discriminate between sources. The date of eruption in which the obsidian was formed is one such parameter, and the fission-track analysis method of age determination was a possible approach. The method, of course, has its limitations: two geographically remote eruptions may have occurred at roughly the same time; or, conversely, successive eruptions may have taken place in a given area over a long time. This is known to be the case in the Lipari islands off the coast of Sicily in the Tyrrhenian Sea, where dated archaeological deposits lie intercalated between obsidian-bearing strata. The same may hold for the Acigöl source, where several obsidian flows are known in the source region. In such cases the determination of a further parameter, the uranium content of obsidian samples, which is an integral part of the fission track analysis technique, may be useful in supplementing other criteria available for source identification. It may be added here that our purpose has not been to date the use of the artefacts (which are made by chipping or flaking, and not melting, of the obsidian). That only becomes possible if the artefact has been heated to high temperatures during its use[4], thus annealing out the then-existing fission tracks and, effectively, re-setting the clock.

Our attention has been focused on obsidians designated as Group 1 on the basis of the existing trace-element classification of Cann and C. R.[5], the aim being to distinguish between the various members of this group (which comprises obsidians from Melos and Giali in the Aegean Sea, as well as those from central Anatolia and the Carpathian Range in Hungary). Samples from these sources, the trace-element composition of which had previously been determined by optical spectrography[2,6], were selected for analysis (Table 1). Furthermore, three samples were chosen from the "mesolithic" (that is, pre-farming) levels at the Franchthi Cave in South-East Greece, excavated by Professor T. W. Jacobsen[7]. Radiocarbon dates for this period at Franchthi, lying in the eighth and seventh millennia BC, establish this as the earliest prehistoric context in the Aegean where obsidian has been found. For instance, stratum H-1, A117, from which obsidian was examined, yielded dates of 7527 BC and 6792 BC on the 5568 year ^{14}C half-life.

---

*Present address: University of Surrey, Guildford.

Table 1 Ages and Uranium Contents of the Obsidians Investigated

Obsidian sample *	Age (m.y.) †	U-content (p.p.m. by weight) ‡	
		Primary detection method	Auxiliary detection method
Hungarian			
Borsod (60)	3.86±0.24	8.6	7.7
Borsod (22)	3.37±0.27	8.9	
Central Anatolian			
Bor (100)	2.29±0.32	2.5	
Acigől (269)	1.95±0.33	3.5	
Acigől (156)	8.14±0.59	9.0	
Aegean			
Giali (390)	2.01±0.26	11.1	
Giali (218)	8.04±0.65	4.1	
Melos, Adhamas (120)	8.95±0.94	4.7	
Melos, Adhamas (25)	8.54±0.73	4.8	
Melos, Dhemenegaki (119)	8.35±0.72	4.9	
Melos, Dhemenegaki (116)	2.36±0.53§	—	9.1
Franchthi cave (S. Greece)			
Sample 6936 (H-1, A117)	8.48±0.55	4.6	
Sample 7077 (H-1, A123)	8.82±0.57	4.1	
Sample 7066 (H-1, A99)	9.33±0.60	4.4	

* The code numbers are in accordance with those in refs. 2 and 6.

† All ages are determined by using equation (1) (see text), based on the primary detection method, except for sample 116. No corrections are necessary for thermal lowering of ages (see Table 2). The quoted uncertainties in ages are made up of statistical (Poisson) errors, background subtraction errors, and fluence uncertainties (±5%).

‡ The U-content is determined in all cases by the primary detection method (that is by counting natural and induced tracks in the sample itself), except in the case of sample 116 where the auxiliary detection method was used (placing an external detector to receive induced fission fragments emanating from the sample). In the case of sample 60, both methods were used for comparison purposes. The uncertainty in uranium content values is estimated to be ~10%.

§ The age is based on natural track counting in conjunction with uranium-content determination by the auxiliary detection method in view of the poor optics of the sample. These two factors result in the unusually high error quoted (±22.5%). It is hoped to make a more precise determination of this age later.

Fission Track Analysis

The fission track analysis method has been used by several authors, both to determine the ages of obsidians (see, for example, refs. 8–11) and to obtain their uranium (and thorium) content[9,11–12]. It can be readily shown (refs. 13 and 14, and unpublished work of H. A. K. and S. A. D.), that the age A (in years), and the uranium content C_u (in p.p.m. by weight) in a material, are given by

$$A=(\rho_n/\rho_i).5.01\times 10^{-8}.F \qquad (1)$$

and C_u (p.p.m.)$=9.445\times 10^{7}\times \rho_i/(D.R_{ap}.\cos^2\theta_c.g.f(t).F)$ (2)

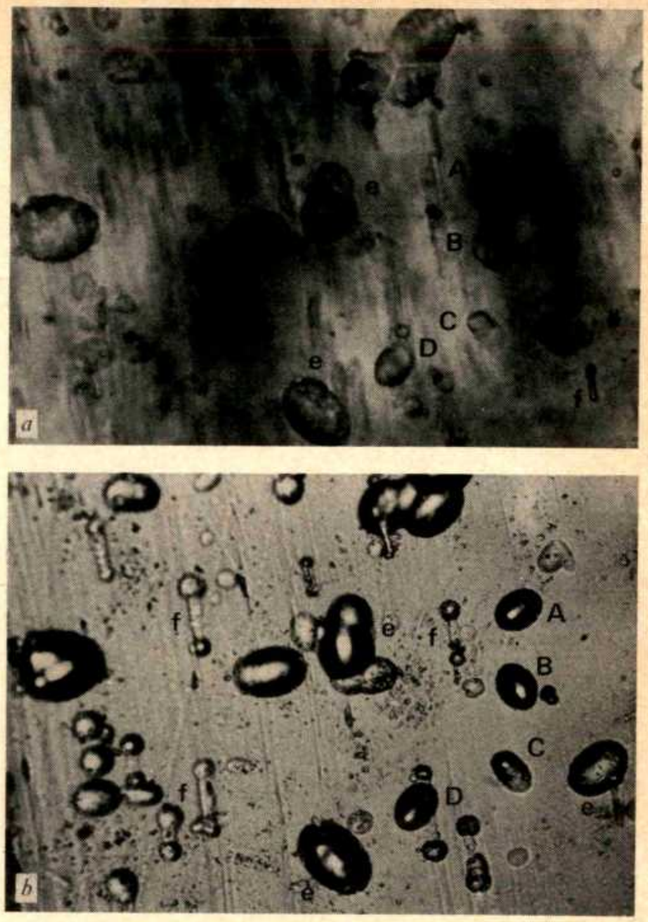


Fig. 1 Contrast between the two modes of microscopic viewing. The same field of view in the Dhemenegaki sample 119 is shown in the two photomicrographs under (*a*) transmitted light mode and (*b*) "epi-illumination" (reflected-light) mode. In *a*, the poor optics of the sample (notice streaks of varying colour and refractive index) results in ill-defined fission etch pits, which are difficult to distinguish from spurious pits and defects. In *b*, epi-illumination brings out the genuine fission etch pits (A, B, C, D) quite clearly. Pits marked e are spurious etch pits on the adopted criteria; configurations marked f are believed to be feldspar crystals the extremities of which appear to offer preferential etching channels to the acid as seen in *b*; (in *a* the feldspar crystals are in deeper layers which have not been etched).

Here ρ_n and ρ_i are the densities of etch pits per cm^2 produced by spontaneous and induced fission, respectively, the latter being induced by a fluence F of thermal neutrons per cm^2, D is the density (g cm^{-3}); R_{ap} the "apparent range" of an average fission fragment; θ_c the "critical angle" of etching; g a geometrical factor; and $f(t)$ the "prolonged etching factor" for the material. We take the value of $D\times R_{ap}$ to be the same as found[14] for a "reference glass" (U-2) containing a known amount of uranium (namely, 2.41 g $cm^{-3}\times 15.2$ μm), and $g=1$ (that is "4π geometry": the contribution of fission fragments from both sides of each etched surface being taken into account). The numerical factors in equations (1) and (2) result from various natural constants; in particular the fission decay constant of ^{238}U has been taken to be 8.42×10^{-17} yr^{-1}.

The critical angle, θ_c, is defined as the minimum inclination, with the surface, of a track that can be successfully etched. We have determined the value of θ_c for (Hungarian) obsidian (unpublished work of H. A. K. and S. A. D.), using an external collimated fission source of ^{252}Cf, to be 26° 00′±30′.

The prolonged etching factor $f(t)$, first introduced by H. A. K. and S. A. D. (unpublished work; see also ref. 14) is defined as the ratio ρ/ρ_0, where ρ is the track density observed on etching the sample for time t, and ρ_0 is the extrapolated value corresponding to zero etching time. The factor cancels out in equation (1) if identical etching conditions are used to determine both ρ and ρ_i, but not in equation (2). The value of $f(t)$ for obsidian is found to be 1.17 at $t=60$ s (on etching in ≈ 40 vol. %HF at 13° C).

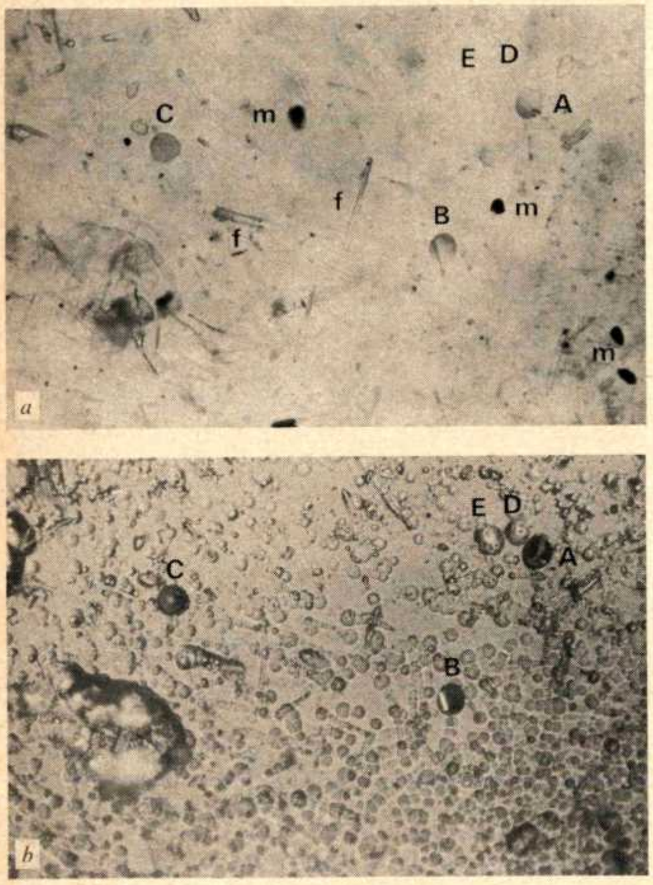


Fig. 2 Acigöl sample 269 observed under (*a*) transmission mode and (*b*) epi-illumination mode of microscopic viewing. In *b* the genuine fission etch pits (A, B, C, D, E) on the top surface are easy to pick out, whereas they remain indistinct in *a* which covers the same field of view. The needle-like objects marked f are feldspar crystals; the black inclusions marked m are believed to be magnetite or other iron ore crystals in the body of the sample (seen in *a*). Such crystals and inclusions, when they cross the top surface, get etched and result in the spurious pits seen in *b*. That most of these pits and other defects are unconnected with fission damage is confirmed by their survival on high temperature annealing (see text). The bright line in etch pit B seen in *b* is the reflexion from a feldspar crystal lying in a different plane.

Although the fission track analysis technique is simple in principle, considerable difficulties were encountered in applying it to obsidians. Several samples were optically very "dirty", containing dark streaks of varying colours and inclusions of crystals of magnetite, feldspar and the like. The latter were found to lie along "flow bands", indicating that feldspar crystals had formed while the obsidian was still partially fluid and in the process of cooling down. On etching in hydrofluoric acid, when a feldspar crystal (or less frequently, a limb of a skeletal magnetite crystal) crossed the etched surface, it seemed to offer a preferential etching channel to the acid, thus yielding an etch pit which was often difficult to distinguish from a genuine fission-fragment etch pit. That most of the pits and other defects were unconnected with fission damage was confirmed by the observation that, in spite of heating the obsidian samples for 1 h at 700° C, the number of etch pits subsequently produced remained largely unchanged. Fleischer and Price have shown[10] that even pure fused silica loses all fission tracks under these annealing conditions (tektite glass requires[10,15] only ~500° C for 1 h for this to happen).

These problems of extraneous, but optically similar, etch pits and enclosed "blisters" and other defects were largely overcome by adopting the "epi-illumination" mode of viewing, instead of the transmitted-light mode used hitherto. The microscope used was a Zeiss 'Photomicroscope'. Figs. 1 and 2 show two pairs of typical photomicrographs under these two viewing conditions.

Ages and Uranium Contents

Table 1 records the ages and uranium contents of obsidian samples from Hungary (Borsod region), central Anatolia, and the Aegean Sea (three different locations). It is interesting to note that although the two samples from Hungary have, within experimental errors, identical ages of formation, the Aegean and the central Anatolian samples show a certain sequential eruption. Thus the two samples (120 and 25) from Adhamas as well as one each from Acigöl (156), Giali (218) and Dhemenegaki (119) all show eruption ages between 8 and 9 m.y., thus suggesting a general volcanic activity in the region at that time. A further simultaneous activity in the whole area (indicated by the central Anatolian samples 100 from Bor and 269 from Acigöl; sample 390 from the island of Giali; and sample 116 from Dhemenegaki on the island of Melos) seems to have taken place about 2 m.y. ago. These conclusions are of considerable potential interest to geologists and petrologists. From our point of view, however, the coincidence of eruption dates (~2 m.y. and ~8 m.y.) in the various locations of interest is a little unfortunate, because it makes clear-cut identification of obsidian sources more difficult. This coincidence is, nevertheless, fortuitous and the principle of the method remains unaffected.

A further variable at our disposal is the uranium content. Here, the two Hungarian samples are found (within experimental errors) to have identical uranium contents as well as eruption ages; the same applies to three of the samples from the island of Melos (120 and 25 from Adhamas, and 119 from Dhemenegaki). The two obsidian samples each from Acigöl, Giali and Dhemenegaki can, however, be clearly differentiated on the basis of both age and uranium content. The uranium content of sample 116 from Dhemenegaki (which was optically very dirty) has been determined by H. A. K. and S. A. D. (unpublished work) using the auxiliary detection method (by irradiating the sample, while in contact with an external plastic detector, in a nuclear reactor). The U-content (9.1 p.p.m.) is quite distinct from the other Dhemenegaki sample (119), as is the age.

In comparing ages, it is necessary to ensure that some of them have not been thermally lowered. It is known (see, for example, refs. 15 and 16) that the application of heat results in track fading, leading to an apparent reduction of age. This can be corrected[14-16] by examining the etch pit diameters (in identical etching conditions) of freshly induced fission and spontaneous (or natural) fission: for fading of tracks is accompanied by a general diminution of the resulting etch-pit diameters. Table 2 shows the results for induced and spontaneous fission in the obsidians studied by us. Application of t test to each of the distributions listed shows that no significant diminution of etch-pit diameters has taken place. The ages recorded in Table 1 can thus be taken to be the true ages.

Franchthi Cave Obsidian

It is clear from the foregoing that, in favourable circumstances, the eruption age and uranium content of obsidian samples can serve as source identification criteria. As a test case, a heuristic assay was made on three obsidian samples from mesolithic levels of the Franchthi Cave in the Argolid, southern Greece. Nearly all the obsidian artefacts and waste flakes at Franchthi show the pearly lustre common in obsidian from both the Melian sources at Adhamas and Dhemenegaki. Melos, lying 120 km south-east of Franchthi, is the nearest known source of workable obsidian. Preliminary spectrographic analyses by Dr J. E. Dixon of the University of Edinburgh place the Franchthi specimens in Group 1 of the existing classification, which is consistent with a Melian origin. The very early date of the Franchthi material, and the lack of other evidence for maritime travel in the eighth or seventh millenium BC made a fresh approach, by fission track analysis, desirable.

Table 2 Comparison of Etch-pit Diameters of Spontaneous and Induced Fission Tracks in Obsidian Samples in Order to Judge whether Thermal Lowering of Ages has Taken Place

Obsidian sample*	Etching time (s)	Mean etch pit diameter (μm) (and the number of pits measured)‡ Spontaneous fission	Induced fission
Hungarian			
Borsod (60)	90	14.16±2.30 (80)	14.39±2.22 (67)
Borsod (22)	60	9.74±1.32 (80)	10.01±1.55 (70)
Central Anatolian			
Acigöl (269)	60	8.81±1.49 (100)	9.06±1.57 (100)
Acigöl (156)	60	8.93±1.46 (120)	9.06±1.37 (120)
Aegean			
Giali (390)	60	8.96±1.23 (103)	8.90±1.34 (112)
Giali (218)	60	8.76±1.16 (89)	8.71±1.05 (81)
Melos, Adhamas (120)	90	11.51±1.28 (103)	11.29±1.29 (98)
Melos, Adhamas (25)	60	8.63±1.10 (90)	8.61±1.12 (88)
Franchthi cave (S. Greece)			
Sample 6936 (H-1, A117)	60	8.56±1.22 (95)	8.70±1.19 (98)
Sample 7077 (H-1, A123)	60	8.86±1.32 (105)	8.85±1.25 (105)
Sample 7066 (H-1, A99)	60	9.00±1.14 (91)	8.98±1.23 (91)

No significant difference in diameters is found in any sample, as verified by the application of *t* test to the means and the standard deviations of the distributions.

* The code numbers are in accordance with those in refs. 2 and 6.

† Etching is always carried out in ≈40 vol. % HF at 13° C.

‡ The numbers of etch pits measured are shown in parentheses immediately after the values of diameters. For elliptical pits, major axis has been measured instead of diameter. The errors shown are standard deviations of "single observations".

Table 1 records the results for age and uranium content from these samples. It will be seen that not only do the ages and U-contents agree between the three samples, but they are also very similar to those for Melian obsidian from Adhamas (samples 120 and 25), and for one of the Dhemenegaki samples (119). They are also compatible with one of Giali samples (218) but not with any of the central Anatolian or Hungarian samples.

The results are thus entirely consistent with an origin in the Melian sources at Adhamas or Dhemenegaki. They exclude a Hungarian or central Anatolian origin, and are particularly useful in reducing the likelihood of an origin in some source in mainland Greece so far unknown, a possibility suggested by some scholars[17]. Although the possibility of unrecorded obsidian sources in mainland Greece could not be excluded, it already seemed unlikely that such a source would fortuitously produce material resembling that of Melos both in appearance and trace element composition. That it should further match the Melian material both in age and uranium content (which the prehistoric finds from Franchthi now do) would be an altogether remarkable coincidence. We therefore suggest that the possibility of such an origin for the Franchthi material, from an unknown source, may now be excluded.

The conclusion that the Franchthi obsidian came from Melos is of great interest. Hitherto, the earliest evidence for a traffic in Melian obsidian was from early neolithic sites at Knossos in Crete, at Sesklo in Thessaly and at Nea Nikomedeia in Macedonia, all a full millenium later. It seemed that in the Aegean, as in the Near East, the obsidian trade began in earnest at the time of the development of farming. Now it seems that the hunters and fishers of southern Greece were already, by the beginning of the seventh millenium BC, and perhaps earlier, reaching Melos by boat. The occurrence of obsidian in successive layers at Franchthi suggests, moreover, that Melos was reached on several occasions. As Jacobsen points out[7], it may be relevant that obsidian first occurs at Franchthi at about the same time that its inhabitants were beginning the intensive exploitation of marine fish. Seafaring and trade may have been the consequence of the intensive use of the sea as a food source.

The Franchthi Cave obsidian is the earliest positive evidence available, from anywhere in the world, of the transport of goods by sea. The very rare obsidian from early neolithic Cyprus, brought by sea from the central Anatolian sources[18], is not found there until 1,500 yr later. The corroboration by fission track analysis of a Melian origin for the Franchthi Cave obsidian thus carries back the history of seafaring by more than a thousand years.

We thank Professor T. W. Jacobsen for allowing C. R. to study the Franchthi Cave obsidian, and for arranging (with the cooperation of the Greek Archaeological Service) that selected samples be made available for analysis; Mr E. D. Lacy for advice on the chemistry of obsidians; and the SRC and the staff of HERALD reactor at AWRE, Aldermaston, for irradiation facilities. H. A. K. and M. T. thank the Pakistan AEC and Colombo Plan authorities, and the Government of Pakistan, for financial support.

Received July 9; revised August 18, 1971.

[1] Cann, J. R., Dixon, J. E., and Renfrew, C., in *Science in Archaeology* (edit. by Brothwell, D., and Higgs, E. S.), 578 (Thames and Hudson, second ed., 1969).

[2] Renfrew, C., Cann, J. R., and Dixon, J. E., *Ann. Brit. School Archaeol. Athens*, **60**, 225 (1965).

[3] Shackleton, N., and Renfrew, C., *Nature*, **228**, 1062 (1970).

[4] Fleischer, R. L., Price, P. B., Walker, R. M., and Leakey, L. S. B., *Nature*, **205**, 1138 (1965).

[5] Cann, J. R., and Renfrew, C., *Proc. Prehist. Soc.*, **30**, 111 (1964).

[6] Renfrew, C., Dixon, J. E., and Cann, J. R., *Proc. Prehist. Soc.*, **32**, 30 (1966).

[7] Jacobsen, T. W., *Hesperia*, **38**, 343 (1969).

[8] Watanabe, N., and Suzuki, M., *Nature*, **222**, 1057 (1969).

[9] Kaneoka, I., and Suzuki, M., *J. Geol. Soc. Japan*, **76**, 309 (1970).

[10] Fleischer, R. L., and Price, P. B., *Geochim. Cosmochim. Acta*, **28**, 755 (1964).

[11] Suzuki, M., *J. Anthropol. Soc. Nippon*, **78** (1), 50 (1970).

[12] Hair, M. W., Kaufhold, J., Maurette, M., and Walker, R. M., *Radiat. Effects*, **7**, 285 (1971).

[13] Durrani, S. A., and Hancock, D. A., *Earth Planet. Sci. Lett.*, **8**, 157 (1970).

[14] Durrani, S. A., and Khan, H. A., *Nature*, **232**, 320 (1971); *Nature Physical Science*, **232**, 175 (1971).

[15] Durrani, S. A., and Khan, H. A., *Earth Planet. Sci. Lett.*, **9**, 431 (1970).

[16] Storzer, D., and Wagner, G. A., *Earth Planet. Sci. Lett.*, **5**, 463 (1969).

[17] Theochares, D. R., *I Argi tis Thessalikis Proistorias*, 79 (Philarchaios Etaireia, Volos, 1967).

[18] Renfrew, C., Dixon, J. E., and Cann, J. R., *Proc. Prehist. Soc.*, **34**, 325 (1968).

Evolution of the Scotia Arc

IAN W. D. DALZIEL & DAVID H. ELLIOT

Department of Geology and Lamont-Doherty Geological Observatory of Columbia University in the City of New York, and Department of Geology and Institute of Polar Studies, Ohio State University

---

In this article the evolution of the Andean–West Antarctic Cordillera, and its subsequent bending, disruption, and fragmentation in the Cenozoic to form the Scotia Arc, is documented by geological evidence.

THE suggestion that the Andean mountain chain reappears in the Antarctic Peninsula[1] (Fig. 1) has in the past been supported by geological similarities[2,3] and the discovery of the discontinuous arcuate submarine Scotia Ridge (Figs. 1 and 2) that joins the two continents[4]. Significant detail has been added in the past thirty years to strengthen this hypothesis.

In earlier models for the evolution of the Scotia Arc, as the whole feature is generally known, the islands of the North and South Scotia Ridges (Fig. 2) were assumed to be parts of an originally straight north–south Cordillera displaced eastward relative to South America and Antarctica during the drift of these continents westward relative to Africa[5–9]. Recently, however, it has been suggested that the present east–west trend of the North and South Scotia Ridges is primary[10–12]. Our review[13] of the published data, and of unpublished data resulting from recent field work[14–17] (also personal communications from G. Grikurov, 1970; M. K. Rickard and K. T. Burns, 1970; J. Stewart, 1971; and E. González P., 1971), leads us to support the concept of an essentially rectilinear Andean–West Antarctic Cordillera. In what follows, we shall reconstruct this Cordillera in some detail. The bending, disruption and fragmentation which formed the Scotia Arc imply less shortening of the Pacific margin than has been assumed[18].

Geological Considerations

The Scotia Ridge system is discontinuous, and locally emergent, forming small, high and extremely rugged islands (Fig. 2). The islands on the east–west trending North and South Scotia Ridges consist mainly of pre-Cenozoic rocks of continental character that can be correlated with those in both southern South America and the Antarctic Peninsula[2,3,5,6,9,13,16,22–24]. The islands of the South Sandwich Ridge, which closes the arc in the east, are wholly volcanic (most are active volcanoes)[25], and lie in a seismically active belt to the west of the South Sandwich Trench (Fig. 1).

The basin of the western Scotia Sea and Drake Passage (47° W to 70° W) are underlain by "normal" oceanic crust[26,27], but in some eastern parts of the basin there is a layer with P-wave velocity 7.5 km s^{-1} at the base of the crust[26–28]. Refraction profiles across the Burdwood Bank and the platform south of the South Orkney Islands (Fig. 2) reveal layers that are interpreted to be continental[26–30]. South Georgia and the South Shetland Islands (Fig. 2) are underlain by approximately 4 km of low velocity rocks (possibly consolidated sediments) over high velocity crustal material that may be either continental or oceanic[26,31].

The structural framework of the Scotia Arc in terms of plate tectonics is not entirely clear. It is generally assumed that the northern boundary of the Antarctic crustal plate lies along the Chile Rise and the South Sandwich Fracture Zone, both apparently consisting of fracture zones connecting short spreading-ridge segments[32,33]. But neither the North and South Scotia Ridges, nor the continental margin of southernmost South America have marked seismicity now (Fig. 1). Consequently the intense seismicity associated with the South Sandwich Trench seems to be anomalous. The problem remains of why there is relative motion between the American and Antarctic plates only along the South Sandwich Fracture Zone and the South Sandwich Arc (Fig. 1). Recently magnetic evidence has been presented for a late Cenozoic–Recent spreading-ridge, trending north-east–south-west in the Drake Passage, and for another situated immediately west of the South Sandwich Islands (Fig. 1) that trends north–south. The presence of the latter, if still active as proposed, helps to account for the seismicity of the South Sandwich Trench[10,12,21]. Reconstructions based on the former have led to an hypothesis that the Andean–West Antarctic Cordillera formed an easterly directed cusp in the area of the Drake Passage prior to 20–25 m.y. ago[10,12].

The geology of the Scotia Arc region can be simplified by emphasizing what seem to us to be the fundamental tectonostratigraphic units. This admittedly involves certain assumptions in correlation[13]. The units are

- Cenozoic sedimentary and volcanic deposits
- Late Mesozoic–Cenozoic plutons (the "Andean" igneous complex)
- Jurassic and Cretaceous sedimentary and volcanic deposits
- Late Palaeozoic sedimentary rocks
- Metamorphic basement complexes (? Palaeozoic; possibly Pre-Cambrian)

The metamorphic basement complexes (mostly low to medium grade paraschists) and the late Palaeozoic sedimentary rocks (mostly graywackes and shales) were both deformed prior to the deposition of the unconformably overlying mid to late Jurassic rocks; the sediments were deformed during the early Mesozoic Gondwanian Orogeny (known elsewhere in Antarctica as the Ellsworth[34] or Weddell[35] Orogeny), and the metamorphics probably earlier[13,22,36,37]. The age relations of the metamorphic basement and the Palaeozoic sediments are not certain, but recent Rb–Sr analysis of cores from the metamorphic basement east of the Andes[38,39] support a pre-late Palaeozoic age for the metamorphism. The Jurassic and Cretaceous rocks, including a thick sequence of mid to late Jurassic calc–alkaline volcanics, were deformed and metamorphosed with variable intensity[13–16] in the late Mesozoic–

earliest Cenozoic Andean Orogeny. They were intruded by pre-, syn-, and post-tectonic plutons of the batholithic "Andean" igneous complex[13,15,16].

Cenozoic sedimentation was scattered and locally interrupted by volcanism. No ages older than 4 m.y. (ref. 25) have been recorded from the South Sandwich Islands. But correlation of the magnetic anomalies immediately west of the islands[10,21] implies that volcanism began at least 8 m.y. ago.

The tectono-stratigraphic units can be traced in South America in continuous belts trending parallel to the Andean Cordillera. They are irregularly distributed in the islands of the Scotia Ridge and the Antarctic Peninsula (Fig. 2). The two main physiographic and tectonic provinces of southern South America are the Andean Cordillera and the extra-Andean lowlands to the east. The lowlands are underlain by the virtually undeformed Mesozoic and Cenozoic sediments and volcanics of the Magallanes Basin (Fig. 2) resting on a Palaeozoic or older metamorphic basement[38,39]. The Cordillera consists of deformed late Jurassic and Cretaceous volcanics and sediments that unconformably overlie the metamorphic basement complex, and are cut by plutons of the batholithic complex. In Tierra del Fuego the Jurassic volcanic deposits are disposed in an anticline with the metamorphic rocks in the core (M. K. Rickard and K. T. Burns, personal communication, 1970). This anticline probably continues northward along the Cordillera (unpublished data of Empresa Nacional del Petroleo, Instituto de Investigaciones Geologicas, and I. W. D. D.), although the structure is complicated by thrusting from the Pacific side[40] (also unpublished data of Empresa Nacional del Petroleo, M. K. Richard and K. T. Burns, and I. W. D. D.). Cretaceous flysch-type sediments flank the High Cordillera, those on the Pacific side being interbedded with mafic volcanics. The "Andean" igneous complex forms much of the Pacific side of the Cordillera. Late Palaeozoic sediments of the Madre de Dios Basin occur on the west coast at 50°–52° S (Fig. 2).

Our detailed reconstruction of the Andean–West Antarctic Cordillera prior to the inception of the Scotia Arc is based largely on the recognition of the tectonic provinces of southern

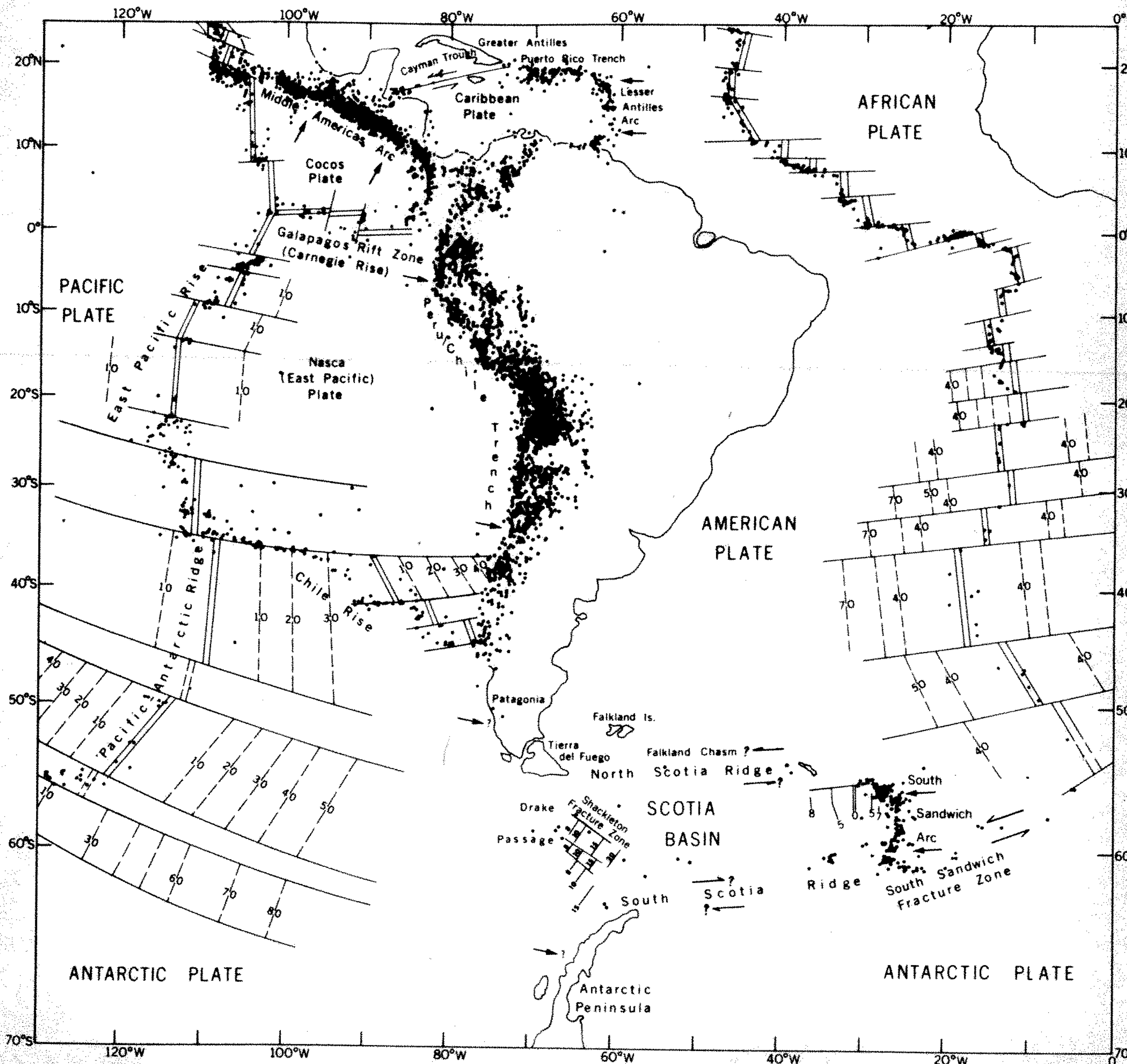


Fig. 1 The geotectonic setting of the Scotia Arc. Epicentres of earthquakes at depths of 0–700 km for the period 1961–1967 (ref. 19) are shown by black dots. The approximate age in millions of years of the floor of the Atlantic and Pacific Oceans, and of the Scotia Sea, as inferred from marine magnetic anomalies[10,20,21] is also shown. Arrows indicate inferred relative motion of the lithospheric plates on which they are drawn.

South America along the North and South Scotia Ridges, and in the Antarctic Peninsula. Extrapolation of the onshore geology (Fig. 2), our interpretation of the seismic refraction profiles[26,29], and dredging[41], indicate that the shallow Burdwood Bank immediately east of Tierra del Fuego is a structural continuation of the extra-Cordilleran Mesozoic–Cenozoic sedimentary basin. Refraction data[26,29] also show higher velocities in the basement of the basin that underlies the bank and forms its southern margin. This (seismic) basement is likely to be the Jurassic volcanics that underlie the sedimentary succession of the extra-Cordilleran basin in Tierra del Fuego[29,42], and which are known along strike as far east as Isla de los Estados (Fig. 2). Lack of appreciable magnetic expression along the southern margin of the bank[12,27] is not surprising, for the volcanics are largely felsic[13,15,16,40,42].

The metamorphic rocks of islets called the Black and Shag Rocks, the flysch-type rocks of early Cretaceous age intruded by an "Andean"-type igneous complex on South Georgia and the "Andean"-type igneous rocks of the Clerke Rocks[43–45] (Fig. 2), all indicate that the platforms supporting these islands also form part of the Cordillera. Moreover, the mafic volcanic rocks interbedded with the flysch-type sediments on South Georgia demonstrate that the island is a fragment of the Pacific side of the Andean Cordillera rather than a complete cross-section of it, as implied by earlier reconstructions[5–7], or part of the Atlantic side, as recently proposed[12]. We believe, therefore, that South Georgia originally adjoined Burdwood Bank, close to Isla Navarino with which it has often been compared geologically[23,24]. Thus the original length of the North Scotia Ridge is unlikely to have been as great as is suggested by earlier reconstructions[5,6,18].

The rocks of the South Orkney Islands, principally schists, amphibolites, and other metamorphic rocks, and intensely deformed, possibly late Palaeozoic sediments, are Cordilleran types[13,16,22], and the platform along whose northern margin they lie apparently constitutes an extra-Cordilleran Mesozoic–Cenozoic basin like the Magallanes Basin[30]. A similar basin is located east of the Antarctic Peninsula[13,16] (Fig. 2), and probably forms much of the submerged platform adjoining the Weddell Sea. The Antarctic Peninsula itself is Cordilleran.

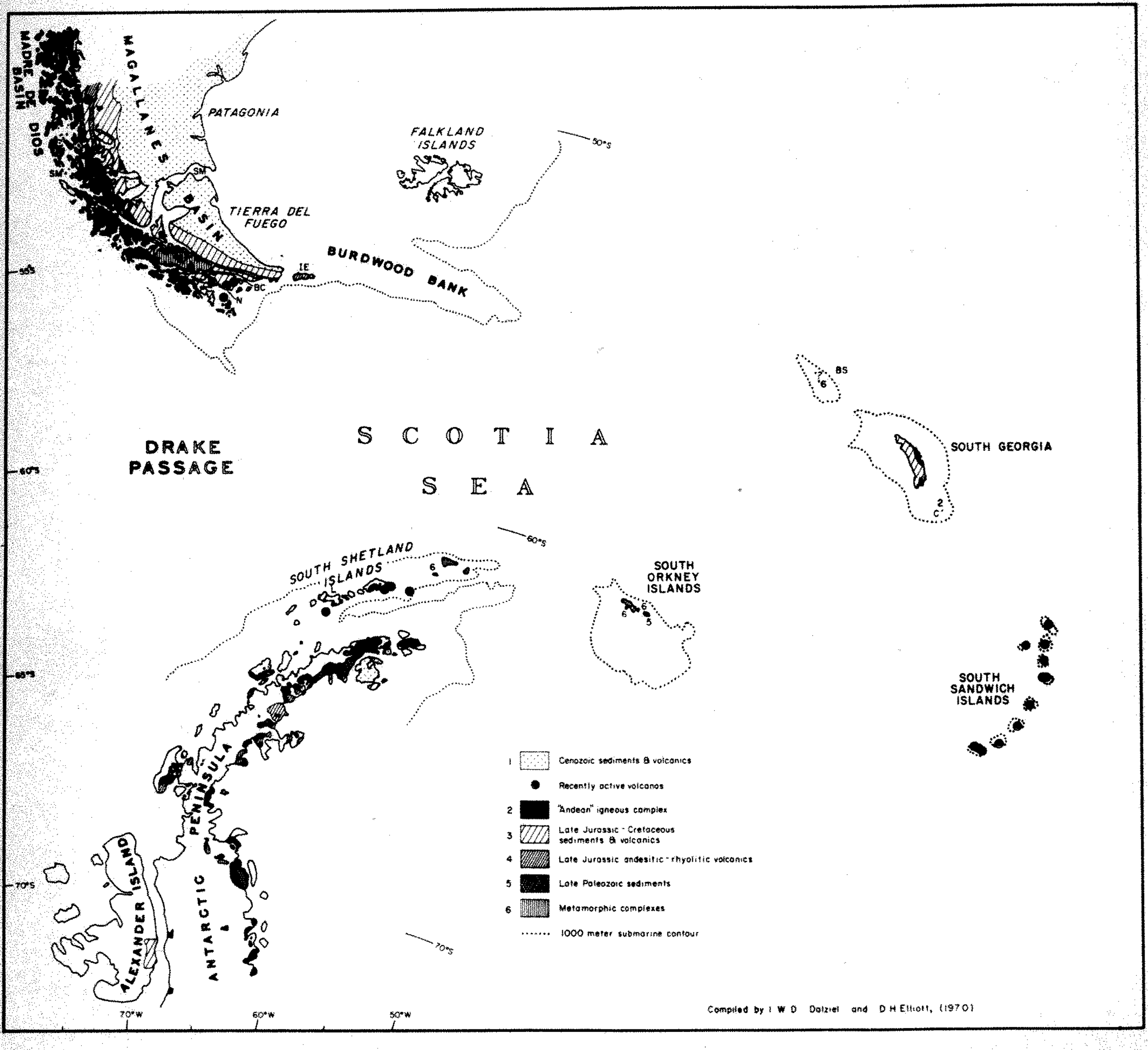


Fig. 2 Geological map of the Scotia Arc region modified after maps by the British Antarctic Survey, the Instituto de Investigaciones Geologicas (Chile), and Direccion Nacional de Geologia y Mineria (Argentina). BC, Beagle Canal; BS, Black and Shag Rocks; C, Clerke Rocks; IE, Isla de los Estados; L, Livingston Island; N, Isla Navarino.

North of 68° S it consists of "Andean" plutons intruding possibly late Palaeozoic sediments and late Jurassic volcanic rocks[22]. Irregular distribution of tectono-stratigraphic units results from the mild deformational effects of the Andean Orogeny in this area. The late Mesozoic rocks are therefore essentially flat-lying, and the outcrop pattern is a function of exposure level[13]. Farther south, however, on the Lassiter Coast (75° S, 67° W), deformed Jurassic clastic sediments are intruded by granitic plutons[47,48] and are analogous to the Andean Cordillera. This may be the case elsewhere south of 68° S in the Antarctic Peninsula, but there is insufficient published data.

Undoubted late Mesozoic sedimentary rocks with interbedded volcanics, comparable with the Pacific side of the Andean High Cordillera, are confined to Livingston Island in the South Shetland group[49,50] and to Alexander Island[51-53] (Fig. 2).

Evolution of the Andean–West Antarctic Cordillera

Southern South America, the North and South Scotia Ridges, and the Antarctic Peninsula are underlain at least in part by continental rocks certainly as old as Palaeozoic[38,39], and possibly Pre-Cambrian[37]. The structure of the basement metamorphic rocks is, however, too complex, and at present too poorly known[13,15-17,54] for any conclusion to be drawn concerning their relationship to the present Cordillera.

Marine magnetic data (W. C. Pitman, III, personal communication, 1971) and the results of the Deep Sea Drilling Programme[55] indicate that southern South America and the Antarctic Peninsula formed a marginal strip of Gondwanaland until the late Jurassic. The sedimentary contact between the late Jurassic lavas and the late Palaeozoic sedimentary sequences[13,17,22,46,54], together with the parallelism of the major fold axes in the Antarctic Peninsula and Miers Bluff Formation in the South Shetlands with the trend of the peninsula and the South Shetland chain[54], suggest that the late Palaeozoic sequences were deformed adjacent to Gondwanaland during the early Mesozoic Gondwanian Orogeny. Therefore it is unlikely that the late Palaeozoic sequences were deposited elsewhere and tectonically emplaced adjacent to Gondwanaland.

Deformation of the late Paleozoic sediments is the first recognizable sign of tectonic instability on the margin of Gondwanaland in the Mesozoic. It occurred not only in what is now the Scotia Arc region, but also in the Ellsworth and Pensacola Mountains of interior West Antarctica, the Cape Fold Belt of southernmost South Africa, and the Sierra de la Ventana of Argentina. In all of these areas (Du Toit's "Samfrau" geosyncline, see Fig. 4*a*) thick sedimentary deposits were highly deformed in the early Mesozoic (late Triassic–early Jurassic). The deformation was characteristically accompanied by at most low grade metamorphism, which may indicate little underthrusting. On the other hand, the extensive mid to late Jurassic calc-alkaline volcanism following uplift and erosion probably represents the surface evidence of a subduction zone that developed along the margin of South America and West Antarctica, as they began to move westward relative to Africa by spreading of the ocean floor. The most recent estimates of the time of opening of the South Atlantic, based on marine magnetic data (W. C. Pitman, III, personal communication, 1971) and the Deep Sea Drilling Programme[55], are consistent with this interpretation.

Deformation and uplift along the axis of the present Cordillera began in the early Late Cretaceous in western South America and was pre-mid Cretaceous at the southern end of the Antarctic Peninsula[47,48], but did not occur until the latest Cretaceous or earliest Cenozoic in the sub-Andean belt on the Atlantic side of the Cordillera in South America[56]. Pre- and syn-tectonic granitic plutons do occur[13,15,16,39,40], and post-tectonic intrusions of latest Cretaceous age[13,15,16,38-40] are common in the Cordillera. One intrusion as young as 12 m.y. (refs. 38 and 57) occurs in the sub-Andean belt between the High Cordillera and the extra-Cordilleran basin. The widespread structural effects of the Andean Orogeny on the rocks of the South Scotia Ridge and the northern Antarctic Peninsula were limited to relatively open folding, faulting and intrusion[13,16,22].

Evolution of the Scotia Arc

The geology and geological history of the region place the following constraints on the evolution of the Scotia Arc:

(1) The similarity of the geology and geological history in southern South America, the continental fragments bordering the Scotia Sea, and the Antarctic Peninsula make it virtually certain that there was once a continuous Andean–West Antarctic Cordillera.

(2) The right-angle bend in the southernmost Andes, the direction of overfolding and overthrusting there (always at right angles to the Cordillera and from the Pacific side), the fact that South Georgia represents a part of the Pacific side of the Cordillera, and limited palaeomagnetic data from the Antarctic Peninsula[7,58] all indicate bending, disruption, and eventual fragmentation of the Cordillera.

(3) Dating of the deformational and intrusive events of the Andean Orogeny indicates that the fragmentation cannot have occurred before the end of the Mesozoic. The striking similarity of Mesozoic tectonostratigraphic provinces around the Scotia Basin seems to demand continuity at least until the end of the Andean Orogeny.

(4) The disruption must have occurred prior to the inception of volcanicity in the South Sandwich Ridge, where the oldest dated rocks are 4 m.y. old (ref. 25). But the magnetic data are interpreted[10,21] to indicate an age of at least 8 m.y. for the South Sandwich Ridge, and that sea floor spreading has occurred from at least mid-Cenozoic (20–25 m.y. ago) until Recent in the Drake Passage. Hence the disruption and fragmentation of the Andean–West Antarctic Cordillera occurred in the early Cenozoic prior to 20–25 m.y. ago, or else accompanied spreading in the Drake Passage. Dott has[9,59] postulated that the fragmentation was entirely late Cenozoic, accompanying a global late Cenozoic tectonic episode.

Fig. 3 shows our concept of the evolution of the Scotia Arc based on these constraints. The Falkland (Malvinas) Platform is regarded as being a stable part of the South American continent at this time, so that bending of the Andean Cordillera resulted in the creation of the Falkland Trough between the North Scotia Ridge and the Falkland Platform.

Note that elimination of the South American and Antarctic Peninsula "oroclines" with the continents held in their present positions (inset) results in considerable and unacceptable overlap. This suggests that despite the spreading in the Drake Passage indicated by Barker's identification of the magnetic anomalies there[12,21], South America and West Antarctica are closer together now than they were when they separated from Gondwanaland (Fig. 3*a*). Some of the late Cenozoic–Recent spreading in the Drake Passage could have been taken up in subduction zones along the North and South Scotia Ridges. This is likely along the South Scotia Ridge that is bordered on its northern side by deep troughs[60], and was the site of andesitic volcanism in the early Cenozoic, prior to the onset of mildly alkaline activity at the beginning of the Pliocene[22,61]. Troughs also occur along the southern margin of the North Scotia Ridge[59], but the only sign of Cenozoic volcanism there is the presence of a few small vents north and west of Cape Horn.

Even if much of the spreading was not taken up in subduction zones, but rather by movement of the continents, the geology does not allow a cusp-shaped original connexion between the continents as suggested by Barker and Griffiths with the Pacific margin of the Cordillera extending no farther east than Cape Horn and Elephant Island[12] (Fig. 2), and with South Georgia on the Atlantic side. South Georgia is clearly part of the Pacific side of the Cordillera[13,23,24]. The geological evidence, however, dictates only a latest Cretaceous maximum age limit for the disruption and fragmentation of an originally near rectilinear Cordillera. It is possible, therefore, that the two continents approached one another more closely than at present during the early Cenozoic. The spreading in the Drake Passage over the past 20 m.y. may document subsequent separation from a cusp-shaped contact if little subduction occurred. Hence the two lines of evidence do not necessarily lead to mutually exclusive models.

The reason for the disruption of the original Cordillera in the Drake Passage is not entirely clear. The predominant factor appears to have been the narrowness of the continental connexion between the two parts of Gondwanaland that are now South America and West Antarctica when the South Atlantic Ocean Basin began to open. In all probability the lithospheric plate underlying the Pacific Ocean tended to bend and disrupt this continental strip rather than continue to be consumed beneath it. The initial bending of the Cordillera may, therefore, have accompanied rather than post-dated the Andean Orogeny. Katz[62] has pointed out that pronounced virgation of folds in the sub-Andean belt of South America occurs at the hinge of the orocline.

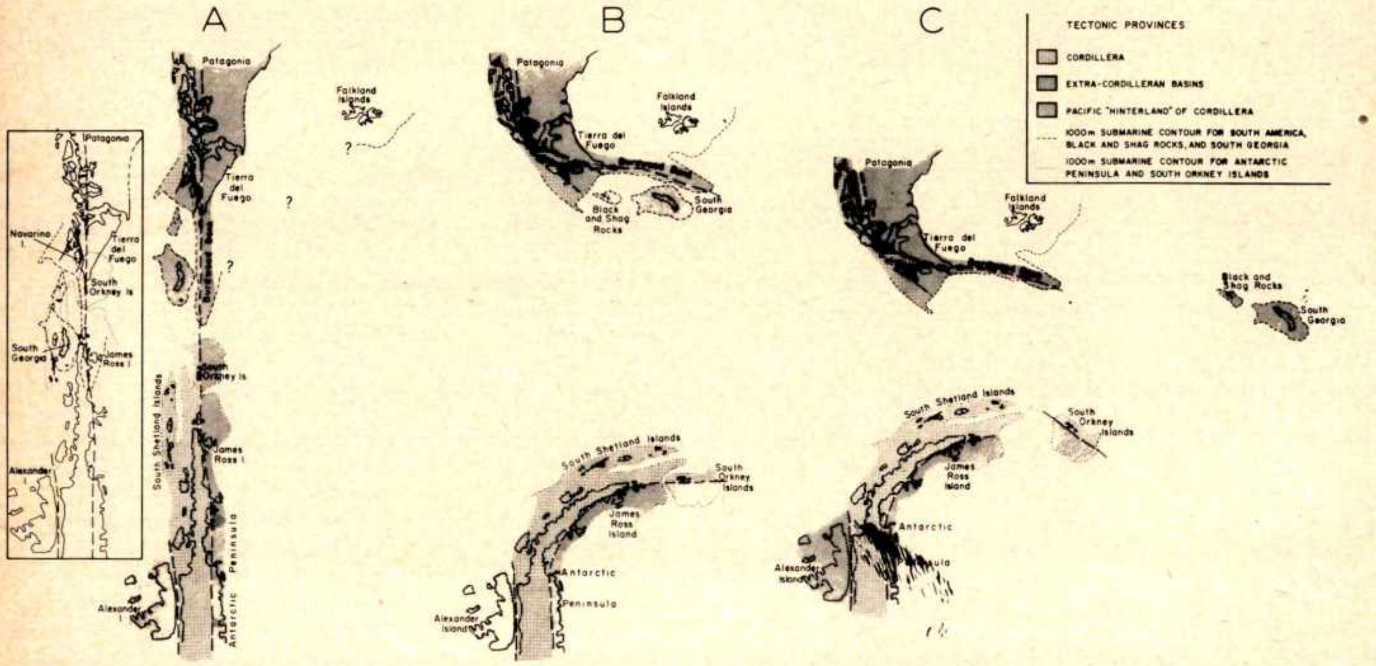


Fig. 3 Evolution of the Scotia Arc. *a*, Andean–West Antarctic Cordillera in the latest Mesozoic following the Andean Orogeny and preceding bending and disruption (insert: restoration of Cordillera assuming no north–south separation of South America and the Antarctic Peninsula). *b*, Scotia Arc in the (?) early Cenozoic following bending and disruption of the Cordillera, but preceding dispersal of continental fragments. *c*, Present distribution of continents and continental fragments around the Scotia Sea.

Reconstruction of Gondwanaland

Attempts to reconstruct the initial configuration of Gondwanaland[63–70] have all encountered difficulty in the area of the Scotia Arc and West Antarctica (Fig. 4). This is probably in part due to the distortion of parts of the continental fragments since their separation from the supercontinent. The main problems in reconstruction have arisen because of unacceptable overlap of the Antarctic Peninsula and Africa[65] (Fig. 4*c*), distortion of the Scotia Arc[64,68], and overlap of South America and Antarctica[70].

Our model demands that an essentially rectilinear Cordillera connected South America and West Antarctica until the end of the Mesozoic. This could be reconciled with the generally accepted "fit" of South America and Africa[71] in several ways—or in a combination of them: (1) anticlockwise rotation of the Antarctic Peninsula relative to East Antarctica following continental separation, (2) right-lateral strike-slip faulting along the margin between East and West Antarctica subsequent to continental separation, (3) a "fit" of East Antarctica and Africa other than those recently proposed[64,65].

There is stratigraphic evidence to support the existence of a sedimentary basin with deep water facies in the South Atlantic area in the late Palaeozoic[65]. This basin need not necessarily have been oceanic in character, nor as large as has been proposed (Fig. 4*d*). Strike-slip displacement and/or rotation of West Antarctica relative to East Antarctica have been proposed elsewhere[65–67] and might well explain the anomalous swing in strike and termination of the early Mesozoic fold structures in the Ellsworth Mountains[68]. Finally, the East Antarctica–Africa "fit" seems to have considerable geological and bathymetric uncertainty.

Final solution of the problem must await further work on the magnetic anomalies from the southern oceans, and a better understanding of the different parts of Du Toit's "Samfrau" geosyncline[63] (Fig. 4*a*). Our model, however, demands a solution close to that of Fig. 4*e* (ref. 67).

To summarize, there seems little doubt that the southernmost Andes once continued into West Antarctica as an essentially rectilinear Cordillera. A proto-Cordillera developed at latest in the early Mesozoic, and the main Cordillera resulted from the Andean Orogeny in late Mesozoic to early Cenozoic time. Oroclinal bending probably began during the Andean Orogeny, but complete disruption and fragmentation of the Cordillera did not ensue until the Cenozoic. The Pacific margin has been shortened in the vicinity of the Scotia Arc, but only by about 1,000 km. Our model places constraints on the reconstruction of Gondwanaland.

We thank R. H. Dott, jun., R. J. Fleck, M. Halpern, W. J. Ludwig and L. R. Sykes for reading the manuscript. The project was supported by the National Science Foundation,

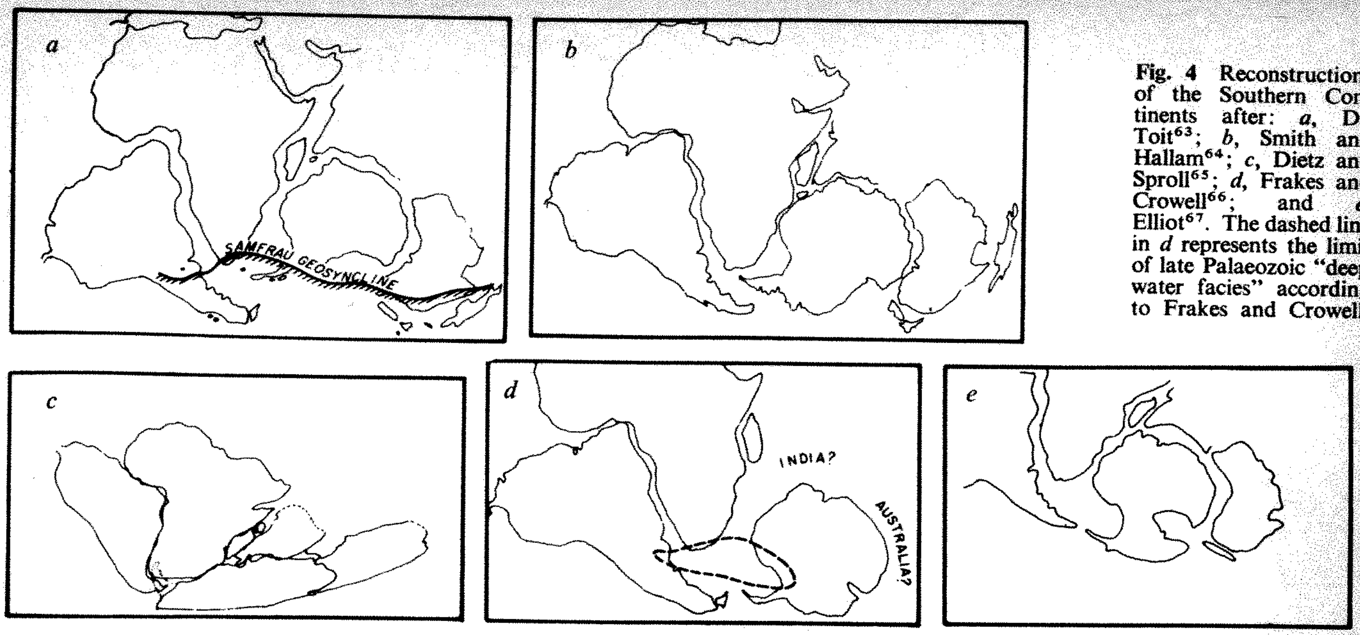


Fig. 4 Reconstructions of the Southern Continents after: *a*, Du Toit[63]; *b*, Smith and Hallam[64]; *c*, Dietz and Sproll[65]; *d*, Frakes and Crowell[66]; and *e*, Elliot[67]. The dashed line in *d* represents the limit of late Palaeozoic "deep water facies" according to Frakes and Crowell.

Office of Polar Programs; Mort D. Turner, Robert L. Dale, and others at the Office of Polar Programs, Empresa Nacional del Petroleo (Chile), the masters and crews of R/V Hero, USCGC Edisto, and USCGC Glacier gave invaluable help.

Received July 6, 1971.

[1] Barrow, J., *Royal Geographical Society*, **1**, 62 (1831).
[2] Arctowski, H., *Bulletin de la Société Geologique de la France, Ser. 3*, **23**, 589 (1895).
[3] Andersson, J. G., *Uppsala University Geological Institute Bulletin*, **7**, 19 (1906).
[4] Herdman, H. F. P., *Report on Soundings taken during the Discovery Investigations*, 1926–32, **6**, 214 (1932).
[5] Matthews, D. H., *Geol. Mag.*, **96**, 425 (1959).
[6] Hawkes, D. D., *Geol. Mag.*, **99**, 85 (1962).
[7] Hamilton, W., *Geological Survey of Canada*, **66-15**, 178 (1966).
[8] Wilson, J. T., *Earth Planet. Sci. Lett.*, **1**, 335 (1966).
[9] Dott, jun., R. H., *Proc. UNESCO-IUGS Symp. on Continental Drift, Montevideo, Uruguay, October 1967* (in the press).
[10] Barker, P. F., *Nature*, **228**, 1293 (1970).
[11] Moores, E., *Nature*, **228**, 837 (1970).
[12] Barker, P. F., and Griffiths, D. H., *Phil. Trans. Roy. Soc., London* (in the press).
[13] Dalziel, I. W. D., and Elliot, D. H., in *The Ocean Basins and Continental Margins*: 1, *The South Atlantic* (edit. by Stehli, F. G., and Nairn, A. E. M.), (Plenum Publishing Corporation, New York, in the press).
[14] Dalziel, I. W. D., *Antarctic Journal of the US*, **4**, 137 (1969).
[15] Dalziel, I. W. D., *Antarctic Journal of the US*, **5**, 99 (1970).
[16] Dalziel, I. W. D., *Antarctic Journal of the US* (in the press).
[17] Dalziel, I. W. D., Price, D. P., and Stirewalt, G. L., *Antarctic Journal of the US*, **5**, 100 (1970).
[18] Deuser, W. G., *Tectonophysics*, **10**, 391 (1970).
[19] Barazangi, M., and Dorman, J., *Seismol. Soc. Amer. Bull.*, **59**, 369 (1969).
[20] Heirtzler, J. R., *Sci. Amer.*, **219**, 69 (1968).
[21] Barker, P. F., in *Antarctic Geology and Geophysics*, (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[22] Adie, R. J., in *Antarctic Research* (edit. by Priestley, R., Adie, R. J., and de Q. Robin, R.), (Butterworths, London, 1964).
[23] Frakes, L. A., *Bull. Geol. Soc. Amer.*, **77**, 1463 (1966).
[24] Katz, H. R., and Watters, W. A., *New Zealand Journal of Geology and Geophysics*, **9**, 323 (1966).
[25] Baker, P. E., *Bull. Volc.*, **32**, 189 (1968).
[26] Ewing, J. I., Ludwig, W. J., Ewing, M., and Eittreim, S. L., *J. Geophys. Res.* (in the press).
[27] Griffiths, D. H., and Barker, P. F., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.) (Universitetsforlaget, Oslo, in the press).
[28] Allen, A., *Brit. Antarct. Surv. Sci. Rep.*, 55 (1966).
[29] Ludwig, W. J., Ewing, J. I., and Ewing, M., *Amer. Assoc. Petroleum Geologists Bull.*, **52**, 2337 (1968).
[30] Harrington, P. K., Barker, P. F., and Griffiths, D. H., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[31] Watters, D. G., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[32] Herron, E. M., and Hayes, D. E., *Earth Planet. Sci. Lett.*, **6**, 77 (1969).
[33] Morgan, W. J., and Johnson, G. L., *Trans. Amer. Geophys. Un.*, **51**, 329 (1970).
[34] Craddock, C., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[35] Ford, A., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[36] Tilley, C. E., *Discovery Reports*, **10**, 383 (1935).
[37] Iltchenko, L. N., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[38] Halpern, M., *Proc. UNESCO-IUGS Symp. on Continental Drift, Montevideo, Uruguay* (in the press).
[39] Halpern, M., and Carlin, G. M., *Antarctic Journal of the US* (in the press).
[40] Kranck, E. H., *Acta Geographica Helsingfors*, **4**, 1 (1932).
[41] MacFadyen, W. A., *Discovery Reports*, **7**, 1 (1933).
[42] Katz, H. R., *Amer. Assoc. Petroleum Geologists Bull.*, **47**, 506 (1963).
[43] Tyrell, G. W., *Discovery Reports*, **23**, 37 (1945).
[44] Trendall, A. F., *Falkland Islands Dependencies Survey Sci. Rep.*, 7 (1953).
[45] Tendall, A. F., *Falkland Islands Dependencies Survey Sci. Rep.*, 19 (1959).
[46] Bibby, J. S., *Brit. Antarct. Surv. Sci. Rep.*, **53** (1966).
[47] Laudon, T. S., Lackey, L. L., Quilty, P. G., and Otway, P. M., *American Geographical Society Antarctic Map Folio Series, Folio 12—Geology, Sheet 3, Eastern Ellsworth Land* (1969).
[48] Williams, P. L., Schneider, D. L., Plummer, C. C., and Brown, L. E., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[49] Aráya, R., and Hervé, F., *Contribucion del Instituto Antarctico Chileno*, 20 (1966).
[50] González-Ferrán, O., Katsui, K., and Taverna, J. J., *Instituto Anarcttico Chileno, Serie Scientifica*, 17 (1969).
[51] Horne, R. R., *Bull. Brit. Antarct. Surv.*, **11**, 1 (1967).
[52] Horne, R. R., *Bull. Brit. Antarct. Surv.*, **17**, 73 (1968).
[53] Horne, R. R., *Bull. Brit. Antarct. Surv.*, **22**, 61 (1969).
[54] Dalziel, I. W. D., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[55] Maxwell, Q. E., Van Herzen, R. P., Hsu, K. J., Andrews, J. E., Saito, T., Percival, jun., S. F., Milow, E. D., and Boyce, R. E., *Science*, **168**, 1047 (1970).
[56] Katz, H. R., *J. Geol.*, **70**, 595 (1962).
[57] Katz, H. R., *Minerales, Santiago de Chile*, **74**, 1 (1961).
[58] Blundell, D. J., *Brit. Antarct. Surv. Sci. Rep.*, 39 (1962).
[59] Dott, jun., R. H., *Science*, **166**, 874 (1969).
[60] Kroenke, L. W., and Woolard, G. P., *Hawaii Institute of Geophysics Report 68-4* (1968).
[61] Baker, P. E., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[62] Katz, H. R., *Geol. Rdsch.*, **54**, 1195 (1964).

[63] Du Toit, A. L., *Our Wandering Continents* (Oliver and Boyd, Edinburgh, 1937).
[64] Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
[65] Dietz, R. S., and Sproll, W. P., *Science*, **167**, 1612 (1970).
[66] Frakes, L. A., and Crowell, J. C., *Nature*, **217**, 832 (1968).
[67] Elliot, D. H., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), (Universitetsforlaget, Oslo, in the press).
[68] Craddock, C., *American Geographical Society Antarctic Map Series Folio 12—Geology, Sheet 23, Gondwanaland* (1970).
[69] Heirtzler, J. R., in *Antarctic Research* (edit. by Porter, H. D.), (Amer. Assoc. Adv. Science, in the press).
[70] Tarling, D. H., *Nature*, **229**, 17 (1971).
[71] Bullard, E. C., Everett, J. E., and Smith, A. G., *Phil. Trans. Roy. Soc. London*, *A*, **258**, 41 (1965).

Andean Orogeny and Ocean Floor Spreading

R. W. R. RUTLAND

Department of Geology and Mineralogy, University of Adelaide, Adelaide, South Australia 5001

The periodicity and eastward migration of the Andean orogeny is explained in terms of periodic loss of marginal continental crust by crustal subduction.

ALTHOUGH there is strong evidence[1-4] that new oceanic crust is being created along the oceanic fracture system at rates of several centimetres per year, the evidence that oceanic crust is being destroyed at similar rates in the continental fracture system is less compelling, and some have rejected this hypothesis in favour of the hypothesis of an expanding Earth[5].

The rate of crustal subduction in the Peru–Chile trench is about 6 cm yr^{-1} (ref. 6), although changes of spreading rate and direction may have occurred in the past 25 million years[7,8]. Even so, calculated rates have always been high (5 cm yr^{-1}), and no pauses in spreading have been detected. The undeformed nature of the sediments in the Peru–Chile trench is a problem[9-13], however, and so is the disappearance of the probable westward extension of the continental crust of Chile[14]. This article gives an account of my view of what has happened, and is based on field studies of the Antofagasta segment in northern Chile (Fig. 1). I suggest that the orogenic history of Chile may be understood in terms of ocean floor spreading if there has been periodic loss of continental crustal material from the continental margin.

The Andes in the Antofagasta segment (Figs. 1 and 2) consist of two main geosynclinal belts, the Cambrian–Devonian East Andean geosyncline and the Jurassic–Cretaceous West Andean geosyncline. On the face of it, this suggests a westward migration of the main trough of sedimentation from the Palaeozoic to the Mesozoic. The West Andean geosyncline, however, was certainly developed on an older continental basement, not on oceanic crust. This basement locally consists of virtually unmetamorphosed Upper Palaeozoic sediments, or more commonly of metamorphic foliated rocks which have usually been regarded as Precambrian but which are probably of Palaeozoic age[15]. Thus the Mesozoic geosyncline probably developed on an outer metamorphosed and deformed Palaeozoic geosynclinal belt parallel to the unmetamorphosed inner belt of the East Andean geosyncline. This interpretation is supported by the fact that a belt of Palaeozoic granites and Triassic volcanics lies along the junction of the East and West Andean geosynclines.

The West Andean geosyncline was divided into a "eugeosynclinal" volcanic (coast range) zone and a "miogeosynclinal" sedimentary (longitudinal valley) zone during the Jurassic (ref. 16, Fig. 5, p. 42), and "infraneritic" and "bathyal" facies have been recognized in the latter zone (ref. 17, Fig. 6, p. 60). The geosyncline was apparently narrower in the Lower Cretaceous and the facies boundary between so-called eugeosynclinal and miogeosynclinal facies was displaced to the east (ref. 16, Parte II, Fig. 4). This was a consequence of the phase of mobility at the end of the Jurassic (chiefly faulting and plutonism) which led to the uplift and erosion of the coast range zone (Fig. 2).

The sea was already excluded from the longitudinal valley zone north of 26° S at the end of the Jurassic and was excluded from the southern part of the West Andean geosyncline before the main phase of mobility at the beginning of the Upper Cretaceous (although marine sedimentation continued still further south in parts of the Magellan geosyncline). Thus it appears that the present difference in elevation between north and south Chile reflects a difference in crustal structure which dates from at least Jurassic time.

Folding in the West Andean geosyncline began with a Cenomanian phase and was continued by later Cretaceous and early Tertiary phases. The Cretaceous folding was not accompanied by regional metamorphism, nor was it characterized by large strains or cleavage development. The folds are generally of large wavelength and amplitude and open in style. In the later phases folding was more localized and generally less intense, giving way to block faulting (with major reverse faults) and acid volcanism. Thick deposits of Upper Cretaceous continental sediments were accumulated in the longitudinal valley zone of the Western Andes in the Upper Cretaceous. The Puna zone also began to develop as a basin and has a thicker Cretaceous sequence than the East Andean zone. Marine incursions in the East Andean zone appear to correspond to the Middle Cretaceous tectonism of the Western Andes.

Following further tectonism in late Cretaceous or early Tertiary time, the Western and Eastern Andes became positive areas subject to repeated uplift and erosion while the Puna and Sub-Andean zones became syn and post-orogenic basins in which the later phases of mobility were concentrated. The western margin of the Puna zone became the site of the main ignimbritic volcanism of the late Miocene and Pliocene and then of the Plio-Pleistocene central volcanoes. The present longitudinal valley and associated topographic features did not develop as basins until Miocene time, when the main uplift of the Puna zone began. Possibly the Peru–Chile trench is of the same age, as discussed later[13].

There is a tendency for the site of the most intense deformation to migrate eastwards with time. Thus the most intense deformation in the coast range zone was at the end of the Jurassic, in the longitudinal valley zone during the Upper Cretaceous and in the Sub-Andean zone during the Pliocene. The Puna zone, however, is similar to the Sub-Andean zone and probably had its main deformation after the East Andean zone. The crustal shortening associated with the orogenic deformations is not easily estimated. If the open folding of concentric style is entirely the consequence of crustal shortening and if some allowance is made for the effect of reversed faulting, the total shortening since mid-Cretaceous time might be as much as 40%. The maximum width of the belt is about 700 km, so that the maximum crustal shortening would seem to be 500 km. The actual amount might be much less, and is therefore at least an order of magnitude less than the amount of crust postulated to have been consumed by crustal subduction in the same period.

Plutonic and volcanic activity also migrated from west to east. Upper Jurassic plutons lie on the eastern margin of the coast range zone, with Upper Cretaceous plutons immediately to the east and scattered smaller Tertiary intrusions further east again. The main volcanism was in the coast range zone in the Jurassic, in the longitudinal valley zone in the Cretaceous and early Tertiary, and in the Puna zone in the later Tertiary.

The present coast of north Chile seems to be a fault scarp modified by marine erosion in Plio–Pleistocene time. The steep coastal cliffs are usually several hundred metres high and display a Jurassic lava sequence dipping gently inland. Clearly the Jurassic volcanic zone originally extended considerably further west. Moreover there is no Mesozoic or Tertiary low temperature, high pressure schist belt in Chile like the Franciscan belt of California or those of island arcs. Only a low pressure, high temperature belt is present, so that if paired metamorphic belts were developed the outer belt either lies offshore or has disappeared. Several mechanisms for the removal of marginal continental crust have been put forward[9,14], and it seems possible that the missing crust has been dragged under the continent where it has been remobilized and has thickened the crust by processes of plutonism and volcanism. The crust in this part of the Andes seems to be thicker than in western North America, for example, where little or no marginal crust has disappeared[17].

The disappearance of marginal crust in this way suggests an explanation for the periodicity and spatial migration of Andean plutonism and volcanism. If the Benioff zone maintains a roughly constant attitude through time, then the site of volcanic and plutonic activity should be a roughly constant distance from the subduction zone. The periodic eastward migration of plutonism and volcanism is then a consequence of periodic loss of marginal continental crust in the subduction zone. The site of Jurassic volcanism was roughly 200 km west of the present site, so it may be supposed that about 200 km of continental crust lay west of the present coast at the beginning of the Jurassic. The three principal phases of granitic plutonism at the end of the Jurassic, in Upper Cretaceous and in Tertiary time may represent three stages in the destruction of the marginal continental crust. The width of the main fault blocks of the present day Chilean Andes (100 km) suggest that each phase of volcanic and plutonic activity is related to the subduction of one fault block.

This hypothesis also suggests that there should be some correlation between tectonism and plutonism. A phase of compressional tectonics causing the subduction of a strip of marginal continental crust should precede each phase of plutonism and a phase of isostatic uplift should accompany the plutonism. This appears to hold for the history of plutonism and tectonism in Chile.

The theory of crustal subduction, including subduction of continental crust, therefore seems to provide a satisfactory model for the development of the Andes of northern Chile, and adjacent Argentina and Bolivia. The chief difficulties, however, are the undeformed nature and the volume of the sediments in the Peru–Chile trench[8–13]. Scholl *et al.*[13] have concluded that: (1) Sediment removal probably occurred at the base of the continental margin and adjacent deep-sea floor in late Cretaceous and perhaps earliest Tertiary time. (2) Uncertainties in denudation–sedimentation budget leave open the question of tectonic removal of sediment in Tertiary time. (3) Reasonable estimates of erosion suggest that late Cenozoic underthrusting at the postulated rate has not taken place or that it has taken place without removing sediment from the trench. They are driven to suggest that sinking of the plate may take place seaward of the trench.

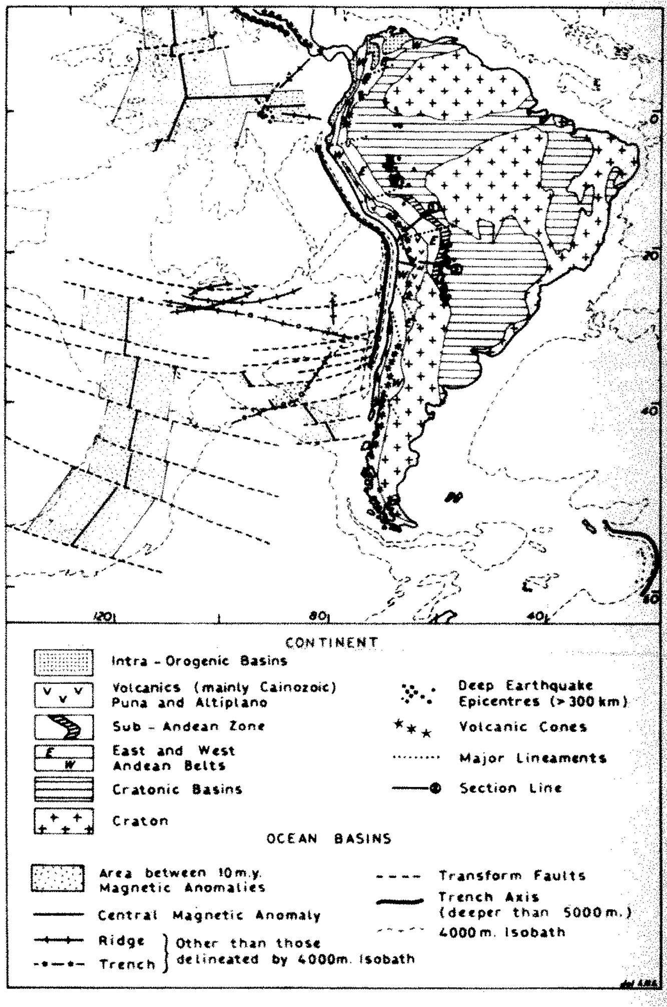

0
20
40
60
120
80
40
CONTINENT
Intra - Orogenic Basins
Volcanics (mainly Cainozoic) Puna and Altiplano
Sub - Andean Zone
East and West Andean Belts
E
W
Cratonic Basins
Craton
Deep Earthquake Epicentres (> 300 km)
Volcanic Cones
Major Lineaments
Section Line
OCEAN BASINS
Area between 10 m.y. Magnetic Anomalies
Central Magnetic Anomaly
Ridge
Trench
Other than those delineated by 4000m. Isobath
Transform Faults
Trench Axis (deeper than 5000 m.)
4000 m. Isobath

Fig. 1 Major tectonic zones of the Andes of South America in relation to major tectonic and topographic features of the East Pacific. Note that the continuity of the spreading system of the East Pacific Rise is well established but a portion has been omitted from the map for lack of available data.

The hypothesis of subduction of marginal continental crust effectively explains the absence of Mesozoic and early Tertiary sediments. The third point is therefore the most crucial. It is partly based on the inference that most of the fill of the trench south of 36° S consists of turbidites deposited in glacial episodes of lowered sea level during the past three million years and that the volume of these sediments is close to their maximum estimate for this period. Yet, on the basis of the present configuration of the trench, the rate of removal of sediment from the trench by crustal subduction should be greater than the rate of supply. For this reason too, the problem becomes more severe if the trench fill is assumed to be of greater age.

The only alternative explanation that suggests itself to me is that denudation rates on land are not directly relevant to the rate of accumulation in the trench, but that rapid submarine erosion of the onshore flanks of the trench is responsible for the fill.

The present relief of the Andes has developed largely in the past ten or possibly twenty million years, and it seems probable that the present submarine relief is partly of the same age. Prior to the development of this relief large quantities of sediment may have accumulated on the onshore side of the subduction zone. As the relief of the onshore flank developed, much of this sediment, with the help of earthquake activity, would become unstable and be transported rapidly into the trench. In this way the erosional products of some tens of millions of years might be transported into the trench in a fraction of that time.

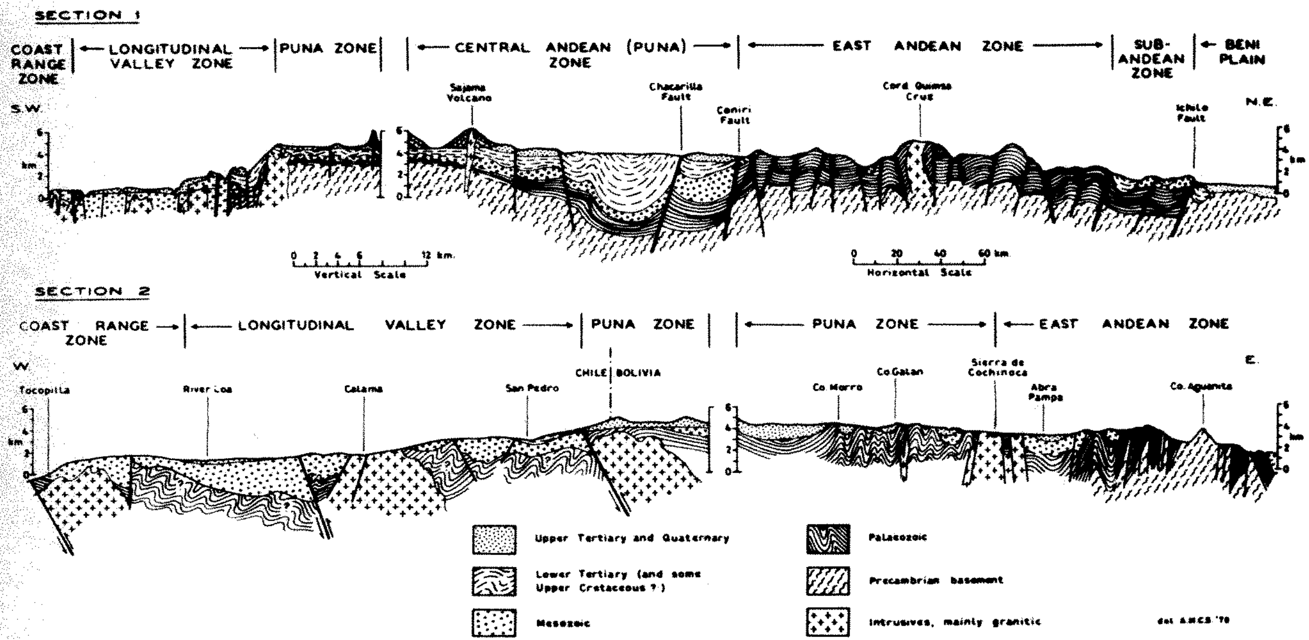


Fig. 2 Sections across the Andean orogenic belt. Section lines are shown on Fig. 1. Section 1 runs north-east from Arica in Chile. The south-western portion is after Salas *et al.* in ref. 16. The north-eastern portion is after Ahlfeld[27]. Section 2 is across the Chilean and Argentinian Andes near 22° S. The eastern portion is interpreted after Turner[28].

The study by Scholl *et al.*[13] did not extend south of 44° S but earlier studies have shown a very different sediment distribution south of the Chile Rise where seismic evidence suggests that crustal subduction has ceased[1] (although more recent studies have tentatively suggested that subduction is continuing at a reduced rate[18]). Sedimentary accumulation is not confined to the southern continuation of the trench but extends westwards for 200 km (ref. 19). The absence of such a wide belt north of the Chile Rise is itself strong evidence for crustal subduction there.

The volume of the sediments in the trench may not therefore mean that crustal subduction is not taking place. Further evidence on the nature of sediments in the trench and their undeformed character comes from an analogy between the trench and onshore fault blocks, which will now be developed.

Contrary to some statements[20] which have been quoted recently[19], the fault-blocks of northern Chile are certainly a consequence of compressive tectonics and are limited by reverse faults[21-23]. These faults are exposed, for example, in gorges on the east side of the Pampa de Tamarugal and on the road from Calama to San Pedro de Atacama on the west side of the Cerros de Purilactis. In both cases the faults dip steeply to the east. A similar fault may lie offshore and may be responsible for the coastal scarp, and yet another may occur in the trench. Movement along such faults may well account for the loss of marginal blocks by crustal subduction although the rate of movement required would be extremely high compared with the observed rates onshore.

The block faulting was initiated in the early Tertiary. In the Antofagasta segment[21-23] filling of the intermontane troughs began in the Oligocene or Miocene with the Tambores, San Pedro and Lower Loa Formations. Intermittent tectonism in the east of the segment then accompanied the deposition of the Pliocene San Bartolo and Upper Loa Formations.

The Loa Formation (probably the Upper part only) representing an ancestral valley of the River Loa is now preserved at a height of about 400 m in the coastal scarp a few kilometres north of the present gorge of the River Loa. Clearly therefore the Loa Formation extended further west and probably continued into the ocean before the uplift of the coast range.

These relations suggest that the Peru–Chile trench is at least as old as the block faulted onshore basins and that the surface of acoustic basement is likely to be the unconformity developed across folded Cretaceous and older rocks. In this case four kinds of fill might be expected in the trench or in sediment traps on its onshore flank in the Antofagasta segment: (1) products of dissection of the block-faulted terrain prior to deposition of the Loa Formation, (2) equivalents of the Loa Formation carried into the ocean by the ancestral River Loa, (3) sediment carried to the ocean during the period when the present River Loa gorge was cut, and (4) material removed from the coastal fault scarp by marine erosion.

The evidence of points (1), (2) and (3) demonstrates that the present climatic zonation has not controlled offshore sedimentation through most of the Tertiary. The large volumes of coarse piedmont deposits of pre-Pliocene age in N. Chile shows that discharge and erosion from the high Andes was sometimes much higher than at present. Periods of piedmont aggradation have alternated with periods of dissection[24,25]. The estimates of denudation rates used by Scholl *et al.*[13] are therefore questionable. If denudation rates were in fact higher than their estimates, their data on sediment volumes suggest that insufficient Tertiary sediment is now to be found offshore in N. Chile. Consumption of sediment in the Benioff zone or its conversion to acoustic basement may therefore have occurred in the Tertiary as required by the hypothesis of ocean floor spreading.

The onshore intermontane basins show that reversed faulting of their eastern margins has proceeded since the Miocene without deformation of their sedimentary fill. If such reversed faults are utilized for the crustal subduction process it seems quite possible that the process could proceed without folding of the trench sediments. The subsidence of the oceanic crust beneath the prow of continental crust can probably be achieved by reversed faults conjugate to the main easterly dipping reversed fault. If normal faults are commonly developed by the downbending of the oceanic crust[24,25] on the offshore flank of the trench, these normal faults would probably be utilized as the conjugate reversed faults as the oceanic crust passes into the Benioff zone beneath the continental flank of the trench. I therefore conclude that although the postulated rate of subduction of oceanic crust is startling in comparison with processes in the adjacent continental crust, the known geology of the trench and adjacent continental margin is not incompatible with the theory of crustal subduction.

The application of the theory of ocean floor spreading, and in particular of crustal subduction therefore offers a possible explanation of the periodicity within the West Andean orogenic cycle and of why sedimentary facies boundaries, deformation, volcanism, and plutonism should have migrated eastwards. Belts in which there has been no loss of the continental margin or in which continental accretion has occurred should show distinctly different relations. It also seems possible that the extremely high percentage of volcanic rocks in the Andean geosyncline and the nonmarine character of the geosyncline during much of its history may be a consequence of isostatic effects resulting from subduction of marginal continental crust.

Studies of the development of the Chile rise in relation to South America and comparative studies of the Andes on either side of the rise clearly provide a fruitful field for further research on the relation of ocean floor spreading and orogenic processes. A superficial study of the geology and of palaeogeographic maps[16] does not reveal the development of startling contrasts in the Tertiary when the Chile Rise, presumably moving north from Antarctica, may have begun to prevent the easterly flow of oceanic crust beneath southern Chile. Young volcanoes, for example, are developed well south of the Chile Ridge in the Andes just as they are further north although petrological differences have been noted. The limitation of the Peru–Chile trench by the Galapagos and Chile ridges is striking, however, and current seismicity appears to be similarly controlled.

In this connexion it may be noted that the concave disposition of the seismic zone towards the Pacific appears to be related to its dip. North and south of the sub-Andean zone (Fig. 1) the epicentres of deep focus earthquakes have colinear distributions at a substantial angle to the orogenic trend. The overall trend of the epicentres is NNW approximately normal to the major ENE lineaments crossing the orogenic belt. Thus it appears that the dip of the seismic zone is at a maximum at the apex of the arc. The symmetry of the pattern also suggests that movements in the subduction zone are in the ENE direction parallel to the major lineaments. It may also be noted that the bathymetric maps of the Pacific reveal a ridge approximately parallel to the orogenic belt, the significance of which is unknown.

The application to the Andean orogenic belt of the hypothesis of ocean floor spreading as developed here does not explain the progressive change in the orogenic belt as a whole. Analogy with older orogenic belts shows that the orogenic cycle is virtually complete but the implications of this for ocean floor spreading are not clear. Perhaps the cycle comes to an end when indigestion in the subduction zone can no longer be relieved by lateral migration of the geosyncline.

But the Mesozoic–Tertiary orogenic cycle shows similar broad features in terms of geosyncline development and subsequent uplift in all areas irrespective of whether the continental margin has been destroyed as in Chile, created as in the Philippines, or is marked by transform faulting as in California. These features are found not only on present continental margins but also in the Alps and Himalayas where two areas of continental crust have been brought together. Rejuvenation of relief in Neogene times also characterizes older Phanerozoic and Late Precambrian orogenic belts. Attempts[26] to explain orogeny in general and vertical movements in particular in terms of constant rate ocean floor spreading therefore appear inadequate at present.

My conclusions are as follows: (1) The Andes of Northern Chile and adjacent Argentina and Bolivia display a periodic eastward migration of volcanism, plutonism and tectonism which can be explained by periodic subduction of blocks of marginal continental crust. Thus the Jurassic coastline was probably 200 km west of the present coast. (2) The hypothesis explains the absence of Mesozoic and Tertiary sediments offshore. (3) The volume and undeformed nature of the young sediments in the Peru–Chile trench provide obstacles to the easy acceptance of the crustal subduction hypothesis. These obstacles may be overcome if rapid submarine denudation of the onshore flank of the trench is accepted along with the analogy between the onshore fault basins and the trench. (4) Further work on the relations north and south of the Chile Rise is needed to relate orogenic processes to the postulated differences in rates of crustal subduction. (5) The late Cenozoic vertical movements in orogenic belts may not be directly dependent on ocean floor spreading.

This discussion is partly based on work in collaboration with the late Professor S. E. Hollingworth, with the support of the United Kingdom Department of Scientific and Industrial Research. Dr M. R. Walter helped with the figures and criticized an early draft.

Received June 7, 1971.

[1] Isacks, B., Oliver, J., and Sykes, L. R., *J. Geophys. Res.*, **73**, 5855 (1968).
[2] Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).
[3] McManus, D. A., and Burns, R. E., *Ocean Industry*, **4.8**, 40 (1969).
[4] Maxwell, A. E., and Von Herzen, R. P., *Ocean Industry*, **4.5**, 64 (1969).
[5] Rickard, M. J., *Tectonophysics* (in the press).
[6] Le Pichon, X., *J. Geophys. Res.*, **73**, 3661 (1968).
[7] Morgan, W. J., Vogt, P. R., and Falls, D. R., *Nature*, **222**, 137 (1969).
[8] Herron, E. M., and Hayes, D. E., *Earth Planet. Sci. Lett.*, **6**, 77 (1969).
[9] Scholl, D. W., von Huene, R., and Ridlon, J. B., *Science*, **159**, 869 (1968).
[10] Seyfert, C. K., *Nature*, **222**, 70 (1969).
[11] Lomnitz, C., *Nature*, **222**, 366 (1969).
[12] Malahoff, A., *J. Geophys. Res.*, **15**, 1992 (1970).
[13] Scholl, D. W., Christensen, M. N., von Huene, R., and Marlow, M. S., *Geol. Soc. Amer. Bull.*, **81**, 1339 (1970 .
[14] Miller, H., *Geol. Rundschau.*, **59**, 927 (1970).
[15] Gonzalez-Bonorino, F., and Aguirre, L., *Geol. Rundschau*, **59**, 979 (1970).
[16] Ruiz, F. C., *Geologia y Yacimientos metaliferos de Chile* (Inst. Invest. Geol., Santiago, Chile, 1965).
[17] Biese, W., *Inst. de Geologia, Universidad de Chile*, *Pub. 19* (1961).
[18] Barker, P. F., *Nature*, **228**, 1293 (1970).
[19] Hayes, D. E., *Marine Geol.*, **4**, 309 (1966).
[20] Oppenheim, V., *Amer. J. Sci.*, **245**, 158 (1947).
[21] Hollingworth, S. E., *Nature*, **201**, 17 (1964).
[22] Rutland, R. W. R., *Int. Council Sci. Unions*, *Upper Mantle Project*, *Second Aust. Progr. Rep.* 1965/67 (Canberra, 1967).
[23] Thomas, N. A., *Geol. Rundschau*, **59**, 1013 (1970).
[24] Sillitoe, R. H., Mortimer, C., and Clark, A. H., *Trans. Instn. Min. Metall.* (*Sect. B*), **77**, B166 (1968).
[25] Henriquez, A. H., *Geologia y Yacimentos Metaliferos de Chile: Sedimentos Cuaternarios* (Inst. Invest. Geol., Santiago, Chile, 1965).
[26] Coney, P. J., *Geol. Soc. Amer. Bull.*, **81**, 739 (1970).
[27] Ahlfeld, F., *Geol. Rundschau*, **59** (1970).
[28] Turner, J. C. M., *Geol. Rundschau*, **59**, 1028 (1970).

LETTERS TO NATURE

PHYSICAL SCIENCES

Variations in the 10 μm Flux from NGC 1068

ALTHOUGH there is considerable evidence of rapid variability of the intense infrared sources in the nuclei of galaxies and quasars, conclusive data have been lacking. NGC 1068 is one of the most powerful of the extragalactic infrared sources ($L_{IR} = 6 \times 10^{45}$ erg s^{-1}) and, with the exception of M82 which is extended, has the highest signal-to-noise ratio at 10 μm. We have therefore monitored NGC 1068 for several years to look for variations. The pertinent observations are summarized in Fig. 1, where data obtained during the past 3 yr have been combined into four points. The results of the individual observations are given in Table 1.

Table 1 Observations of NGC 1068 at 10 μm

Julian date	Flux level (10^{-26} W m^{-2} Hz)	Standard deviation (10^{-26} W m^{-2} Hz)	Field of view (arc s)
2440167	20.9	1.7	20
2440171	26.8	3.0	20
2440230	25.1	2.5	20
2440509	28.1	1.2	6
2440568	25.2	1.1	6
2440594	26.2	1.3	6
2440868	21.1	2.5	20
2440873	23.0	1.6	20
2440882	25.5	1.4	20
2440889	22.9	1.6	6
2440910	24.6	1.7	6
2440963	18.6	1.2	6
2440993	18.5	1.9	20
2440996	17.9	0.9	6
2440997	17.2	1.0	6
2441033	20.3	1.1	6

The error bars used in Fig. 1 and throughout this discussion are to be interpreted as one standard deviation. They include statistical errors determined from an analysis of the data for each observation and an overall estimate of non-statistical errors determined from the non-repeatability of observations of bright stars. Thus they include all the known errors of observation except an uncertainty of 15% in the absolute calibration of the photometric system. Our discussion is concerned only with measures relative to certain standard stars and so the absolute calibration is not relevant.

The older data on NGC 1068 (refs. 1, 2) have been reanalysed to insure a uniform reduction procedure and to refer all observations to the same standard star, α Tau. Reference to α Tau could usually be made directly but for cases in which secondary standards were used, the estimated errors were increased accordingly. Our observations show that α Tau has remained constant relative to other standard stars to within 4% during this time. The observations were also carefully re-examined; all data for which there is reasonable doubt about the standardization or observational procedure have been rejected.

The evidence for variability is considerably strengthened by the observations since Julian Date 2440950. Most of these data were obtained with a new photometer; it is important, therefore, to examine whether the observations were affected by the change in instrumentation. We know of only two ways in which this change could affect our results: a shift in the filter passband, and, if the source is polarized, the polarizing effect of a 45° diagonal mirror used to deflect the radiation onto the detector. The filters used in the two photometers have been examined carefully and the only significant difference is that the new filter cuts on at about 7.6 μm whereas the old one cuts on near 7.8 μm. According to unpublished data, the atmospheric transmission from 7.6 to 7.8 μm at our site averages less than 15%. From these measurements, we can place a conservative upper limit of 2% on the effect of the change in filters on our observations. The polarizing effect of a front-aluminized, 45° diagonal mirror at 10 μm is less than 1% (H. M. Dyck, private communication). To make sure that the change in instrumentation does not influence our conclusions, all flux levels from NGC 1068 observed with the new photometer have been increased by 3%, the combined upper limit to the two effects.

The conclusion that variations have occurred does not depend solely on the data obtained with the new instrument. There is evidence for variability in the data obtained before JD 2440950. In addition, the measurement on JD 2440993 was made with the old instrument and is in excellent agreement with other measurements made at about the same time. This observation alone differs from the mean of the observations before JD 2440950 by 3.3 standard deviations. The work on JD 2440910 was done with the same filter used in the new photometer; it differs from the mean of the measurements made since JD 2440950 by 3.5 standard deviations.

Most kinds of systematic error would appear equally in observations of all objects with similar spectra. For this reason, it is customary to present observations of a comparison star together with evidence for variations of an astronomical object. Such a procedure is, however, meaningless in this case, because the spectra of stars and galactic nuclei are quite different at 10 μm. The only reasonable comparison object is another galactic nucleus. Sufficient data to permit such a comparison are available for NGC 1275 and NGC 4151 (refs. 1, 3) although the ratio of signal to noise is much lower for these objects than for NGC 1068; the data are shown in Fig. 1. A detailed analysis of these data shows that the fluxes measured with our new photometer average 0.98 ± 0.08 times those measured with the old one, a result which tends to confirm our conclusion that the observed variation in NGC 1068 is not an instrumental effect.

NGC 1068 and NGC 1275 have frequently been observed on the same nights. It is therefore possible to compare these galaxies directly, without any reference to standard stars. Such a comparison should be independent of most kinds of systematic error. Data are available for four nights near JD 2440550 and for three nights near JD 2441000. They show that the flux from NGC 1068 decreased by 30 ± 8% relative to that from NGC 1275 during this period of time.

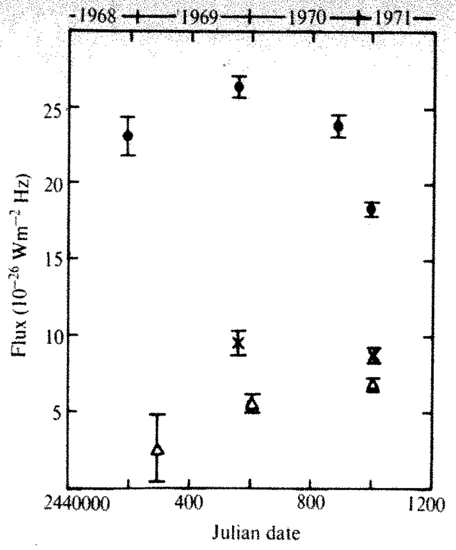


Fig. 1 Variation in the 10 μm flux from the nucleus of NGC 1068. The error bars include all known sources of error except for an uncertainty in the absolute calibration; they are to be interpreted as one standard deviation. The observations of NGC 1275 and NGC 4151, which are included as comparison objects, have been arbitrarily renormalized. ●, NGC 1068; ×, NGC 1275; △, NGC 4151.

Similar but larger variations during an earlier period of time have been described for 3C273 and NGC 4151 (ref. 1). The evidence for these variations was not conclusive because the observations had a low signal-to-noise ratio and were made in conditions which made a detailed study of the errors impossible. Neither of these problems affects the data on NGC 1068; the evidence of variation is compelling. The variability of NGC 1068, combined with the similarities between it and NGC 4151 and 3C273 in other respects, greatly strengthens the argument that these sources also vary.

Although the most complete observations have been made at 10 μm, it seems that the 5 μm flux from NGC 1068 has also decreased. Before JD 2440800, the average flux at this wavelength was $7.3 \pm 0.6 \times 10^{-26}$ W m^{-2} Hz; since then, a level of $5.5 \pm 0.6 \times 10^{-26}$ W m^{-2} Hz has been observed. Our observations are consistent with a similar variation at 3.5 μm. At this wavelength, the observed fluxes are $2.9 \pm 0.5 \times 10^{-26}$ W m^{-2} Hz before JD 2440800 and $2.4 \pm 0.2 \times 10^{-26}$ W m^{-2} Hz since then. At 2.2 μm, the flux from the central region of NGC 1068 is predominantly due to stars. Observations of variations in the nuclear source would be extremely difficult at this wavelength[2].

Terrell[4] has considered the limits which can be placed on the sizes of astronomical objects from observations of variations in flux. Sizes considerably greater than cT (where c is the speed of light and T is the time scale of the fluctuations) are possible if the source consists of a large, optically thick sphere which re-radiates the emission from a varying central source. An optically thick shell with a diameter of the order of 3 pc and containing dust at a temperature of about 400 K would be consistent with our observations of the nucleus of NGC 1068, although the spectrum at wavelengths longer than 15 μm would have to be attributed to a larger, cooler component.

More generally, if the varying source is not optically thick or is not of an appropriate shape, the upper limit on its diameter would be of the order of $cT = 0.3$ pc. The source would have to radiate at least 7×10^{-26} W m^{-2} Hz at 10 μm, the amount of the observed variation. It would have insufficient surface area to radiate this level of flux thermally unless its temperature were greater than 1200 K. A temperature of this order is acceptable only if the emissivity of the radiating dust grains decreases rapidly towards shorter wavelengths so that the estimated flux agrees with that observed at 2.2, 3.5 and 5 μm.

The observations reported here thus place strong constraints on models for the generation of the far infrared flux from NGC 1068 by thermal means. Many other galactic nuclei also radiate strongly in the infrared[1,3]; the similarity of their spectra to that of NGC 1068 suggests that these constraints hold for them also.

Radio and optical variability have already been observed in the nuclei of Seyfert galaxies[5-7] such as NGC 1068. The fluxes modulated in these regions of the spectrum are, however, relatively small when compared with the total emission of the nuclei, most of which is in the infrared[8]. The observations reported here are the first strong evidence that a significant proportion of the nuclear emission is modulated. In the 10 μm atmospheric window alone, we have observed a change in luminosity of 2.5×10^{43} erg s^{-1} which is comparable with the total luminosity of our galaxy and about 0.5% of the total luminosity of the nucleus of NGC 1068. It can be inferred that the total change in luminosity was much greater when allowance is made for unobserved regions in the infrared spectrum.

F. J. Low
G. H. Rieke

University of Arizona,
Tucson, Arizona

Received August 30, 1971.

[1] Kleinmann, D. E., and Low, F. J., *Astrophys. J. Lett.*, **159**, L165 (1970).
[2] Garmire, G., Neugebauer, G., Rieke, G. H., and Low, F. J., *Astrophys. J. Lett.*, **166**, L45 (1971).
[3] Kleinmann, D. E., and Low, F. J., *Astrophys. J. Lett.*, **161**, L203 (1970).
[4] Terrell, J., *Astrophys. J.*, **147**, 827 (1967).
[5] Fogarty, W. G., Epstein, E. E., Montgomery, J. W., and Dworetsky, M. M., *Astron. J.*, **76**, 537 (1971).
[6] Fitch, W. S., Pacholczyk, A. G., and Weymann, R. J., *Astrophys. J. Lett.*, **150**, L67 (1967).
[7] Zaitseva, G. V., and Lyutyi, V. M., *Astron. Zh.*, **46**, 237 (1969).
[8] Low, F. J., and Aumann, H. H., *Astrophys. J. Lett.*, **162**, L79 (1970).

Comments on the Evolution of the North-East Atlantic

It has been proposed[1-3] that the opening of the Bay of Biscay occurred by rotation about a pole situated near Paris (50.0° N, 3.3° E) and not about the pole proposed by Bullard *et al.*[4] (43.6° N, 1.0° E). The rotation about the first pole results in strike-slip motion along the North Pyrenean Fault during the pre-Upper-Cretaceous opening, while the rotation about the second pole results in compression there. The second rotation is not compatible with geological data[5] which indicates extension prior to Upper Cretaceous in the Pyrenean region.

We wish to show how these two hypothetical rotations agree with the magnetic data of Williams and McKenzie[6].

Fig. 1 shows a rotation of the Iberian peninsula (contour at the 1,000 fathom isobath) by 23° about the first pole. Intermediary rotated positions every 5° are also shown. Similarly, Fig. 2 shows a rotation of the Iberian peninsula by 27.8° about the Bullard *et al.* pole. If the 1,000 fathom contour represents the limit of the continent, most of the area between anomaly 32 and the western limit of Portugal (except for the southernmost part presumably related to movement of Africa) would have been created by the relative rotation of the Iberian peninsula in Fig. 1, but not in Fig. 2. The first rotation would thus seem to be in better agreement with Williams and McKenzie's data. In addition, this first rotation explains the shape of anomaly 32 as reflecting the pre-drift position of the Iberian peninsula.

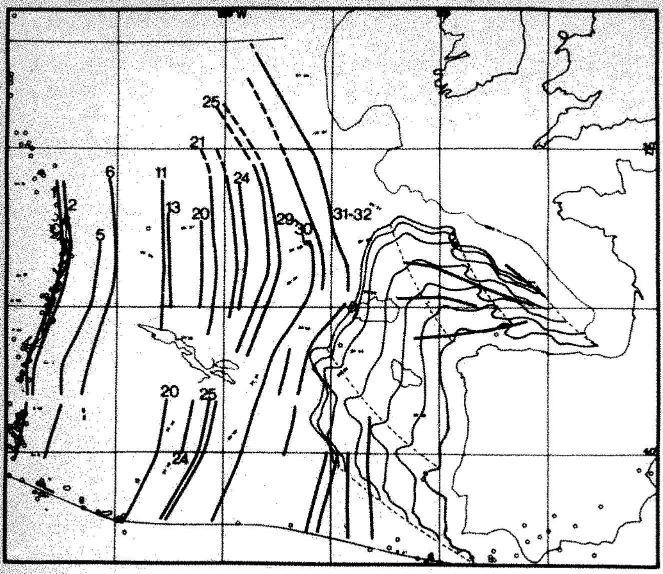


Fig. 1 Magnetic anomalies after Williams and McKenzie[6]. The Iberian Peninsula has been rotated about a pole at 50.0° N, 3.3° E. Several flow-lines are shown in dashed lines.

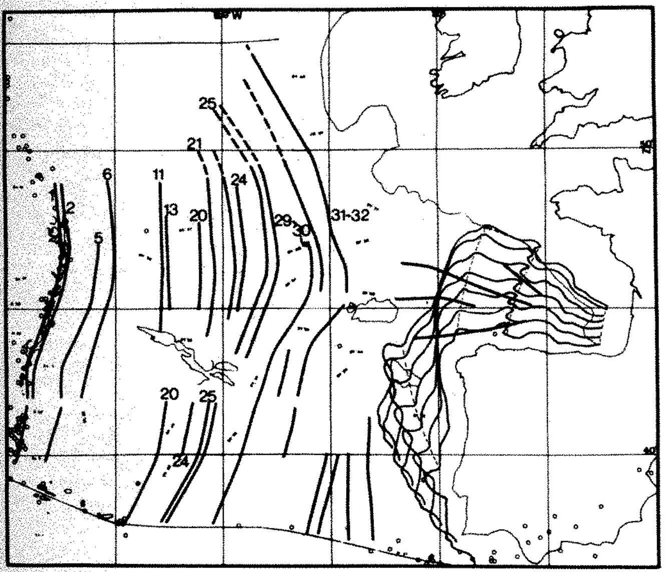


Fig. 2 Magnetic anomalies after Williams and McKenzie[6]. The Iberian Peninsula has been rotated about the Bullard *et al.* pole at 43.6° N, 1.0° E. Several flow-lines are shown in dashed lines.

But even in Fig. 1 the detailed agreement between measured and predicted magnetic anomalies is not good. This may result from inaccurate identification of the anomalies east of 32 by Williams and McKenzie, as a detailed 10 km spaced systematic aeromagnetic survey of the Bay of Biscay (ref. 7 and unpublished work of E. Le Borgue, J. Le Mouël and X. Le P.) has shown the anomalies there to be less fan-shaped than indicated by their map. In any case, an extension toward the west of the systematic detailed survey would enable a definite choice to be made between these two possible rotations.

Xavier Le Pichon
Jean-Claude Sibuet

Centre Océanologique de Bretagne,
BP 337, 29*N. Brest*

Received August 11, 1971.

[1] Le Pichon, X., Bonnin, J., and Sibuet, J. C., *CR Acad. Sci.*, **271**, 1941 (1970).
[2] Le Pichon, X., Bonnin, J., Francheteau, J., and Sibuet, J. C., Une hypothèse d'evolution tectonique du golfe de Gascogne. Symp. sur l'histoire structurale du golfe de Gascogne, Rueil-Malmaison (in the press).
[3] Le Pichon, X., and Sibuet, J. C., *Earth Planet. Sci. Lett.* (in the press).
[4] Bullard, E. C., Everett, J. E., and Smith, A. G., *Phil. Trans. Roy. Soc.*, A, **258**, 41 (1965).
[5] Mattauer, M., *Earth Planet. Sci. Lett.*, **7**, 87 (1969).
[6] Williams, C. A., and McKenzie, D., *Nature*, **232**, 168 (1971).
[7] Le Borgne, E., and Le Mouël, J., *CR Acad. Sci.*, **271**, 1167 (1970).

Implied Superheavy Element Decay Lifetime from Meteorites

Schramm and Fowler[1] demonstrated that superheavy elements could be synthesized in the *r*-process. In the light of these calculations it is interesting to look at the anomalous heavy Xe isotopic composition of carbonaceous chondrites. Rowe[2] has shown that these isotopic anomalies could be caused by the fission of some unknown nucleus. The Xe isotopic composition for this carbonaceous chondrite fission (CCF) spectrum is very different from the ^{244}Pu fission spectrum measured by Alexander *et al.*[3] and observed in other types of meteorites[4-6]. A reasonable candidate for this unknown fissioning nucleus would be a long lived element[7-10].

Assuming CCF is due to the fission of a superheavy element (SH) it is possible to estimate the half life of SH using the production rates calculated by Schramm and Fowler[1] and the interval, Δ, between the termination of nucleosynthesis and the time of Xe gas retention. The value of Δ has been estimated[11,12] from ^{129}I–^{129}Xe and ^{244}Pu–Xe measurements. A current best estimate for Δ would be 1.07×10^8 yr for a "smooth" slowly varying, time-dependent model of nucleosynthesis and 1.56×10^8 yr for nucleosynthesis models with a large "spiked" event just before solar system separation. These values of Δ were calculated using the techniques of Schramm and Wasserburg[11] with the parameters shown in Table 1. Although the parameter values in Table 1 differ slightly from those used in ref. 11, the range of uncertainty estimated[11] is still valid (though possibly overcautious). Therefore the possible range of values for Δ of

$$7.5\times10^7\ \text{yr}\le\Delta\le2.5\times10^8\ \text{yr} \tag{1}$$

given by Schramm and Wasserburg will be used here. This range includes the maximum effect of large time-dependent variations in the production function as well as any uncertainties in parameter values. Carbonaceous chondrites are generally regarded as more primitive than other meteorites (compare ref. 13). Their I–Xe ages seem, however, to be within $\sim10^6$ yr of other meteorites[14,15]. Thus the limits on Δ given above should apply to the carbonaceous chondrites.

The implied abundance ratio of element SH relative to ^{244}Pu can be calculated from the values given in Table 1, *B* and *C*. For Type I carbonaceous chondrites the ratio is

$$\mathrm{SH}/^{244}\mathrm{Pu}\approx0.0012/F \tag{2}$$

where F is the ^{136}Xe fission yield for element SH. The branching ratio of alpha decay to spontaneous fission decay for SH is unimportant because all alpha decays will eventually lead to a spontaneously fissioning nucleus (see ref. 1). There may be an uncertainty of a factor of 2 at most in the constant 0.0012. That uncertainty is, however, small compared with the uncertainty in *F*. At present it is not known whether SH will fission symmetrically, asymmetrically or even tertially, nor is the precise size of the fission yield known.

Asymmetric fission was shown by Schramm and Fowler to enable a fit to the rare Earth *r*-process abundance hump. To fit the CCF Xe spectrum with asymmetric fission it would be necessary for the low mass asymmetric peak to be at $A \geq 135$ and, if the high mass peak is at $A \sim 155$, this would imply a lower average neutron multiplicity than predicted by Nix[16]. For asymmetric fission the peak yield can be estimated from systematics (see Hyde[17]) as $\sim 6\%$. Recently John *et al.*[18] found that ^{257}Fm spontaneously fissions with a small symmetric component. If this effect is extrapolated to the superheavy region (a somewhat risky procedure) it tends to imply that element SH fissions symmetrically. The symmetric peak would be at $A \sim 142$, depending on the average neutron multiplicity and the number of alpha decays preceding the fissioning of SH. For symmetric fission the $^{136}\mathrm{Xe}_f$ yield might be as high as 10%. The yield might, however, also be low ($\sim 1\%$) if the symmetric peak was far above Xe. Nix[16] has also pointed out the possibility that tertiary fission may be the dominant mode for superheavy nuclei. If this is true it would be necessary for Xe to lie on the low mass side of one of the mass peaks in order to fit the CCF spectrum. In view of the uncertainties, calculations have been carried out for

$$0.01 \leq F \leq 0.10 \tag{3}$$

allowing a full order of magnitude uncertainty in F.

The implied half life, τ_{SH}, for the superheavy element can be calculated from the equation used by Schramm and Fowler[1]. For a production function with a terminal "spike"

$$\tau_{\mathrm{SH}} = \frac{\tau_{244}}{1 - \left[\dfrac{\tau_{244}\, ln\ R\,(244,\,\mathrm{SH})}{0.693\ \Delta}\right]} \tag{4a}$$

and with a "smooth" production function

$$\tau_{\mathrm{SH}} = \frac{\tau_{244}}{1 - \dfrac{\tau_{244}}{0.693\ \Delta}\left\{ ln\,[R\,(244,\,\mathrm{SH})] + ln\left(\dfrac{\tau_{244}}{\tau_{\mathrm{SH}}}\right)\right\}} \tag{4b}$$

where (using the notation of ref 11)

$$R\,(244,\,\mathrm{SH}) = \left(\frac{\mathrm{SH}}{^{244}\mathrm{Pu}}\right)\cdot\left(\frac{P_{244}}{P_{\mathrm{SH}}}\right)$$

It was found that τ_{SH} implied from CCF is always much less than the duration of *r*-process nucleosynthesis, T_{G} ($T_{\mathrm{G}} >$ the mean age of the elements $\sim 3 \times 10^9$ yr, see ref. 11) and terms varying as $\exp[-ln(2)\,T_{\mathrm{G}}/\tau_{\mathrm{SH}}]$ have been neglected in equation (4). (This assumption might not be valid for $Z_{\mathrm{SH}} \leq 114$, because then P_{SH}/P_{244} can become small, necessitating a large τ_{SH} to fit CCF data. Experimental searches[19,20] for superheavy elements, however, indicate $\tau_{\mathrm{SH}} < 10^9$ yr.)

Table 1 Input Data (Notation defined in Ref. 11)

	Reference
(*A*) *r*-process production ratios	
$P_{129}/P_{127} \approx 1.52$	12
$P_{244}/P_{232} \approx 0.49$	11
$0.75 \gtrsim P_{\mathrm{SH}}/P_{244} \gtrsim 0.20$*	1
(*B*) Observed abundance ratios (4.6×10^9 yr ago)	
$^{129}\mathrm{I}/^{127}\mathrm{I} = 1.07 \times 10^{-4}$	24
$^{232}\mathrm{Th}/^{238}\mathrm{U} \approx 2.42$ (=3.9 now)	11
$^{244}\mathrm{Pu}/^{232}\mathrm{Th} \approx 0.0054$ ($^{244}\mathrm{Pu}/^{238}\mathrm{U} = 0.013$)	25
(*C*) Mean carbonaceous chondrite abundances	
$\langle ^{238}\mathrm{U}\rangle$ ~17 p.p.b. (now) = ~34 p.p.b. (4.6×10^9 yr ago)	26,27,28
$\langle ^{136}\mathrm{Xe}_f\rangle$ CC I $\approx 5 \times 10^{-11}$ CC STP/g $\langle ^{136}\mathrm{Xe}_f\rangle$ CC I $\approx 4 \times 10^{-11}$ CC STP/g	29

* This lower bound assumes $Z_{\mathrm{SH}} = 110$; if $Z_{\mathrm{SH}} = 114$, the lower bound goes to ~ 0.006.

Values for P_{SH}/P_{244} are given in Table 1 and values for $\mathrm{SH}/^{244}\mathrm{Pu}$ are given by equation (2) using relation (3). Thus the range taken for $R(244,\mathrm{SH})$ is

$$0.016 \leq R(244,\mathrm{SH}) \leq 0.60 \tag{5}$$

A best estimate for $R(244,\mathrm{SH})$ would probably be ~ 0.032. Using the values for Δ given previously, this yields a best estimate of $\tau_{\mathrm{SH}} \sim 2.3 \times 10^7$ yr for a spiked model and $\tau_{\mathrm{SH}} \sim 2.4 \times 10^7$ yr for a smooth production model. If the entire range of values given by relations (1) and (5) are considered, the following limits can be placed on τ_{SH} implied from CCF Xe

$$1.65 \times 10^7\ \mathrm{yr} \leq \tau_{\mathrm{SH}} \leq 6.80 \times 10^7\ \mathrm{yr} \tag{6}$$

This narrow range seems consistent with the implied $\tau_{\mathrm{SH}} \sim 3.5 \times 10^7$ yr calculated in ref. 1 from the fossil track experiment of Bhandari *et al.*[21]. Carver and Anders[22], however, have mentioned that a chemical fractionation factor of $\sim 10^4$ should be used in estimating the implied $\mathrm{SH}/^{244}\mathrm{Pu}$ ratio for the solar system from the results of ref. 21. Such a factor changes the half life implied from fossil tracks to $\sim 1.6 \times 10^8$ yr. The range of uncertainty of this number is, however, large enough to intersect the range given in relation (6). It is also important to note that Bhandari *et al.*[21] found a high superheavy element track density in the meteorite Angra dos Reis and yet Hohenberg[23] found the fission Xe spectrum to be that of ^{244}Pu not CCF. This implies that the tracks observed by Bhandari *et al.*[21] do not come from the same source as CCF, thus equality between the implied half lives would be purely coincidental. It should be mentioned that the validity of the experiment of Bhandari *et al.* is in serious question on several points (E. A. Carver and F. Anders, to be published; and P. B. Price, R. S. Rajan and E. K. Shirk, private communication) which will not be discussed here.

The half life implied in relation (6) for a superheavy element producing CCF Xe is long enough to ensure that there would be sufficient SH remaining at the time of track retention ($\sim 3.8 \times 10^8$ yr after Xe retention[4]) to produce a countable fossil fission track density. It is therefore important for observers to look at the fossil tracks in carbonaceous chondrites. Unfortunately, this is very difficult to do because of the small grain size in this meteorite class.

The half life implied from CCF Xe is within an order of magnitude of the Nilsson *et al.*[23] estimate for $^{294}110$ but it is many orders of magnitude longer than Nilsson half life estimates for other superheavy elements produced in the *r*-process. In particular elements $Z = 112$ to 119 correlated by Anders and Heymann[7] with CCF Xe, are predicted by Nilsson to be relatively less stable than $^{294}110$. Although the estimates of Nilsson *et al.* may be uncertain by several orders of magnitude, estimates of relative stability should be fairly reliable.

I thank E. Anders and R. Ganapathy for focusing my interest on this problem. I also acknowledge enlightening comments from R. Walker, C. Hohenberg, J. C. Huneke, D. S. Burnett and J. B. Blake. This work was supported in part by the National Science Foundation.

DAVID N. SCHRAMM

California Institute of Technology,
Pasadena, California

Received August 23, 1971.

[1] Schramm, D. N., and Fowler, W. A., *Nature*, **231**, 103 (1971).
[2] Rowe, M. W., *Geochim. Cosmochim. Acta*, **32**, 1317 (1968).
[3] Alexander, E. C., Lewis, R. C., Reynolds, J. H., and Michel, M. C. *Science*, **172**, 837 (1971).
[4] Wasserburg, G. J., Huneke, J. C., and Burnett, D. S., *Phys. Rev. Lett.*, **22**, 1198 (1969).
[5] Wasserburg, G. J., Huneke, J. C., and Burnett, D. S., *J. Geophys. Res.*, **74**, 4221 (1969).
[6] Hohenberg, C. M., Munk, M. N., and Reynolds, J. H., *J. Geophys. Res.*, **72**, 3139 (1967).
[7] Anders, E., and Heymann, D., *Science*, **164**, 821 (1969).
[8] Srinivasan, B., Alexander, E. C., Manuel, O. K., and Troutner, D. E., *Phys. Rev.*, **179**, 1166 (1969).
[9] Dakowski, M., *Earth Planet. Sci. Lett.*, **6**, 152 (1969).
[10] Rao, M. N., *Nucl. Phys.*, *A*140, 69 (1970).
[11] Schramm, D. N., and Wasserburg, G. J., *Astrophys. J.*, **162**, 57 (1970).
[12] Fowler, W. A., in *Cosmology, Fusion, and Other Matters: A Memorial to George Gamow* (edit. by Reines, F.) (Colorado Associated University Press, Boulder, in the press, 1971).
[13] Anders, E., *Space Sci. Rev.*, **3**, 583 (1964).
[14] Podosek, F. A., and Hohenberg, C. M., *Earth Planet. Sci. Lett.*, **8**, 443 (1970).
[15] Podosek, F. A., *Geochim. Cosmochim. Acta*, **34**, 341 (1970).
[16] Nix, J. R., *Phys. Lett.*, **30*B***, 1 (1969).

[17] Hyde, E. K., *Nuclear Properties of the Heavy Elements III* (Prentice-Hall, Englewood Cliffs, New Jersey, 1964).
[18] John, W., Hulet, E. K., Lougheed, R. W., and Wesolowski, J. J., *Phys. Rev. Lett.*, **27**, 45 (1971).
[19] Nilsson, S. G., Thompson, S. G., and Tsang, C. F., *Phys. Lett.*, **28*B***, 458 (1969).
[20] Grimm, W., Hermann, G., and Schössler, H. D., *Phys. Rev. Lett.*, **26**, 1040 (1971).
[21] Bhandari, N., Bhat, S., Lal, D., Rajagopalan, G., Tamhane, A. S., and Venkatavaradan, V. S., *Nature*, **230**, 219 (19171).
[22] Hohenberg, C. M., *Geochim. Cosmochim. Acta*, **34**, 184 (1970).
[23] Nilsson, S. G., Tsang, C. F., Sobiczewski, A., Szymanski, Z., Wycech, S., Gustafoson, C., Lamm, I. C., Möler, P., and Nilsson, B., *Nucl. Phys.*, *A***131**, 1 (1969).
[24] Hohenberg, C. M., Podosek, F. A., and Reynolds, J. H., *Science*, **156**, 202 (1967).
[25] Podosek, F. A., *Earth Planet. Sci. Lett.*, **8**, 183 (1970).
[26] Morgan, J. W., and Lovering, J. F., *Nature*, **213**, 873 (1967).
[27] Lovering, J. F., and Morgan, J. W., *J. Geophys. Res.*, **69**, 1979 (1964).
[28] Reed, jun., G. W., Kigoshi, K., and Turkevich, A., *Geochim. Cosmochim. Acta*, **20**, 122 (1960).
[29] Mazor, E., Heymann, D., and Anders, E., *Geochim. Cosmochim. Acta*, **34**, 781 (1970).

Isotopic Composition of Xenon from a Greenland Anorthosite

FOLLOWING a study of the data resulting from the lunar α-scattering experiment of Turkevich *et al.*[1], Olsen[2] pointed out that the rocks in the Mare Tranquillitatus region were similar in oxide composition to certain terrestrial anorthosites. When lunar rocks became available for examination after the Apollo 11 mission, O'Hara[3] drew attention to the possibility that terrestrial anorthosites might be re-worked slices of the Earth's primordial crust. Windley[4], in particular, supported this view proposing that early Pre-Cambrian Group III anorthosites are fragments of layered differentiates formed early in the evolution of the Earth. Romey[5], on the other hand, does not like the comparison between lunar and terrestrial anorthosites and suggests that theories of lunar petrogenesis are developing too fast.

Nevertheless Windley's Group III anorthosites do seem to be of extreme age (≃3,500 m.y.) and to represent samples from an early phase of the Earth's geological history. If positive evidence concerning the events which occurred during this period is to be found, then this class of rock may provide a source of useful information. With this approach in mind, and being keenly aware of some of the major isotopic problems present in noble gas studies, it seemed reasonable to obtain samples of these perhaps very special rocks and to study the isotopic compositions of the noble gases "trapped" within them.

Six samples of Group III anorthosites from the West Greenland Fiskenaesset region were obtained from Dr B. F. Windley of the Greenland Geological Survey. It was decided to examine only the xenon in these rocks in the first instance.

Early measurements demonstrated the presence in rock No. 86943 (Windley classification) of xenon with an isotopic composition similar to that found in North American gas wells by Butler *et al.*[6] and Clarke and Thode[7] and discussed by Bennett and Manuel[8]. This, in itself, is an observation of some interest when considered in the light of the measurements of Srinivasan *et al.* (private communication) on a New South Wales iodyrite (International Mineralogical Association classification: iodargyrite) who also report the presence of gas well type xenon. This xenon may be present on a world wide scale in suitable trapping sites.

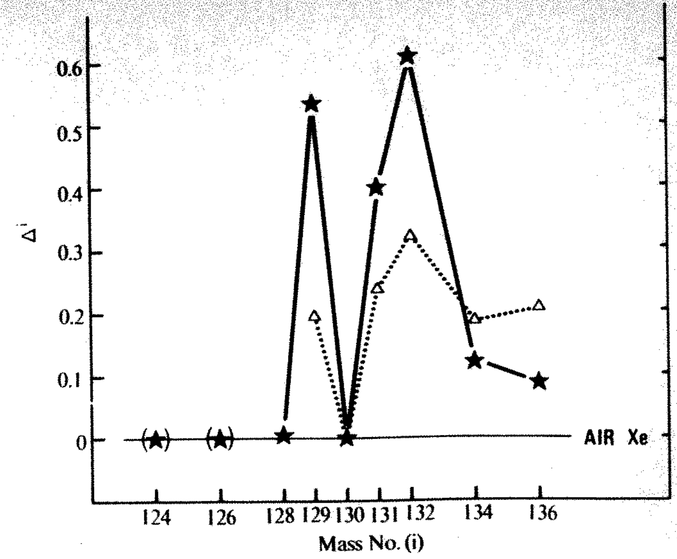


Fig. 1 Xenon ratio differences, $\Delta^i = (Xe^i/^{130}Xe)_{Sample} - (Xe^i/^{130}Xe)_{Air}$, as a function of mass number (i) for 86943 anorthosite fines (>700° C) and C-16 gas well xenon[8]. ★, 86943F (>700° C); △, C-16.

Further studies on rock No. 86943 and its mineral concentrates have resulted in the observation of xenon with an isotopic composition which seems to be necessary if the gas well pattern is to be understood. Both Bennett and Manuel[8] and Reynolds[9] have pointed out that gas well xenon must contain a contribution from ^{238}U spontaneous fission xenon, so that by subtracting this contribution from the gas well pattern it is possible to predict the isotopic composition of the remaining xenon. The isotopic composition of xenon observed in the fines of rock No. 86943 seems to approximate to that required for this component. In fact the presence of the gas well pattern in the total rock tends to support this view. The fines were produced as the result of the separation of the mineral concentrates from the total rock.

The xenon isotopic pattern observed in the fines (>700° C) from rock No. 86943 is shown in Fig. 1 together with the gas well xenon pattern as typified by C 16 (ref. 8). Here the data are presented in terms of ratio differences from air xenon using ^{130}Xe as the normalizing isotope.

In Table 1 the isotopic ratios of the fines xenon, 86943F (>700° C), are listed relative to ^{130}Xe. An estimate of the amount of anomalous ^{132}Xe in the fines, based on the assumption that all the ^{130}Xe is due to air xenon, is included. Blanks (<10% of total xenon) and air standards were run with every sample. Xenon from a number of samples of total rock No.

Table 1 Isotopic Composition of 86943F (>700° C) Xenon *

124	126	128	129	130	131	132	134	136
(0.022)	(0.021)	0.477±0.020 †	7.024±0.090	1.000	5.596±0.085	7.210±0.07	2.683±0.035	2.266±0.020

* Anomalous ^{132}Xe per gram of fines ≃ 2×10^{-12} cc NTP—assuming all ^{130}Xe due to air.
† All errors are one standard deviation.

86943, its mineral concentrates and fines has been examined. The data reported here are the best obtained so far, although the ^{124}Xe and ^{126}Xe ratios are ill defined extrapolated values.

All my attempts to produce a model to synthesize the fines xenon isotopic pattern in terms of xenon components of proven isotopic composition have been unsatisfactory. A model of particular interest would be one based on linear mass fractionation of the type of xenon found in the 1,000° C fraction of the meteorite Pesyanoe (ref. 10) but the low 134 and 136 abundances relative to the 132 mass abundance in the fines makes such a model untenable. Addition of ^{130}Xe to atmospheric xenon, but not to rock xenon, by a mechanism due to Cameron[11] is not an acceptable model for the same reason. No nuclear reaction has yet been proposed which can produce the anomalous pattern *in situ* assuming that the ^{129}Xe, ^{131}Xe and ^{132}Xe abundances are well correlated. The assumption of good correlation seems to be reasonable on the basis of the widespread observations of the gas well pattern. The low abundances of the ^{134}Xe and ^{136}Xe relative to ^{132}Xe in the fines xenon would be difficult to account for if a fission process was considered in order to explain the ^{129}Xe, ^{131}Xe and ^{132}Xe excesses.

On present evidence *in situ* production of the fines xenon component does not seem as likely as a model which accounts for it in terms of sampling, at least 3,210 m.y. ago (K/Ar age cited by Windley[4]), from a reservoir containing xenon with an isotopic composition similar to that of the fines xenon. The isotopic composition of such reservoir xenon could be the result of thorough mixing of more than one xenon component thus avoiding the difficulty associated with ascribing it to a single component. Sampling, at different periods throughout geological time, from this reservoir could account for all the gas well type xenon so far observed if suitable additions to the isotopic pattern are made by the inclusion of xenon from ^{238}U spontaneous fission.

The absence of any trace of the gas well pattern in xenon extracted from rocks of extreme age, or of supposedly deep seated origin, such as eclogites, lherzolite nodules and peridotites would then be considered to be due to their thorough outgassing during periods of metamorphism.

Thus, to summarize, first the West Greenland anorthosite No. 86943 contains gas well type xenon. Not all samples show this pattern, which is not surprising when the mineral heterogeneity of the bulk sample is considered. Second, the fines, produced during the concentration of the major minerals in the rock, contain a xenon component which seems to be essentially gas well type xenon with most of its ^{238}U spontaneous fission xenon component removed. Third, the origin of the fines xenon component is unknown . It is suggested that on the basis of present evidence it seems more likely to have originated from sampling of a suitable reservoir than to have been produced *in situ*.

The demonstrated presence of gas well type xenon in North America, Australia and Greenland indicates that it may be present on a global scale in suitable trapping sites. This observation of anomalous xenon in the fines of a Group III anorthosite substantiates previous suggestions concerning the origin of the gas well isotopic pattern. It does not, however, answer the vital question of how the anomalous xenon was produced. For example, have Group III anorthosites simply provided suitable trapping sites for the fines xenon component or has their possible presence in a primitive crust of the Earth some further significance in relation to the present day isotopic composition of gas well and atmospheric xenon?

I thank L. Tilbury, R. Hay, J. G. Kay and W. Smeed for help. This research was supported by the Research Grants Committee of the University of Western Australia and the Australian Research Grants Committee.

P. M. JEFFERY

Department of Physics,
University of Western Australia

Received August 16, 1971.

[1] Turkevich, A. L., Franzgrote, E. J., and Patterson, J. H., *Science*, **165**, 277 (1969).
[2] Olsen, E., *Science*, **166**, 401 (1969).
[3] O'Hara, M. J., *Nature*, **225**, 321 (1970).
[4] Windley, B. F., *Nature*, **226**, 333 (1970).
[5] Romey, W. D., *Science*, **172**, 292 (1971).
[6] Butler, W. A., Jeffery, P. M., Reynolds, J. H., and Wasserburg, G. J., *J. Geophys. Res.*, **68**, 3283 (1963).
[7] Clarke, W. B., and Thode, H. G., in *Isotopic and Cosmic Chemistry* (edit. by Craig, H., Miller, S. L., and Wasserburg, G. J.), 471 (North-Holland, Amsterdam, 1964).
[8] Bennett, G. A., and Manuel, O. K., *Geochim. Cosmochim. Acta*, **34**, 593 (1970).
[9] Reynolds, J. H., *Proc. Intern. Conf. Mass Spec. Kyoto*, 594 (1970).
[10] Marti, K., *Science*, **165**, 1263 (1969).
[11] Cameron, A. G. W., *Icarus*, **1**, 13 (1962).

Carbon-13 NMR Study of the Stereochemistry of Steranes from Oil Shale of the Green River Formation (Eocene)

THE presence of steranes and triterpanes in oils and sediments is especially significant as they possess the appropriate chemical stability for preservation throughout terrestrial diagenesis. They also contain a high degree of molecular order in their carbon skeleton and so the probability of their being synthesized by abiological processes is minimized. The high level of latent information in the structure and stereochemistry of these compounds renders them ideal biological markers[1-6].

The determination of true steric configuration is important in the study of both linear and cyclic fossil isoprenoids. For example, the structural information gathered on these compounds could be used in an analogous manner to the chemotaxonomic conclusions of recent flora. The biosynthesis of steroids and triterpenoids has now been shown to follow the biogenetic isoprene rule by squalene epoxide[7-11]. In steroid and triterpenoid biosynthesis it has been established that the role of the enzyme is critical in specifying the unique chain folding which allows the annelation steps to proceed along one path—a path not favoured thermodynamically[12,15]. The study of the stereochemistry of fossil steroids and isoprenoids is expected to lead to the understanding of the evolution of enzyme systems governing biosynthesis of their precursors as well as to the unravelling of the nature and kinetics of diagenetic processes in carbonaceous sediments.

The occurrence and the significance of steranes and triterpanes in the bitumen of Green River Shale has been well documented[16-22]. Gross identification of the individual biogenic compounds presented in these works was achieved on the basis of combined data from gas chromatography and mass spectrometry or X-ray crystallography[23-25].

In our study milligram quantities of high purity C_{27}, C_{28} steranes were isolated from Green River Shale using a preparative gas-liquid chromatography system developed in this laboratory for milligram-scale automatic separation of individual components from complex mixtures (see refs. 26 and 27 for details). The ^{13}C nuclear magnetic resonance (NMR) spectra of the isolated steranes were determined on a Varian HR-60 NMR spectrometer, modified for high resolution pulsed Fourier transform spectroscopy. Equipment and operational details have been described elsewhere[28,29]. Proton decoupling was performed both in the noise mode (simultaneously decoupling all protons) or off-resonance in the coherent mode to assist in spectral identification. The sample probe accommodated 5 mm sample tubes, and contained separate 15.08 MHz ^{13}C r.f. pulse coils and 60 MHz ^{1}H (proton) decoupling coils. For some spectra, the receiver coil was double tuned to allow internal locking on deuterium

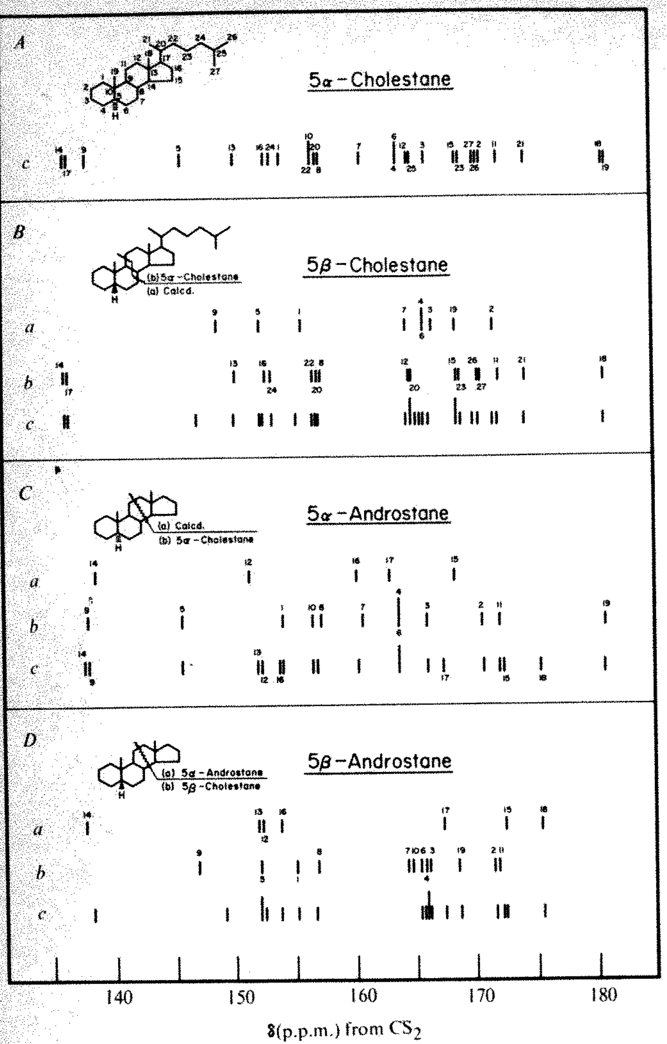


Fig. 1 Line diagram of the proton-decoupled ^{13}C resonances. *a*, Lines: resonance values calculated by additive parameters. *b*, Lines: chemical shifts taken from the appropriate part of the reference compound. *c*, Lines: experimental values. *A*, 5α-Cholestane 29 mg (Sigma Chemical Company). *B*, 5β-Cholestane 30 mg (Sigma Chemical Company). *C*, 5α-Androstane 30 mg (Mann Research Laboratory). *D*, 5β-Androstane 10 mg (Mann Research Laboratory).

(9.211 MHz). For the rest of the spectra, the ^{13}C transmitter coil was double tuned to allow coherent decoupling at the solvent deuterium frequency for a sharp reference peak, and an external ^{7}Li lock (23.321 MHz) was used which gave a limiting ^{13}C spectral resolution of approximately 0.03 p.p.m. $CDCl_3$ was used as the solvent in all cases. Due to the small quantity of the collected terpanes available (5–15 mg), both 30 µl. spherical microbulbs and normal 5 mm NMR tubes were used for spectra. The microbulbs were found to give increased sensitivity at the expense of resolution. For each spectrum, free-induction decays following 400 W r.f. pulses were accumulated on a Fabri-Tek model 1074 signal averager with a 4096 word memory. The accumulated signal was then Fourier transformed into a magnitude spectrum electronically using a Spectrum Analyser, or into an absorption spectrum numerically by a CDC 6600 computer.

The spectra were evaluated on the basis of the ^{13}C NMR behaviour of authentic tetracyclic steranes, model compounds and published data. The chemical shift assignments were aided by the extensive use of the qualitative rule of structural additivity derived for perhydrophenanthrenes[30], steroids, terpanes and carotenes[31,32].

Line diagrams of the noise decoupled ^{13}C NMR spectra of the stereoisomers cholestane (5α-cholestane) and coprostane (5β-cholestane) are shown in Fig. 1 (*A* and *B*, *c* lines). Both of these compounds have been tentatively identified in the Green River shale[21]. The difference between the two isomers lies in the manner of fusion of the A and B rings whereby the bridgehead carbons are *trans* or *cis*-substituted. To aid structural assignment, two additional model compounds, 5α-androstane and 5β-androstane, were selected and served as additive basic structural building units (*C* and *D*).

Our identification of ^{13}C resonances of cholestane followed the assignment reported by Rich *et al.*[31]. These assignments formed the basis of our extended calculations, where a part of the structure of cholestane was utilized (*b* lines). The calculations of the ^{13}C chemical shifts for the remaining portion of the molecule were performed by using the additive methyl substituent parameters obtained by factor analysis of methyl cyclohexanes by Dalling (ref. 30; *a* lines).

Although the spectrum of coprostane (5β-cholestane) seems different from cholestane, closer examination reveals that the effect of the A ring inversion on the chemical shifts can easily be traced to simple gauche-anti spatial orientation changes of the C-2, C-3 and C-4 carbons of the A ring relative to γ carbons in the B ring. As expected, the C_{19} methyl has been deshielded (−) and its resonance shifted downfield by about 12 p.p.m. The C-9 and C-7 carbons were affected the strongest by the C-2 and C-4 carbons respectively which folded into a close gauche position causing 8 and 5 p.p.m. shifts upfield. The resonances of the C-2, C-3 and C-4 carbons moved slightly upfield due to the sum of deshielding (−) and shielding (+) effects of the neighbouring carbons. The bridgehead carbons of C-5 and C-10 could be assigned with a high degree of confidence on the basis of their carbon–proton coupling behaviour shown in the coherent decoupled spectra.

The designation of the carbons of the five membered ring of the androstanes presented a particular problem because no parametric values are available to calculate ^{13}C resonances in perhydroindanes. Recourse was made to another set of parameters called "hydrocarbon parameters" developed by Dalling[30] to approximate the chemical shifts of those particular carbons. The values obtained are shown in Fig. 1, *C*/*a* lines.

Table 1 ^{13}C Chemical Shifts* and Assignments for the ^{13}Spectra of Steranes

Carbon	5α-Andro-stane	5β-Andro-stane	5α-Chol-estane†	5β-Chol-estane	5α-Ergostane
1	154.9	154.9	154.0	155.1	154.0
2	170.5	171.3	170.5	171.4	172.2
3	165.9	165.7	165.9	166.1	166.1
4	163.6	165.6	163.6	165.6	163.6
5	145.6	152.2	145.7	152.1	145.6
6	163.6	165.6	163.6	165.4	163.6
7	160.2	165.1	160.6	164.3	160.5
8	156.8	156.4	157.2	156.9	157.2
9	138.0	148.9	137.9	146.9	137.9
10	156.4	165.3	156.5	164.7	156.4
11	171.8	171.8	171.9	171.8	173.9
12	152.2	151.8	164.5	164.7	164.5
13	151.9	151.8	150.1	150.0	150.2
14	137.6	138.0	136.1	136.2	136.2
15	172.2	172.1	168.5	168.4	168.4
16	153.7	153.5	152.6	152.3	152.5
17	167.2	167.1	136.4	136.4	136.5
18	175.2	174.2	180.6	180.6	180.2
19	180.5	168.4	180.7	168.4	180.4
20			156.9	156.7	156.4
21			174.1	174.0	178.3
22			156.1	156.5	158.9
23			168.8	168.8	161.9
24			153.1	153.1	153.5
25			164.7	165.1	175.1
26			170.2	169.8	161.3
27			169.9	170.2	170.6
28					171.9

* In p.p.m. upfield from CS_2.
† Slightly different values have been reported by Roberts *et al.*[31].

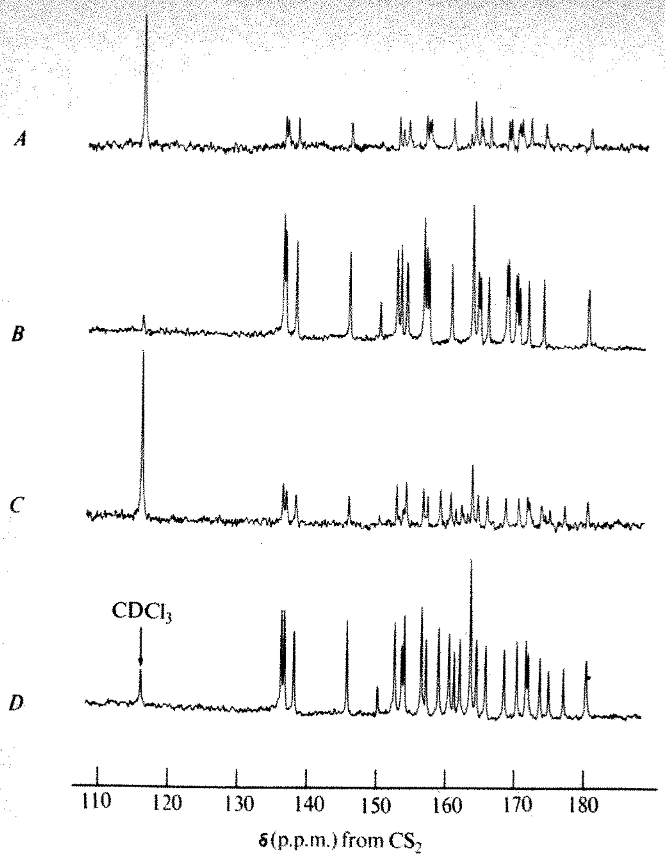


Fig. 2 Noise decoupled spectra of steranes. *A*, 12 mg sample of C_{27} sterane from Green River Shale. *B*, Authentic 5α-cholestane (Sigma). *C*, 8 mg sample of C_{28} sterane from Green River Shale. *D*, 5α-ergostane prepared from ergosterol (R & K Labs Inc.). Samples dissolved in 0.3–0.5 ml. CDCl$_3$ and placed in 5 mm sample tubes. Spectral conditions: ^{1}H noise decoupled, solvent deuterium decoupled, ^{7}Li external lock, ^{13}C r.f. pulse flip angle 50°, pulse interval 1.0 s, sample temperature 23° C, 5 kHz data sampling rate after r.f. pulse. 15,000–140,000 accumulated free-induction decays numerically fourier transformed.

Carbon resonances belonging to C-18, C-13 and C-14 positions were uniquely identified with the assistance of their proton coupling showing a quartet, a singlet and a doublet respectively.

The designation of carbon shifts of 5β-androstane provides a fine demonstration of the validity of the structural additivity principle. All assignments were derived using only the appropriate parts of 5α-androstane and 5β-cholestane molecules. ^{13}C NMR spectra possess fingerprint qualities corresponding to the multitude of factors affecting shifts, intensity and fine structure of the NMR signal. Both substituent[33] and conformational[34] effects have been shown to be very important. This quality of the ^{13}C NMR signal was demonstrated on the C_{27} and C_{28} steranes isolated from the hydrocarbon fraction of the oil shale bitumen of the Green River Formation. Their ^{13}C NMR noise decoupled spectra are present in Fig. 2 with the spectra of authentic 5α-cholestane and 5α-ergostane. There is no doubt that the spectra of the unknown steranes are identical with the spectra of the appropriate reference compounds.

On the basis of this spectral identity and study of model steranes we can state that the mode of ring fusions (all *trans*) and the geometric configuration of the side chain (17α[H]) in the isolated C_{27} and C_{28} steranes follow unequivocally from the ^{13}C NMR data. The assignment of carbon resonances of 5α-ergostane was accomplished by using the assignments of 5α-cholestane for the cyclic portion of the molecule and aliphatic additive parameters[35] to calculate the side-chain shifts. The experimental ^{13}C chemical shift values of the investigated steranes are compiled in Table 1.

We thank Dr Emilio Gelpi and Miss P. C. Wszolek for help, Dr M. P. Klein of the Biodynamics Laboratory, University of California, Berkeley, for computer facilities, the Department of Chemistry, University of California, Berkeley, for the use of the NMR spectrometer. We also thank NASA for support. A. L. B. was a John Simon Guggenheim Memorial Fellow 1970–71.

B. BALOGH
D. M. WILSON
A. L. BURLINGAME

Space Sciences Laboratory, University of California, Berkeley, California 94720

Received August 18, 1971.

[1] Bendoraitis, J. G., Brown, G. L., and Hepner, L. S., *Anal. Chem.*, **34**, 49 (1962); Bendoraitis, J. G., Brown, G. L., and Hepner, L. S., *Sixth World Petrol. Cong.*, Frankfurt (June, 1963).

[2] Eglinton, G., Scott, P. M., Belsky, T., Burlingame, A. L., Richter, W. J., and Calvin, M., *Adv. Org. Geochem.*, 41 (Pergamon Press, London, 1964).

[3] Gelpi, E., Han, J., Nooner, D. W., and Oro, J., *Geochim. Cosmochim. Acta*, **34**, 965 (1970).

[4] Johns, R. B., Belsky, T., McCarthy, E. D., Burlingame, A. L., Haug, P., Schnoes, H. K., Richter, W. J., and Calvin, M., *Geochim. Cosmochim. Acta*, **30**, 1191 (1966).

[5] McCarthy, E. D., and Calvin, M., *Nature*, **216**, 642 (1967).

[6] Studier, H. M., Hayatsu, R., and Anders, E., *Geochim. Cosmochim. Acta*, **34**, 965 (1970).

[7] Eschenmoser, A., Ruzicka, L., Jeger, O., and Arigoni, D., *Helv. Chim. Acta*, **38**, 1890 (1955).

[8] Ruzicka, L., *Proc. Chem. Soc.*, 341 (1959).

[9] Clayton, R. B., *Quart. Rev.*, **19**, 168 (1965).

[10] Van Tamelen, E. E., Willet, J. D., Clayton, R. B., and Lord, K. E., *J. Amer. Chem. Soc.*, **88**, 4752 (1966).

[11] Johnson, W. S., *Accounts of Chem. Research*, **1**, 1 (1968).

[12] Van Tamelen, E. E., Sharpless, K. B., Willet, J. D., Clayton, R. B., and Burlingame, A. L., *J. Amer. Chem. Soc.*, **89**, 3920 (1968).

[13] Van Tamelen, E. E., Sharpless, K. B., Hanzlik, R., Clayton, R. B., Burlingame, A. L., and Wszolek, P. C., *J. Amer. Chem. Soc.*, **89**, 7150 (1967).

[14] Van Tamelen, E. E., Hanzlik, R. P., Sharpless, K. B., Clayton, R. B., Richter, W. J., and Burlingame, A. L., *J. Amer. Chem. Soc.*, **90**, 3283 (1969).

[15] Van Tamelen, E. E., Hanzlik, R. P., Clayton, R. B., and Burlingame, A. L., *J. Amer. Chem. Soc.*, **92**, 2137 (1970).

[16] Burlingame, A. L., Haug, P., Belsky, T., and Calvin, M., *Proc. US. Nat. Acad. Sci.*, **54**, 1406 (1965).

[17] Murphy, M. T. J., McCormick, A., and Eglinton, G., *Science*, **157**, 1040 (1967).

[18] Hills, I. R., Whitehead, E. V., Anders, D. E., Cummins, J. J., and Robinson, W. E., *Chem. Comm.*, 752 (1966).

[19] Henderson, W., Wollrab, V., and Eglinton, G., *Chem. Comm.*, 710 (1968).

[20] Henderson, W., Wollrab, V., and Eglinton, G., *Advances in Organic Geochemistry* (edit. by Schenk, P. A., and Havenaar, I.), 181 (Pergamon Press, 1968).

[21] Gallegos, E. J., *Div. Petrol. Chem.*, **15**, (Chicago, September 1970).

[22] Anders, D. E., and Robinson, W. E., *Geochim. Cosmochim. Acta*, **35**, 661 (1971).

[23] Hills, I. R., Smith, G. W., and Whitehead, E. V., *Div. Petrol. Chem.*, **13**, B5 (Atlantic City, New Jersey, September, 1968).

[24] Burlingame, A. L., and Schnoes, H. K., in *Mass Spectrometry in Organic Geochemistry* (edit. by Eglinton, G., and Murphy, M. T. J.), 89 (Springer, New Jersey, 1969).

[25] Schnoes, H. K., and Burlingame, A. L., in *Topics in Organic Mass Spectrometry* (edit. by Burlingame, A. L.), 369 (Wiley–Interscience, New York, 1970).

[26] Gelpi, E., Wszolek, P. C., Yang, E., and Burlingame, A. L., *J. Chrom. Sci.*, **9**, 147 (1971).

[27] Gelpi, E., Wszolek, P. C., Yang, E., and Burlingame, A. L., *Anal. Chem.*, **43**, 864 (1971).

[28] Jones, D. E., thesis, Univ. California, Berkeley (1970).

[29] Wilson, D. M., thesis, Univ. California, Berkeley (1971).

[30] Dalling, D. K., thesis, Univ. Utah, Salt Lake City (1969).

[31] Reich, H. J., Jautelat, M., Messe, M. T., Weigert, F. J., and Roberts, J. D., *J. Amer. Chem. Soc.*, **91**, 7445 (1969).

[32] Jautelat, M., Grutzner, J. B., and Roberts, J. D., *Proc. US Nat. Acad. Sci.*, **65**, 288 (1970).

[33] Cheney, B. V., and Grant, D. M., *J. Amer. Chem. Soc.*, **89**, 5319 (1967).

[34] Grant, D. M., and Cheney, B. V., *J. Amer. Chem. Soc.*, **89**, 5315 (1967).

[35] Grant, D. M., and Paul, E. G., *J. Amer. Chem. Soc.*, **86**, 2984 (1964).

BIOLOGICAL SCIENCES

Cellular Lipid Inclusions in the White Matter in Multiple Sclerosis

WE have obtained evidence to support the suggestion that fatty acid[1-4] and glycolipid[4-8] metabolism is defective in the white matter outside acute plaques of multiple sclerosis. Histochemical techniques show two types of intracellular lipid inclusions in such apparently normal areas.

Seventeen plaques from eight cases of multiple sclerosis and white matter from sixteen control patients, who had died from other neurological diseases, were fixed in routine saline-formol and transferred to 1% calcium acetate—10% formalin. Frozen sections were stained with haematoxylin-eosin, Sudan black, Nile blue sulphate[9], osmium tetroxide-α naphthylamine[9], 'oil red O', modified periodic acid-Schiff[9], gold-hydroxamic acid, acid haematein[9], perchloric acid-naphthoquinone[9], Holczinger's copper-rubeanic acid[9], diazotization-coupling[10], p-dimethylaminobenzaldehyde-nitrite[11], oxidized tannin-azo[12], trypan blue[13], Hale's dialysed iron[14], toluidine blue, Feulgen[15] and methyl green-pyronin[15]. Sections were also stained with 'Luxol'-fast blue either with the standard technique[15] or with the dye dissolved in 60% ethanol. Some tissue sections were extracted with acetone (4° C), chloroform-methanol (22° C; 2 : 1 v/v), or ethanol (22° C) for 1 h.

Plaques of multiple sclerosis were designated as: acute (six from two cases), showing perivenular infiltration with lymphocytes and plasma cells as well as hypercellularity throughout the lesion; active established chronic (five from five cases), with a rim of proliferating neuroglia at the edge; or inactive established chronic (six from four cases), with no evidence of cellular activity.

Two types of lipid inclusions were found in the white matter at least 2 cm away from acute plaques. The first consisted of granular or globular, isotropic droplets usually near neuroglial nuclei. The cells involved usually appeared to be oligodendroglia rather than astrocytes. These droplets were always stained with 'Luxol'-fast blue in 60% ethanol (Fig. 1) and, less frequently, with copper-rubeanic acid (Fig. 2), but not by the other methods used. They remained colloidal-iron negative after extraction with ethanol (see below). Extraction with chloroform-methanol extinguished staining with both Luxol-fast blue and copper rubeanic acid: acetone eliminated only the latter staining. The precise nature of the lipid in this type of inclusion is uncertain; the fatty acid reaction could be due to hydrolysis of phospholipids.

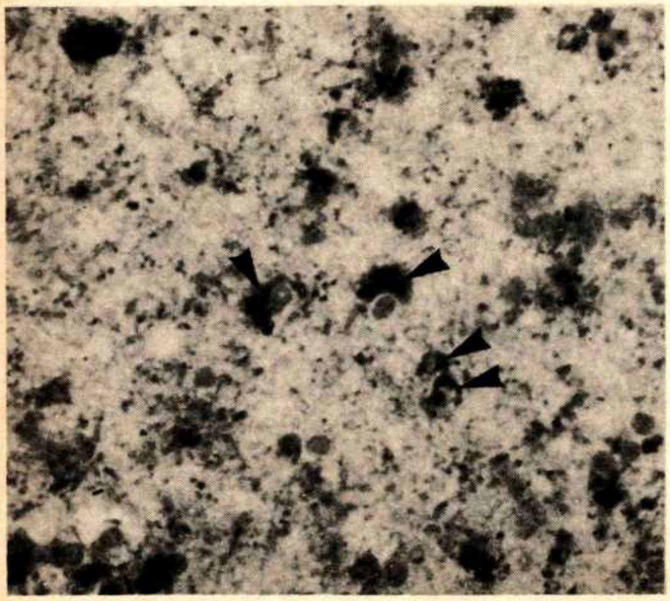


Fig. 1 Type 1 inclusion in cells resembling oligodendroglia (arrows) in the white matter around an acute plaque of multiple sclerosis. 'Luxol'-blue in 60% ethanol (× 960).

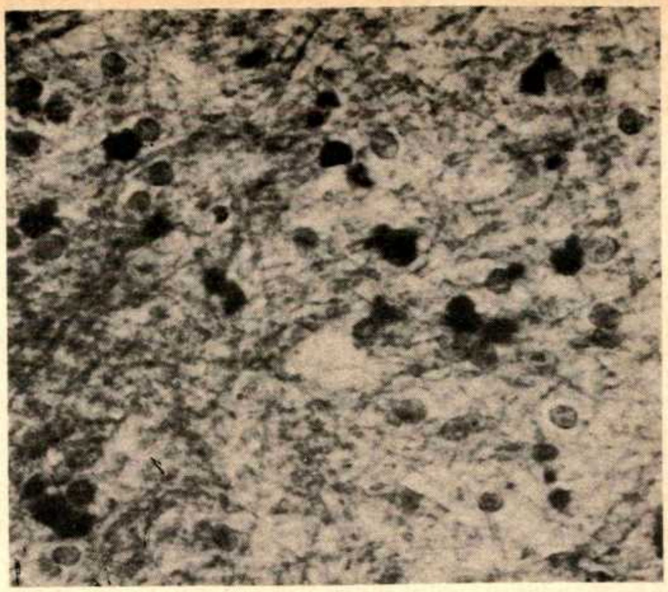


Fig. 2 As for Fig. 1 but stained by Holczinger's copper-rubeanic acid method for free fatty acids (× 960).

The second lipid inclusion in the white matter around acute plaques was a homogeneous relatively large mass usually close to or surrounding a cell nucleus. The cell type involved could not be clearly identified. The lipid mass was stained metachromatically pale orange-brown with cresyl violet (Fig. 3) and thionin (indicating sulphatide), metachromatically red with toluidine blue, moderately with 'Luxol'-fast blue, perchloric acid-naphthoquinone (cholesterol) and Alcian blue, and weakly with the modified PAS method (indicating a little cerebroside). These reactions were prevented by preliminary extraction with chloroform–methanol, but only that with perchloric acid–naphthoquinone was extinguished by acetone. The colloidal iron reaction became positive after extraction with ethanol, but not with acetone or ethanol followed by chloroform–methanol. This lipid inclusion was unstained by the other lipid methods used, the protein techniques and the methods for nucleic acids. With the exception of 'Luxol' preparations, however, it could always be identified in stained sections by its characteristic crystalline anisotropism (Fig. 3). Thus, this second inclusion seems to contain sulphatide, cholesterol and a little cerebroside, but no detectable mucosubstances. The colloidal iron reaction after ethanol recalls the staining of myelin lipids after acetone extraction[17]; the reversibility of both phenomena by chloroform–methanol extraction suggests that an acidic lipid (much as sulphatide) had been unmasked by ethanol or acetone.

Both lipid inclusions were frequently seen around acute plaques, but only occasionally around active and inactive chronic lesions. The first inclusion was never seen in control brains. Inclusions resembling the second type were noted in three control brains but were only occasionally related to cell nuclei. The nature of such extracellular inclusions is obscure[18-20]; possibly they are derived from intracellular lipid accumulations[21]. Neither type of lipid inclusion in acute multiple sclerosis resembled the Rosenthal fibre[22]: their negative reaction for protein, DNA and RNA indicates that they were not viral inclusion bodies.

The lipid inclusions within neuroglial cells are consistent with reports of abnormal proportions of lipids in white matter in multiple sclerosis[1-8]. In particular, the second lipid inclusion may be responsible for the raised sulphatide/cerebroside ratio in multiple sclerosis white matter[8].

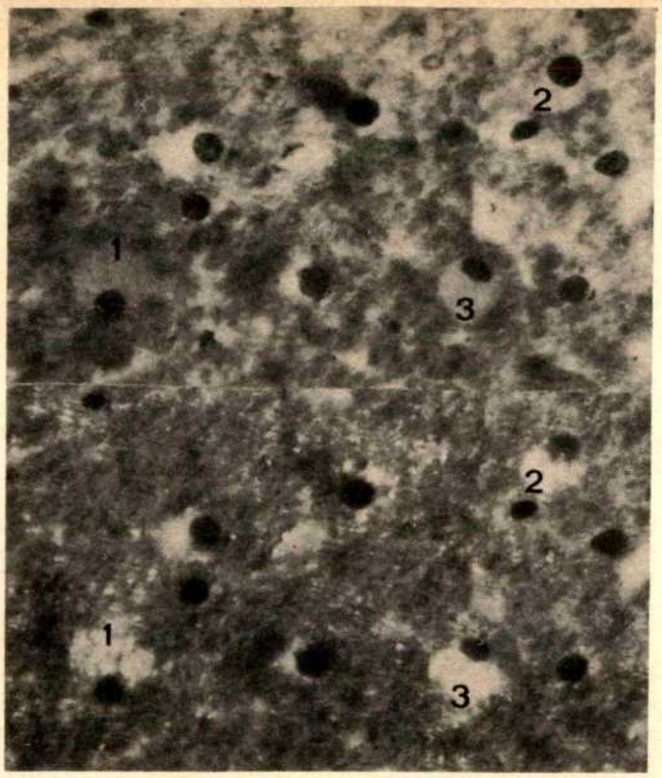


Fig. 3 Top: type 2 inclusion in the white matter around an acute plaque of multiple sclerosis. Note the intracellular site of these inclusions (1, 2, 3), which were stained metachromatically orange-brown in the section. Cresyl violet (× 960). Bottom: same field viewed in polarized light.

The metabolic interpretation of the neuroglial lipid inclusions in multiple sclerosis is speculative. They could result from defective cerebroside synthesis and increased production of sulphatide or, possibly, by failure in extrusion of membrane from the myelin–formative cell, so that lipid accumulates intracellularly.

It is unlikely that the inclusions reflect remyelinating activity, because they occur in apparently normal white matter distant from the demyelinating edge and, moreover, their histochemical characteristics do not correspond with those of the lipids that temporarily appear within interfascicular oligodendroglia during myelination[23–26].

We thank Dr David Haler, Mr Douglas High, Dr Keith Mant, Professor Keith Simpson and Dr Peter Sylvester for brain samples.

O. B. BAYLISS
C. W. M. ADAMS

Department of Pathology,
Guy's Hospital Medical School,
*London SE*1

Received April 29; revised July 9, 1971.

[1] Gerstl, B., Kahnke, M. J., Smith, J. K., Tavaststjerna, M. G., and Hayman, R. B., *Brain*, **84**, 310 (1961).
[2] Baker, R. W. R., Thompson, R. H. S., and Zilkha, K. J., *Lancet*, i, 26 (1963).
[3] Stallberg-Stenhagen, S., and Svennerholm, L., *J. Lipid Res.*, **6**, 146 (1965).
[4] Gerstl, B., Eng, L. F., Tavaststjerna, M., Smith, J. K., and Kruse, S. L., *J. Neurochem.*, **17**, 677 (1970).
[5] Cumings, J. N., *Brain*, **76**, 551 (1953).
[6] Davison, A. N., and Wajda, M., *J. Neurochem.*, **9**, 427 (1962).
[7] Einstein, E. R., Dalal, K. B., and Csejtey, J., *J. Neurol. Sci.*, **11**, 109 (1970).
[8] Cumings, J. N., and Goodwin, H., *Lancet*, ii, 664 (1968).
[9] As set out in Adams, C. W. M., *Adv. Lipid Res.*, **7**, 1 (1969).
[10] Glenner, G. G., and Lillie, R. D., *J. Histochem. Cytochem.*, **7**, 416 (1959).
[11] Adams, C. W. M., *J. Clin. Pathol.*, **10**, 56 (1957).
[12] Dixon, K. C., *Amer. J. Pathol.*, **35**, 199 (1959).
[13] Adams, C. W. M., and Bayliss, O. B., *J. Histochem. Cytochem.*, **16**, 110 (1968).
[14] Hale, C. W., *Nature*, **157**, 802 (1946).
[15] As set out in Pearse, A. G. E., *Histochemistry: Theoretical and Applied*, third ed. (Churchill, London, 1968).
[16] Heslinga, F. J. M., and Déierkauf, F. A., *J. Histochem. Cytochem.*, **10**, 704 (1962).
[17] Adams, C. W. M., and Bayliss, O. B., *J. Histochem. Cytochem.*, **16**, 119 (1968).
[18] Smith, M., *J. Neurol. Neurosurg. Psychiat.*, **12**, 100 (1949).
[19] Wolman, M., *Confin. Neurol.*, **20**, 36 (1960).
[20] Ibrahim, M. Z. M., and Levine, S., *J. Neurol. Neurosurg. Psychiat.*, **30**, 545 (1967).
[21] Ungar, H., Hine, C. H., Kodama, J. K., and Anderson, H. H., *Arch. Pathol.*, **60**, 139 (1955).
[22] Herndon, R. M., Rubinstein, L. J., Freeman, J. M., and Mathieson, G., *J. Neuropathol. Exp. Neurol.*, **29**, 524 (1970).
[23] Adams, C. W. M., and Davison, A. N., in *Neurohistochemistry* (edit. by Adams, C. W. M.), 332 (Elsevier, Amsterdam, 1965).
[24] Adams, C. W. M., and Leibowitz, S., in *Structure and Function of Nervous Tissue* (edit. by Bourne, G. H.), **3**, 309 (Academic Press, New York, 1969).
[25] Mickel, H. S., and Gilles, F. H., *Brain*, **93**, 337 (1970).
[26] Adams, C. W. M., *Research on Multiple Sclerosis* (Thomas, Springfield, Illinois, in the press).

Nature of the Nerve Lesion caused by a Pneumatic Tourniquet

THE use of a tourniquet around a limb may be followed by damage to peripheral nerves. This lesion has been investigated experimentally in cats[1,2] and the results suggested that in most cases the nerve damage was limited to the portion of the nerve under the tourniquet. Nerve fibres conducted normally distal to this level, with either a conduction block or a reduced conduction velocity at the site of the tourniquet. Histological studies showed localized demyelination under the tourniquet with preservation of axonal continuity. A similar pathological change is believed to occur in many forms of human pressure palsy, and the conclusion that the nerve damage is due to ischaemia has been widely accepted.

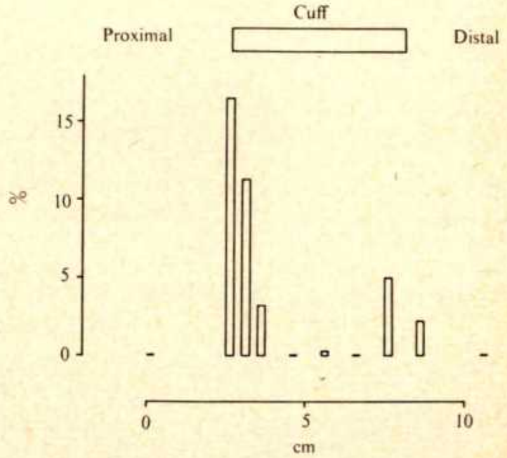


Fig. 1 Histogram to show proportion of abnormal fibres in transverse sections of medial popliteal nerve at different levels in relation to site of tourniquet (5 cm cuff inflated to 1,000 mm Hg for 75 min). Approximate position of cuff shown above. The specimen was taken 3 months after tourniquet. Nerve was fixed by perfusion with buffered glutaraldehyde and post-fixed with osmium tetroxide, 1 µm transverse sections were stained with toluidine blue, photographed, and counts made from enlarged prints[5]. Abnormal fibres (axon diameter 5 µm or greater, myelin thickness less than 1 µm) are expressed as a percentage of the large fibre population.

We have recently done some further experiments using a sphygmomanometer cuff inflated to 1,000 mm Hg around the hind limb of the baboon for 1–3 h. A brief preliminary account of the technique and of the resulting changes in nerve conduction has been published[3]. In the course of the experiments, it became clear that the role of direct pressure in producing nerve damage had been underestimated by previous workers. Two pieces of evidence for this can be cited.

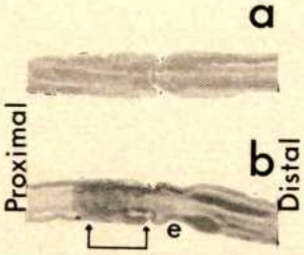


Fig. 2 Single fibres from medial popliteal nerve 1–4 days after tourniquet (5 cm cuff at 1,000 mm Hg for 90 min), to show different stages of invagination at nodes of Ranvier. Fibres were taken from a portion of nerve under the proximal edge of the cuff. Perfusion-fixation was carried out with buffered glutaraldehyde followed by post-fixation with osmium tetroxide; single fibres teased apart in 'Epon'. Symbol in *b* is as in Fig. 4.

(1) With the relatively wide (5 cm) cuff used, it has been found consistently that the nerve damage is maximal under the edges of the cuff or is restricted to these regions. This has been true of all stages of the pathological change in nerves studied for up to 6 months after the tourniquet had been applied. It has been demonstrated in single teased fibres and in transverse sections (Fig. 1). (2) The nature of the pathological change was best appreciated by examining single teased fibres by light and electron microscopy. In essence, the early change consisted of a dislocation of the nodes of Ranvier of the large-diameter myelinated fibres. The mildest form was a slight invagination of one paranode into the adjacent one (Figs. 2*a* and 3). Deeper invagination is illustrated in Figs. 2*b* and 4; it is accompanied by infolding of the myelin of the ensheathing paranode, with movement of the nodal axoplasm and axonal membrane away from the point of junction of the two Schwann cells. The dislocated nodal part of the axon could still be identified by the thickening of the axonal membrane and by the presence of characteristic transverse bands. The junction of adjacent Schwann cells was identified by their microvilli. If one assumes that the original site of the nodal axon was at the point of junction of the two Schwann cells, there must have been movement of the axon relative to the Schwann cell junction of approximately 30 µm, accompanying the invagination of the paranodal myelin.

A penetration of one paranode by another deeper than that shown in Fig. 4 may occur. In longitudinal sections of single fibres, it has not been unusual to see invagination and movement of the nodal axon which has proceeded for 100–200 µm. In such cases there is partial or complete rupture of myelin lamellae in the invaginated region.

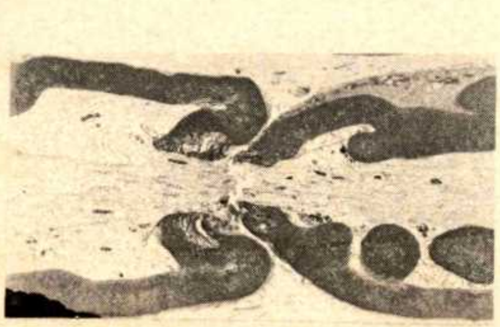


Fig. 3 Electron micrograph (LS) of nodal region shown in Fig. 2*a*.

All these changes are seen within the first few hours after the tourniquet and, by the end of the first week, invagination of myelin is beginning to be followed by demyelination. In our experience, the demyelination has usually been restricted to the region within 200 µm of the node of Ranvier. Demyelination of whole internodal segments has been rare. When teasing single nerve fibres, the striking feature of these lesions is that the changes described above have a clear polarity, the direction of invagination always being away from the cuff towards uncompressed tissue.

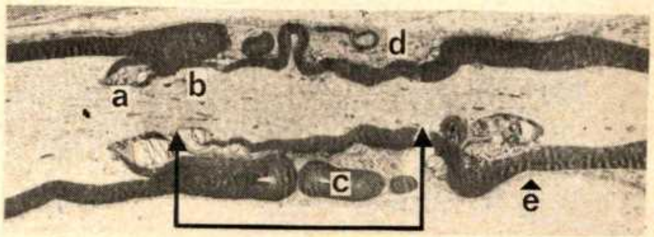


Fig. 4 Electron micrograph (LS) of nodal region shown in Fig. 2*b*. *a*, Terminal myelin loops of ensheathing paranode; *b*, terminal myelin loops of ensheathed paranode; *c*, myelin fold of ensheathing paranode cut tangentially; *d*, Schwann cell cytoplasm; *e*, microvilli indicating site of Schwann cell junction. Large arrows show length of ensheathed paranode (approximately 20 µm).

From the nature of the lesion, its polarity, and its distribution under the cuff, we therefore conclude that it is due to direct pressure and not to ischaemia. The principal evidence cited by previous authors against the possibility of direct pressure producing nerve damage is the experiment described by Grundfest[4], in which the pressure in an oxygenated chamber enclosing excised frog nerve was increased to approximately 1,000 atm before conduction was abolished. But this is a different situation from that which occurs when a pneumatic cuff is inflated around a limb. In the latter case there is a pressure gradient in the tissues between the portion under the cuff and that beyond its edge. Our results indicate that in these circumstances, longitudinal movement of the axon and its myelin occurs with respect to the Schwann cell. This change precedes demyelination.

This work was supported by the MRC and by the Muscular Dystrophy Group. J. O. is a Wellcome senior research fellow in clinical science.

J. OCHOA
G. DANTA
T. J. FOWLER
R. W. GILLIATT

Institute of Neurology,
Queen Square,
London WC1N 3BG

Received August 18, 1971.

[1] Denny-Brown, D., and Brenner, G., *Arch. Neurol. Psychiat.*, **51**, 1 (1944).
[2] Mayer, R. F., and Denny-Brown, D., *Neurology*, **14**, 714 (1964).
[3] Danta, G., Fowler, T. J., and Gilliatt, R. W., *J. Physiol.*, **215**, 50P (1971).
[4] Grundfest, H., *Cold Spring Harbor Symp. Quant. Biol.*, **4**, 179 (1936).
[5] Swallow, M., *J. Neurol. Neurosurg. Psychiat.*, **29**, 205 (1966).

Local Origin of Fibroblasts deduced from Sarcomas induced in Chimaeras by Implants of Pliable Disks

"THE origin of fibroblasts in healing wounds has been debated for over 100 years" according to Ross, Everett and Tyler[1], whose recent experiment indicated local connective tissue to be the source. Barnes and Khrushchov[2] had already concluded that in murine radiation-chimaeras precursor cells arose in bone marrow. We deduced from this that implants of pliable disks of adequate size, which give rise to local malignant growths (sarcoma), should in similar radiation-chimaeras produce sarcomas derived from the donor (myeloid) elements of the chimaeras and have examined such sarcomas induced by cellulose or silicone rubber. In the chimaeras the donated marrow was distinguishable from the host tissue by the presence in mitotic cells of *T6T6* chromosomes in one or other phase. The sarcomas were analysed cytologically; also, tumours arising in allogeneic chimaeras were tested genetically by their capacity for continued growth in mice of both pure strains. Both tests indicate that the tumours arose from the native host cells and not from the donated myeloid cells or their progeny.

The radiation-chimaeras used were of two kinds CBA/CBA-*T6T6* and CBA-*T6T6*/A produced as in a previous experiment[3].

Five to six weeks after the establishment of chimaerism, the mice were anaesthetized with ether. An incision was made in the mid-line of the shaved skin of the back and into subfascial pockets on each side a disk was inserted, the wound being closed with Michel clips.

In one series the disks were of 'Millipore R' filter, 25 mm diameter and 0.10 µm pore size. In the other series the disks were of silicone rubber (supplied by Dow Corning Center for Aid to Medical Research, Midland, Michigan)—2 cm in diameter and 1 mm thick[4]. Tumours once diagnosed were allowed to grow to an estimated diameter greater than 0.5 cm. Then the mouse was injected with 'Colcemid' and killed 4–5 h later. A portion of the tumour was macerated to produce cells suitable for chromosome analysis[3]. In a few mice the tumour presented not as a solid mass but as a fluctuant cyst around a rubber disk. At necropsy the cyst was aspirated of its bloodstained fluid which was then processed as a cell suspension. In each case a tumorous thickening of part of the cyst wall was identified.

Portions of solid tumour about 1 mm^3 were passed by means of a Bashford needle (or cyst fluid by syringe and needle) to suitably compatible mice. Thus tumours in syngeneic chimaeras were passed to (CBA × CBA-*T6T6*) F_1 hybrids, where the single *T6* chromosome of dividing cells of the second host would distinguish cells of the second host from tumour cells, which should contain no *T6* chromosomes if the tumour arose from the natural cells of the primary host or two or more *T6* chromosomes if the tumour derived from the donated cells. Tumours in allogeneic chimaeras were analysed by passage to CBA, A and (CBA-*T6T6* × A) F_1 mice: tumours of host origin should grow in CBA and F_1 mice only, whereas those stemming from the donated bone marrow should grow in A and F_1 mice only. A single case of a subcutaneous sarcoma arising in a syngeneic chimaera of another experiment was analysed cytologically.

The fate of fifty-five chimaeras investigated is given in Table 1.

All tumours were analysed cytologically as of host origin (Table 2). In all but five a diagnosis was made on the primary tumour (or cyst fluid). In four of the five exceptions absence or paucity of mitoses in the primary tumour was the cause of doubt. Repetition on the first passage of the tumour confirmed that the primary tumour was of host origin. In the fifth animal (A390) a small tumour was diagnosed only at necropsy; passage was successful and the passaged tumour shown (M6251) to have arisen from the host tissue of the original chimaera.

All twenty-one tumours arising in allogeneic chimaeras behaved in the genetic test of passage as host type tumours, killing CBA and (CBA-*T6T6* × A) F_1 recipients but not A strain mice.

Table 1 Fate of Radiation Chimaeras with Implants

Implant	Type of chimaera	Early death	Killed—tumour	Late death—no tumour
Cellulose	Syngeneic	4	7	0
	Allogeneic	0	13	2
Silicone rubber	Syngeneic	0	11	3
	Allogeneic	2	8*	5

*One died undiagnosed.

Table 2 Sarcomas in Subcutaneous Tissue

Type of chimaera	Code No.	Origin of tumour	Confirmation on passage Code No.	Origin
(*a*) Induced by 'Millipore' filter				
Allogeneic	M5595	—	M5625	Host
Allogeneic	M5616	Host		
Syngeneic	M5617	? Host	M6115	Host
Allogeneic	M5618	Host		
Syngeneic	M5631	Host		
Allogeneic	M5633	Host		
Allogeneic	M5634	Host		
Syngeneic	M5639	Host		
Syngeneic	M5640	Host		
Allogeneic	M5642	Host		
Allogeneic	M5648	Host		
Allogeneic	M5650	Host		
Allogeneic	M5653	Host		
Allogeneic	M5660	Host		
Syngeneic	M5664	Host		
Allogeneic	M5668	Host		
Allogeneic	M5675	Host		
Syngeneic	M5676	Host		
Syngeneic	M5679	Host		
Allogeneic	M5691	Host		
(*b*) Induced by silicone rubber				
Allogeneic	M5638	—	M5649	Host
Syngeneic	M5692	Host		
Syngeneic	M5695	Host		
Syngeneic	M5696	Host		
Syngeneic	M5702	Host		
Syngeneic	M5728	Host		
Allogeneic	M5733	Host		
Allogeneic	M5767	Host		
Allogeneic	M5768	Host		
Syngeneic	M5785	Host		
Syngeneic	M5786	—	M5864	Host
Syngeneic	M5791	Host		
Syngeneic	M5795	Host		
Syngeneic	M5796	Host		
Allogeneic	M5805	Host		
Allogeneic	M5825	Host		
Syngeneic	M5865	Host		
Allogeneic	M5915	—	(M6106) (M6153)	Host Host
Allogeneic	A390	—	M6251	Host
(*c*) Spontaneous				
Syngeneic	M5579	? Host	M5623	Host

The one spontaneous sarcoma in a syngeneic chimaera was also typed cytologically as of the host.

The results indicate that sarcomas induced in the subcutaneous connective tissues of radiation chimaeras originate in cells of the host rather than in immigrant cells derived from the donated bone marrow. Similar results had been obtained for osteogenic connective tissue[3]: sarcomas induced in bone after injection of strontium-90 were also of host-type in spite of the immediate propinquity of donor-type bone marrow.

In this experiment none of forty tumours arose from the donated tissue. Long experience in this laboratory with chimaeras induced after this lethal dose of radiation (1,000 rads) to CBA mice indicates that little, if any, reversion of myeloid or lymphoid tissues occurs, so that virtually all leucocytes were of donor origin[5]. The deduction to be made, therefore, is that these tumours, most of which were of spindle cells (fibrosarcoma), arose from native connective tissue cells, not immigrant leucocytes.

Ross *et al.*[1] came to a similar conclusion for the origin of normal fibroblasts in healing wounds of skin. This was deduced from experiments in which ^{3}H-thymidine was given to one partner of an irradiated parabiotic pair of rats dependent for their marrow function and survival on the shielded femur of the animal that was later injected. Labelled leucocytes were found in skin wounds of both but labelled fibroblasts only in the animal exposed to ^{3}H-thymidine.

The thesis of Barnes and Khrushchov[2] on which the present experiment was planned rested on the observation that, after 5–7 days' duration of a glass disk *in situ*, the predominant cell attached to the glass had the general microscopic appearance of a young fibroblast. Ryan and Spector[6] apparently consider the cells on the glass to be a continuously maintained population of macrophages derived from blood monocytes, dividing after a latent interval of several days and departing. Spector[7] concludes that macrophages, though capable of change to epithelioid cells in tissue, do not convert to fibroblasts.

The controversy that has lasted for more than a century on the origin of the fibroblast may still not be resolved and proponents of both options may be right. We deduce from the present study and that of Ross *et al.*[1] that malignant and normal fibroblasts can be derived from local precursors in connective tissue, but this does not exclude that in appropriate circumstances fibroblasts may be derived from myelogenous cells.

We thank Dr Austin Brues for advice on materials for implantation and D. Stone, M. Bland and Mrs C. Smith for technical assistance.

D. W. H. BARNES
E. P. EVANS
J. F. LOUTIT

Medical Research Council
Radiobiology Unit,
Harwell,
Didcot,
Berkshire

Received May 17; revised July 9, 1971.

[1] Ross, R., Everett, N. B., and Tyler, Ruth, *J. Cell Biol.*, **44**, 645 (1970).
[2] Barnes, D. W. H., and Khrushchov, N. G., *Nature*, **218**, 599 (1968).
[3] Barnes, D. W. H., Carr, T. E. F., Evans, E. P., and Loutit, J. F., *Intern. J. Radiat. Biol.*, **18**, 531 (1970).
[4] Brues, A. M., Auerbach, H., De Roche, Georgia M., and Grube, D. D., *Argonne National Laboratory, Biological and Medical Research Division, Annual Report, ANL-7535* (1968).
[5] Micklem, H. S., and Loutit, J. F., *Tissue Grafting and Radiation*, Chapter 4 (Academic Press, New York, 1966).
[6] Ryan, G. B., and Spector, W. G., *Proc. Roy. Soc.*, B, **175**, 269 (1970).
[7] Spector, W. G., *Series Haematologica 3*, **2**, 132 (1970).

Induction of Zona Reaction in Golden Hamster Eggs by Cortical Granule Material

BRADEN *et al.*[1] have shown that the zona pellucida of mammalian eggs changes after fertilization so that penetration of further spermatozoa is greatly reduced. Austin and Braden[2] inferred that this zona reaction was due to the action of the cortical granule material which is released from the egg into the perivitelline space shortly after sperm entry into the egg cytoplasm. We wish to present experimental evidence to support this inference.

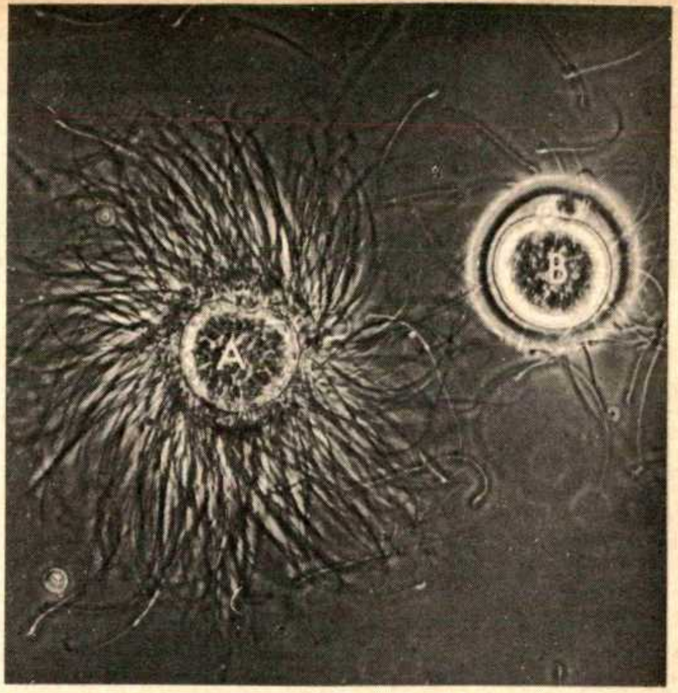


Fig. 1 Two eggs recovered from a hamster female 5 h after artificial insemination and incubated for 5 min with capacitated spermatozoa. One of the eggs (*A*) has many spermatozoa attached to the zona pellucida (negative zona reaction), while the other egg (*B*) does not have spermatozoa firmly attached to the zona (positive zona reaction) (× 450).

Adult female hamsters were induced to ovulate by injection of human chorionic gonadotropin on day 3 of the oestrous cycle[3], counting the day of the post-oestrous discharge[4] as day 1. Fourteen hours after the injection, they were artificially inseminated with cauda epididymal spermatozoa and killed at various times after insemination, from 5 to 72 h. Eggs were flushed from the oviducts with Tyrode solution and cumulus cells, if present, were removed by treatment with 0.1% bovine testicular hyaluronidase (300 USP units/mg, Nutritional Biochem. Corp.) in Tyrode solution. Cumulus-free eggs thus obtained were placed in 100 μl. of an incubation medium[5] under mineral oil in a sterile plastic dish. Spermatozoa used to inseminate these eggs were capacitated with detoxified human blood serum[6,7]. Five minutes after the eggs and spermatozoa had been mixed together, two types of eggs could be distinguished (Fig. 1). One type had a cloud of actively moving spermatozoa strongly attached itself to the surface of the zona pellucida. When these eggs were cleaned by pipetting them through a small bore pipette and examined with a phase contrast microscope, none contained a spermatozoon either in the perivitelline space or in the vitellus. Clearly no egg of this type had been penetrated by any spermatozoon before the time of recovery from the oviducts. In the other type of eggs, no spermatozoa were found firmly attached to the zona pellucida in spite of their continuous collisions with the zona surface. Examination of these eggs with a phase contrast microscope revealed that each had already been penetrated by a spermatozoon and cortical granule breakdown had taken place. When the two types of eggs were separated and incubated for 1 to 2 h with capacitated spermatozoa, the zona pellucida of already fertilized eggs was not penetrated by extra spermatozoa while unfertilized eggs became highly polyspermic. A series of observations (to be reported in full later) have indicated that the refractory state of the zona pellucida "zona reaction" develops less than 15 min after cortical granule breakdown before sperm chromatin dispersion can be seen. A similar strong zona reaction was observed throughout the cleavage stages, indicating that this is a permanent change of the zona pellucida.

If cortical granule material is responsible for the development of the zona reaction, it should be possible to induce this reaction by exposing the zona pellucida of an unfertilized egg to the cortical granule material released from fertilized eggs. The following experiments were designed to determine whether this was the case. For each experiment, 200–300 unfertilized eggs were flushed from the oviducts of females injected with human chorionic gonadotropin 14 h previously. After dispersing the cumulus oophorus cells with 0.1% bovine testicular hyaluronidase in Tyrode solution, the eggs were treated with 0.1% hog pancreatic trypsin (1–300, Nutritional Biochem. Corp.) in Tyrode solution containing 0.1% bovine serum albumin fraction V (Armour). Within 1 min the zona pellucida of all eggs was completely digested. After three thorough washings with Tyrode–albumin, the eggs were placed in 25–50 µl. of incubation medium[5], under mineral oil, and inseminated with capacitated spermatozoa suspended in 1–2 µl. of a sperm-capacitating medium (detoxified human serum). Ten minutes later (our preliminary observations had shown us that the cortical granules of such zona-free eggs break down completely with 2.5 to 5 min after insemination), the incubation medium was drawn into a capillary tube (1.4 mm inside diameter), one end of which was then sealed with flame. The tube was centrifuged at 7,000*g* for 10 min, causing eggs and spermatozoa to sediment at the bottom of the tube in a volume of approximately 1 µl. Supernatant solution (10–25 µl.) containing cortical granule material was placed under mineral oil in a dish (group *A*). Also, incubation medium with capacitated spermatozoa but no eggs (that is no cortical granule material) was centrifuged and 10–25 µl. of the supernatant were placed under mineral oil in another dish (group *B*). Seven to eighteen fresh unfertilized, cumulus-free eggs in 1–2 µl. of Tyrode solution were added to each drop Both groups of eggs were incubated for 30 min at 37° C, then inseminated with capacitated spermatozoa and incubated for an additional hour. Cumulus-free unfertilized eggs and capacitated spermatozoa were mixed concomitantly with 10–25 µl. of the supernatant solution containing cortical granule material and incubated under mineral oil for 1 h at 37° C (group *C*). At the end of the incubation, all eggs were examined with both a high power dissecting microscope and a phase contrast microscope for evidence of zona reaction.

The results (Table 1) showed that all eggs of group *A* displayed a characteristic zona reaction, while none of the eggs of groups *B* and *C* showed the reaction. The zona pellucida of every egg in these two groups (*B* and *C*) was heavily penetrated by spermatozoa.

Table 1 Effect of Cortical Granule Material in the Induction of the Zona Reaction

Group	Exp. No.	No. of eggs	No. of eggs with zona reaction	No. of eggs without zona reaction
A	1	15	15	0
	2	7	7	0
	3	15	15	0
B	1	10	0	10
	2	7	0	7
	3	18	0	18
C	1	9	0	9
	2	8	0	8
	3	18	0	18

The results reported here clearly demonstrate the zona reaction in hamster eggs and indicate that it is elicited by cortical granule material. The apparent effectiveness of this material in spite of great dilution suggests that the active agent is a very potent substance. The chemical nature of the active agent and the mode of its action on the zona pellucida must await further investigation.

This study was supported by grants from the Ford Foundation, the Population Council, the Rockefeller Foundation, and the US Public Health Service. C. B. thanks Professor Vincent J. DeFeo who made possible his appointment as a visiting professor at the University of Hawaii, and the Catholic University of Chile for leave of absence. We thank Mrs Cherrie A. Mahi for assistance in the preparation of the manuscript.

C. Barros[*]
R. Yanagimachi

Department of Anatomy,
University of Hawaii School of Medicine,
Honolulu, Hawaii 96822

Received May 27; revised June 26, 1971.

[*] Permanent address: Embryology Laboratory, Institute of Biological Sciences, Catholic University of Chile, Santiago, Chile.

[1] Braden, A. W. H., Austin, C. R., and David, H. A., *Austral. J. Biol. Sci.*, 7, 391 (1954).
[2] Austin, C. R., and Braden, A. W. H., *J. Exp. Biol.*, 33, 358 (1956).
[3] Yanagimachi, R., *J. Reprod. Fertil.*, 18, 275 (1969).
[4] Orsini, M. W., *Proc. Animal Care Panel.*, 11, 193 (1961).
[5] Biggers, J. D., Whitten, W. K., and Whittingham, D. G., in *Methods of Mammalian Embryology* (edit. by Daniel, J. C.) (Freeman, San Francisco, 1971).
[6] Barros, C., and Garavagno, A., *J. Reprod. Fertil.*, 22, 381 (1970).
[7] Yanagimachi, R., *Biol. Reprod.*, 3, 147 (1970).

Postnatal Malnutrition and Brain Cholinesterase in Rats

Severe malnutrition, especially protein–calorie restriction, early in the life of rats[1] and pigs[2] can cause behavioural abnormalities which persist long after nutritional rehabilitation. The physiological or biochemical basis for these abnormalities is not known, although long term, irreversible alterations in total DNA[3] and brain lipid content have been found to result from early malnutrition. A part of the brain which has been demonstrated to be involved in the control of behaviour is the central cholinergic mechanism[5], and changes in brain acetylcholinesterase (AChE) and cholinesterase (ChE) have been reported to be correlated with behavioural changes in animals subjected to different environmental situations[6]. Furthermore, Sereni *et al.* found that undernutrition during the first 3 weeks of life decreased the activity of AChE in the brains of rats until they were 2 weeks old. We have now investigated the effects of early protein–calorie restriction on the activity of ChE in the brain.

Pregnant Holtzman rats were fed a purified diet containing 25% casein until the birth of their young. Eight newborn male rats were assigned to each litter and during suckling, lactating dams were divided into an experimental group fed a low protein diet (12% casein) to decrease milk production, and a control group fed on optimum protein diet (25% casein). After a 3 week nursing period, rats on the low milk intake were weaned to a low protein diet (3% casein) for the next 4 weeks. The control group received the optimal protein diet. After the restriction period all animals were fed the optimal diet until they were 38 weeks old.

ChE, DNA and nitrogen in the brain were measured at 9 days, 3 weeks, 7 weeks and 39 weeks of age. The colorimetric method[8] was used for the ChE assay and nitrogen was determined by the microkjeldahl method. To determine DNA, homogenization of brain tissues followed by hydrolysis of tissue lipids were accomplished as described by Penn and Suwalski[9]. The nucleic acid extracts were obtained by Schneider's procedure[10]. Finally, a modified method developed by Burton[11] was used for colour development.

Table 1 shows that both specific and total ChE activity increased with age. The rate of increase was most marked during the first 7 weeks of life. At 9 days malnourished rats

Table 1 Brain Cholinesterase Activities of Malnourished Rats and of Rehabilitated Rats

Age (weeks)	Group	ChE/g wet brain weight (R* × 10⁶)	Specific activity ChE/100 mg N (R × 10⁶)	ChE/mg DNA (R × 10⁶)	Total activity ChE/brain (R × 10⁶)
1+ (9 days)	Control (8)	3.92±0.22	28.5±1.17	5.21±0.70	3.71±0.34
	Experimental (8)	4.20±0.17†	29.1±1.29	5.17±0.44	3.65±0.20
3	Control (9)	6.92±0.51	37.9±2.87	9.16±1.08	11.1±0.92
	Experimental (11)	6.61±0.91	37.1±5.62	8.28±1.61	8.43±1.32‡
7	Control (13)	9.51±0.35	43.0±1.40	10.5±0.91	19.0±1.12
	Experimental (10)	11.8±0.89‡	51.2±3.18‡	10.1±0.84	15.4±1.15‡
38	Control (5)	8.51±0.35	47.0±2.45	7.31±0.26	16.7±0.86
	Experimental (5)	9.76±0.53‡	50.7±3.04*	8.61±1.01*	17.7±1.32

The numbers of animals in each group are shown in parentheses.
All data are given as mean ± s.d.
* R is rate in moles substrate hydrolysed per minute.
† Significant difference ($P<0.05$) from control.
‡ Significant difference ($P<0.005$) from control.

had an increased specific activity of ChE (ChE/g wet brain weight), the mean difference from the control value being significant at the level of 0.05. When activity was expressed as either ChE/100 mg nitrogen or ChE/mg DNA, there were no differences between the two groups, which does not agree with the report[7] of lowered brain AChE activity (CμM/30 m/mgN) in undernourished rats at 6, 8 and 14 days, and no differences at 21 days. At 3 weeks of age the total ChE activity per brain in the malnourished rats was significantly lower ($P<0.005$) than that of control animals. There were no differences in the specific activity of the enzyme between the two groups, which agrees with Sereni's results on 21 day old rats[7]. On the other hand, Adlard *et al.*[12] reported that the brain AChE activity (per gram wet tissue weight) at 21 days was lower (11%) in rats undernourished during gestation and lactation.

At 7 weeks of age the total enzyme activity per brain in the malnourished group was still lower ($P<0.005$) than the control value. But the specific activity of the enzyme (ChE/g wet brain weight and ChE/100 mg nitrogen) was significantly increased ($P<0.005$) in the malnourished group. The specific activity expressed as ChE/mg DNA did not differ between the two groups. The increased specific activity of brain ChE in the 7 week old malnourished rats persisted after the animals had been given a normal diet for 31 weeks. The total enzyme activity per brain did not differ significantly between the two groups at 38 weeks. This is attributable to the smaller brain weights of the previously malnourished rats. Thus, severe protein–calorie deficiency in early life resulted in long lasting increases in the specific activity of brain ChE in rats.

It is interesting that these long lasting increases in brain ChE parallel the long term increases in the emotional reactivity observed in adult rats given identical nutritional treatment[1]. Moreover, the long term effects of early handling give similar results in that both emotional reactivity[13] and subcortical ChE[14] decrease.

This research was supported in part by funds provided through the State University of New York and by a Public Health research grant from the National Institute of Child Health and Human Development.

HAE SOOK IM
RICHARD H. BARNES
DAVID A. LEVITSKY

Graduate School of Nutrition,
Cornell University,
Ithaca, New York 14850

Received June 1; revised July 1, 1971.

[1] Levitsky, D. A., and Barnes, R. H., *Nature*, **225**, 468 (1970).
[2] Barnes, R. H., Moore, A. U., and Pond, W. G., *J. Nutrit.*, **100**, 149 (1970).
[3] Winick, M., and Nobel, A., *J. Nutrit.*, **89**, 300 (1966).
[4] Cully, W. J., and Lineberger, R. O., *J. Nutrit.*, **96**, 375 (1968).
[5] Russell, R. W., *Fed. Proc.*, **28**, 121 (1969).
[6] Rosensweig, M. R., Krech, D., Bennett, E. L., and Diamond, M. C., *J. Comp. Physiol. Psychol.*, **55**, 429 (1962).
[7] Sereni, F., Principi, N., Perletti, L., and Sereni, L. P., *Biol. Neonat.*, **10**, 254 (1966).
[8] Ellman, G. L., Courtney, K. D., Andres, jun., V., and Featherstone, R. M., *Biochem. Pharmacol.*, **7**, 88 (1961).
[9] Penn, N. W., and Suwalski, R., *Biochem. J.*, **115**, 563 (1969).
[10] Schneider, W. C., *J. Biol. Chem.*, **161**, 293 (1945).
[11] Burton, K., *Biochem. J.*, **62**, 315 (1956).
[12] Adlard, B. P. F., Dobbing, J., and Smart, J. L., *Biochem. J.*, **119**, 46P (1970).
[13] Denenberg, V. H., and Morton, J. R. C., *Animal Behav.*, **12**, 11 (1964).
[14] Tapp, J. T., and Markowitz, H., *Science*, **140**, 486 (1963).

Stereochemical and Vibrational Theories of Odour

ALTHOUGH many theories of odour specificity have been proposed, only two have been supported by quantitative measurements with statistical analysis. My stereochemical theory correlates the odours of various compounds with the sizes and shapes of their molecular models[1]. Wright's vibrational theory relates the odours to certain absorption peaks in their far infrared spectra[2]. It would be interesting to compare the relative success of our two theories directly, but so far this has not been possible, because we had used different test compounds and different statistical criteria.

Accordingly I have decided to apply my theory to the same set of compounds that Wright discussed. These are twenty-six homologues of benzaldehyde and nitrobenzene, originally prepared by Klouwen and Ruys[3], who reported their odours in qualitative terminology, for example, bitter almond, weak note of sassafras. As a basis for comparing our theories, however, it would be preferable to obtain quantitative measurements of the degree to which each compound resembles in odour a single standard, for example, benzaldehyde itself (which is the principal constituent of bitter almond oil).

For this purpose I used a panel of twenty-nine observers who were experienced in olfactory tests but unfamiliar with most of these compounds[4]. About 0.1 g of each odorant was presented in coded 10 ml. glass-stoppered Erlenmeyer flasks, for comparison with a standard flask containing 0.1 g benzaldehyde. The observers were instructed to ignore any differences in intensity, and to concentrate on odour quality. The scale of odour similarity runs from 0 for no similarity up to 8 for extreme similarity. The odorants were presented in daily batches of eight in randomized sequence, and each odour appeared twice. Hence the odour similarities given in the middle column of Table 1 represent the averages of more than fifty individual judgments. These odour similarity values constitute the experimental olfactory data which each theory must seek to explain.

The starting point for my stereochemical theory is a set of three orthogonal silhouette photographs of a scale model of the benzaldehyde molecule. Using the PAPA scanning computer[1], the molecular silhouette of each homologous compound was compared with the benzaldehyde silhouette as the standard. The centre of gravity of each molecular silhouette was taken as the datum point. The computer-generated scale of molecular shape resemblance runs from 120 for complete identity down to 90 or less for very dissimilar molecules. The detailed results are given at the right of Table 1.

Table 1 Olfactory and Stereochemical Similarities to Benzaldehyde

Compound	Odour similarity *	Molecular shape †
Benzaldehyde (standard)	(7.17) ‡	(120.6) ‡
o-Methylbenzaldehyde	3.79	109.0
m-Methylbenzaldehyde	4.35	106.8
p-Methylbenzaldehyde	3.62	108.7
o-Ethylbenzaldehyde	2.98	103.1
m-Ethylbenzaldehyde	1.34	101.6
p-Ethylbenzaldehyde	2.98	102.5
o-Isopropylbenzaldehyde	—§	102.7
m-Isopropylbenzaldehyde	1.54	101.0
p-Isopropylbenzaldehyde	1.44	100.3
o-tert. Butylbenzaldehyde	0.84	100.8
m-tert. Butylbenzaldehyde	1.40	97.5
p-tert. Butylbenzaldehyde	1.25	97.1
Nitrobenzene	6.40	112.5
o-Methylnitrobenzene	3.87	108.1
m-Methylnitrobenzene	5.76	105.2
p-Methylnitrobenzene	4.36	107.5
o-Ethylnitrobenzene	1.69	103.3
m-Ethylnitrobenzene	1.73	101.1
p-Ethylnitrobenzene	2.17	102.6
o-Isopropylnitrobenzene	1.20	101.5
m-Isopropylnitrobenzene	1.09	99.0
p-Isopropylnitrobenzene	1.59	100.5
o-tert. Butylnitrobenzene	0.81	98.8
m-tert. Butylnitrobenzene	1.31	99.7
p-tert. Butylnitrobenzene	1.27	98.3
Benzonitrile	6.29	112.0

* Similarity to odour of benzaldehyde.
† Resemblance to molecular silhouette of benzaldehyde.
‡ Omitted from statistical calculations.
§ Sample unobtainable.

These data are easier to comprehend in the form of a graph (Fig. 1). Clearly there is a strong correlation between odour quality and molecular shape. The more closely a compound resembles benzaldehyde in the shape of its molecule, the more similar it is to benzaldehyde in odour. (The only exception is *m*-methylnitrobenzene which smelled much closer to benzaldehyde than its molecular shape would indicate.) The pair of regression lines diverge mildly, indicating a relatively high correlation coefficient, $r = 0.90$. With $N = 25$ compounds, the probability was extremely low that this correlation could be fortuitous, $P \ll 0.001$. The molecular silhouette photographs were also run through the PAPA computer a second time, using the functional group (CHO or NO_2) of the molecular silhouette as datum point instead of the centre of gravity. The results were not quite so good, $r = 0.71$, but the probability level remained highly significant, $P < 0.001$. The experimental data evidently provide strong support for the stereochemical theory of odour.

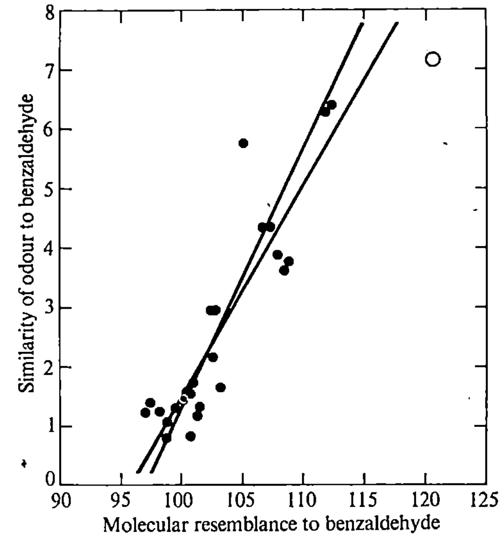


Fig. 1 The almond odour is correlated with molecular shape for 25 homologues of benzaldehyde and nitrobenzene. $r = 0.90$. (Two regression lines are shown because both variables are subject to experimental error; the open circle represents the benzaldehyde standard.)

Now it is the turn of the vibrational theory to explain the observed olfactory values for this series of compounds. In the study reported earlier[2], Wright and Robson did not have quantitative olfactory data available, so they treated the sixteen almond-like odorants as a group, contrasting them with an external control group of thirty-one miscellaneous compounds with other odours. Thus there is no basis on which to compare their χ^2 analysis with my regression analysis, even though their final probability levels appear much the same as mine.

If our two theories are to be realistically compared, it is necessary for Wright to devise a method for computing, from the infrared spectral frequencies, a quantitative measurement of how closely the spectrum of each compound resembles the spectrum of benzaldehyde itself, or some composite spectrum based on several compounds having an almond odour. This procedure would enable him to add his column of vibrational data to Table 1, and draw his graph corresponding with Fig. 1. Then, and only then, could we compare correlation coefficients, and obtain a direct measure of which theory fits the olfactory data more precisely.

I have developed one aspect among three quantitative comparisons of our theories that are in progress[5]. I thank Dr F. Rijkens of the Chemische Fabriek "Naarden" who supplied most of these compounds, and Drs R. H. Wright and J. R. Hughes for samples. Many of the aldehydes were freshly regenerated here from their semicarbazones by Dr Marvel-Dare Nutting. The measurements with the PAPA computer were furnished by Miss Silvana Vallerga at the University of Genoa.

J. E. Amoore

Western Regional Research Laboratory,
Agricultural Research Service,
US Department of Agriculture,
Albany, California

Received January 19; revised April 16, 1971.

[1] Amoore, J. E., Palmieri, G., and Wanke, E., *Nature*, **216**, 1084 (1967).
[2] Wright, R. H., and Robson, A., *Nature*, **222**, 290 (1969).
[3] Klouwen, M. H., and Ruys, A. H., *Parfum. Cosmét. Savons*, **6**, 6 (1963).
[4] Amoore, J. E., and Venstrom, D., *J. Food Sci.*, **31**, 118 (1966).
[5] Amoore, J. E., in *Gustation and Olfaction* (edit. by Ohloff, G., and Thomas, A. F.), 147 (Academic Press, London, 1971).

Evidence for Inhibition by Brain Serotonin of Mouse Killing Behaviour in Rats

THERE is some doubt about the role of brain serotonin in aggression. In mice made aggressive by prolonged isolation, there is a decrease in brain serotonin turnover[1], while increased aggressiveness has been noted in cats[2] and rats[3] given *p*-chlorophenylalanine (PCPA), which inhibits serotonin synthesis[4], although the experiments with cats[2] were not confirmed later[5]. Dominguez and Longo[6] reported that PCPA attenuates irritability and viciousness in rats with septal lesions, and Welch and Welch[7] observed decreased aggressiveness in isolated mice given PCPA. The explanation for these contradictory results may be that different models of aggression are not mediated through the same areas of the brain or by the same biochemical mechanisms.

We have therefore investigated the effect of modifying brain serotonin metabolism on mouse killing behaviour induced in isolated rats with or without lesions in the anterior olfactory area[8,9]. Two operations were performed on male Wistar rats (200–240 g, Morini). In the first (bulbectomy), the cortex of the olfactory bulb and the anterior part of the olfactory nucleus were removed on both sides of the brain. A glass cannula was inserted at a right angle to the lamina cribrosa of the ethmoid bone through a hole in the frontal bone. In the second operation (bulbotomy) both olfactory stalks were cut with a needle inserted by the same route as the cannula. Cuts were caudal to the olfactory nucleus, so that most of the rostral end of the olfactory tubercle was sectioned. Sham operations were performed by inserting a needle as in the other two cases but without sectioning the olfactory stalk.

After surgery, rats were isolated in cages (26 × 20 × 14.5 cm) in a regime of light from 0700 to 1900 h. Mouse killing activity was tested after 10–15 days by putting a white male mouse into the rat's home cage. We recorded the number of rats which killed their mouse within 1 h unless otherwise stated. All tests were made at 2000 h. Killing was minimal in the middle of the dark or light period.

Table 1 Induction by PCPA of Mouse Killing Behaviour in Intact and Olfactory Bulb-Lesioned Rats

	No. of mouse-killers/No. of animals treated				
Rats	Saline	*P**	PCPA	*P*†	*P**
Intact	6/50		18/40	<0.02	
Sham operated	2/25	ns	8/20	<0.02	ns
Bulbectomized	4/40	ns	34/40	<0.001	<0.01
Bulbotomized	28/40	<0.001	35/35	<0.001	<0.001

DL-PCPA methylester HCl was injected intraperitoneally as a single dose of 400 mg/kg, 36 h before tests for mouse killing behaviour.
Tests were performed at the tenth day of isolation.
For statistical analysis chi-square test was used.
* Compared with the correspondent intact group.
† Compared with the correspondent group injected with saline.

The behaviour of the sham operated rats did not differ significantly from that of untreated controls. After 10 days of isolation, about 10% of rats in both groups were killing mice (Table 1). PCPA increased the percentage of mouse killers to 45% and 50% in the control and in the sham operated group, respectively. Contrary to previous results[10], bilateral removal of the olfactory bulb by suction induced no muricidal behaviour. PCPA, however, caused 85% of these rats to kill mice.

Sectioning the olfactory stalk produced 70% of mouse killers and PCPA increased this to 100%. Moreover, treatment with PCPA always induced killing in rats which had not been affected by bulbotomy (Table 1).

In about 30% of these animals mouse killing induced by PCPA ceased within 10 days of the last administration of the drug; once established in the remaining animals it persisted indefinitely, but the killing response occurred after a longer latency. Since PCPA, in addition to its effect on serotonin, produces a small but significant depletion of brain catecholamines[4], we investigated the effect of selective inhibition of catecholamine synthesis on mouse killing. Ten non-killer, bulbectomized rats were treated with D,L-α-methyl-tyrosine, methyl ester (HCl 250 mg/kg given intraperitoneally) and tested for mouse killing behaviour at 6 h intervals for 24 h. None of these animals turned into mouse killers.

Table 2 Inhibition by 5-HTP and Pargyline of Mouse Killing Behaviour

	No. of killers*/No. of animals treated			
Treatment	Intact	*P*†	Bulbotomized	*P*†
Saline	38/38		25/25	
5-HTP	10/20	<0.001	16/28	<0.001
Pargyline	7/30	<0.001	6/28	<0.001

Drugs were injected intraperitoneally; L-5-HTP at a dose of 30 mg/kg 20 min before test; pargyline at a dose of 100 mg/kg 8 h before test.
* Animals who killed the mouse within 15 min from presentation.
† Compared with the correspondent saline-injected group.

Since these experiments suggested that mouse killing was potentiated by selective depletion of brain serotonin, we investigated the effect of increasing this substance by treatment with L-5-hydroxytryptophan (5-HTP), its immediate precursor, or parygyline, a monoamine oxidase inhibitor. 5-HTP (30 mg/kg) or pargyline (100 mg/kg) were administered intraperitoneally 20 min and 8 h respectively, before the test, to rats which had become killers as a result of isolation or bulbotomy.

As Table 2 shows, 15 min after the mouse had been presented, only 50% of the animals treated with 5-HTP and 23% of those treated with pargyline killed the mouse. Moreover, none of the pargyline-treated animals, which had killed the mouse 12 h after presentation, ate their victims, although before treatment they ate up part or all of the mouse within 1 h.

Our studies support the idea that inhibitory influences on the mouse killing by rats originate in the anterior olfactory area[9]. Our findings may differ from those of Vergnes and Karli[10] because they removed the olfactory bulb by inserting a suction cannula in a fronto-caudal direction. This could have removed a more extensive portion of the olfactory nucleus and/or part of the olfactory tubercle.

Since in our experiments bulbectomy eliminated all the olfactory cortex but only the anterior part of the olfactory nucleus, which in turn is eliminated after bulbotomy, these results suggest that the olfactory nucleus is involved in the inhibition of mouse killing. Some inhibitory influences, however, must originate within the olfactory bulb for PCPA produced many more killers among bulbectomized than sham operated animals.

The fact that either bulbectomy or PCPA alone induced mouse killing in only a percentage of animals, while the combination of the two was effective in 100% of cases, suggests that the two procedures act through different mechanisms. PCPA probably removes serotoninergic inhibitory influences. This idea is supported by the finding that mouse killing is antagonized or suppressed in animals whose brain serotonin has been increased by 5-HPT or pargyline.

The fact that PCPA potentiates mouse-killing behaviour in rats but antagonizes other types of aggressiveness, indicates that the former is not only behaviourally[11] but also biochemically different from the others.

We thank A. Frongia for examining the histological sections, G. Puddu for making statistics and G. L. Gessa for encouragement and excellent advice. Pargyline was a gift from Guy Everett (Abbott Laboratories).

G. DI CHIARA
R. CAMBA
P. F. SPANO

Institutes of Pharmacology and Criminal Anthropology,
University of Cagliari

Received March 22; revised June 21, 1971.

[1] Giacalone, E., Tansella, M., Valzelli, L., and Garattini, S., *Biochem. Pharmacol.*, **17**, 1315 (1968).
[2] Ferguson, J., Henriksen, S., Cohen, H., Mitchell, G., Barchas, J., and Dement, W., *Science*, **161**, 499 (1970).
[3] Sheard, M. H., *Brain Res.*, **15**, 524 (1969).
[4] Koe, B. K., and Weissman, A., *J. Pharmacol. Exp. Therap.*, **154**, 499 (1966).
[5] Zitrin, A., Beach, F. A., Barchas, J. D., and Dement, W. C., *Science*, **170**, 868 (1970).
[6] Dominguez, M., and Longo, V. G., *Physiol. Behav.*, **4**, 1031 (1969).
[7] Welch, A. S., and Welch, B. L., *Biochem. Pharmacol.*, **17**, 699 (1968).
[8] Vergnes, M., and Karli, P., *CR Soc. Biol.*, **163**, 2704 (1969).
[9] Vergnes, M., and Karli, P., *CR Soc. Biol.*, **159**, 972 (1965).
[10] Vergnes, M., and Karli, P., *CR Soc. Biol.*, **157**, 1061 (1963).
[11] Barnett, S. A., in *Aggressive Behaviour* (edit. by Garattini, S. E., and Sigg, E. B.), 3 (Excerpta Medica Foundation, Amsterdam, 1969).

Retraction and Expansion of the Dendritic Tree of Motor Neurones of Adult Rats induced *in vivo*

Cerf and Chacko[1] found that the number and diameter of motor neurone dendrites decreased after axotomy in the adult bullfrog. The purposes of the investigation reported here are to confirm and extend these observations in a mammal, to determine whether the dendritic changes are due to loss of axoplasm or loss of effective neuromuscular transmission, to see if dendrites recover after nerve regeneration has restored contact between the nerve and the originally innervated muscle mass, and if they recover after the regenerating nerve makes contact with different denervated muscle. Quantitative assessments of the dendritic spread of developing rat and human cortical neurones have been made by Eayrs and Goodhead[2], and Schadé and van Groenigen[3]. Schapiro and Vukovitch[4] found that the number of dendritic spines of rat cortical neurones could be influenced by variety of experience during development; Jones and Thomas[5] and Matthews and Powell[6] reported a decrease in the number and length of dendrites after deafferentation. Possible neuronal plasticity in the central nervous system of adult rats has been reported by Raisman[7].

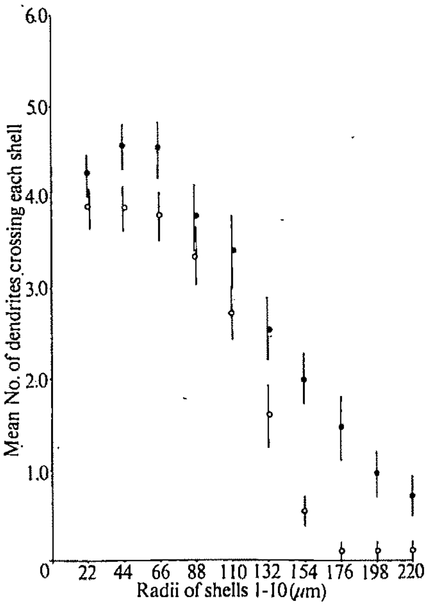


Fig. 1 The number of dendrites crossing concentric shells is expressed graphically against the radius of the shell. ●, Normal right side; ○, treated left side of a rat 6 weeks after botulinum toxin. Twenty neurones were measured on each side and the mean values are shown, with standard errors.

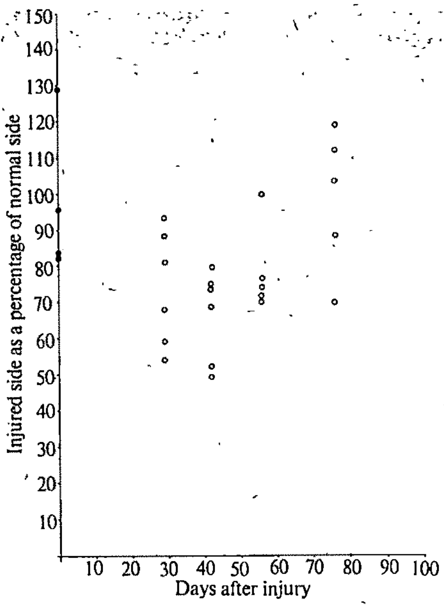


Fig. 2 Dendritic spread of hypoglossal neurones is expressed against the time since hypoglossal nerve transection; each point was obtained from twenty normal and twenty abnormal neurones of one rat. Values found between 4 weeks and 8 weeks after injury are significantly different from normal ($P<0.02$; >0.01). ●, Normal rats; ○, experimental rats.

Sholl's[8] "concentric shell" method was used to assess the dendritic fields of hypoglossal neurones in rat medullae prepared by the Golgi–Cox[8] method with 6 weeks' impregnation. Two hundred μm lateral ventricular nucleus transverse and coronal sections were used to measure the dendritic spread of twenty impregnated neurones from the left and from the right hypoglossal nucleus of each rat; the mean number of dendrites crossing each of the concentric shells was determined and expressed graphically against the radius of the shell. Fig. 1 shows mean values obtained from the normal right hypoglossal nucleus, and from the left hypoglossal nucleus 6 weeks after injecting botulinum toxin into the left side of the tongue (experimental series 2, see below). The smaller number of dendrites found passing through peripheral "shells" after injection suggests that terminal dendrites have retracted or degenerated, or failed to impregnate in these neurones. The mean dendritic spread of neurones of one hypoglossal nucleus was expressed by summing all the means from shell 2 to shell 10 (Fig. 1); this sum of means obtained from the nucleus containing the abnormal neurones was then calculated as a percentage of the sum of means obtained similarly from neurones of the opposite, unoperated, nucleus. Only in this way could variation between rats in impregnation by the Golgi–Cox method be overcome. No correction was made for planar projection of the dendrites which showed no preferred plane of radiation.

There were five experimental series of male rats aged 3 months, running in part concurrently. In the first, the left hypoglossal nerve was transected at the point where it crossed the carotid bifurcation[9], and regeneration proceeded; in the second, 250 pg of botulinum toxin type A was injected into the left half of the tongue[10] to prevent local neuromuscular transmission; in the third, the left hypoglossal nerve was transplanted into the normally innervated left sternomastoid[11]; in the fourth, the left hypoglossal nerve was transplanted into

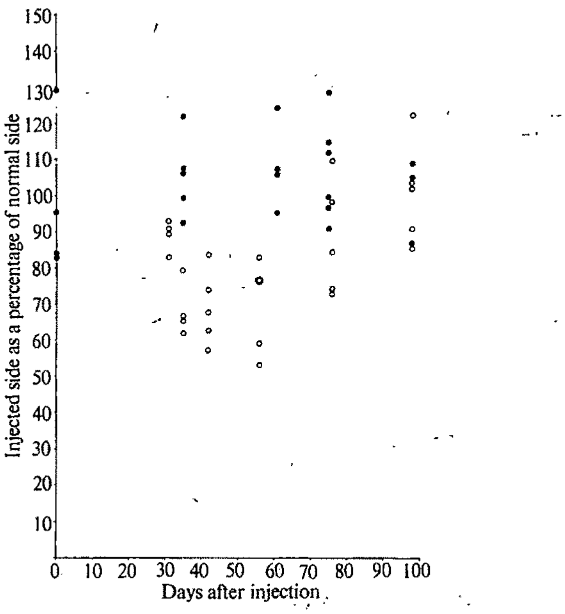


Fig. 3 Dendritic spread of hypoglossal neurones is expressed against the time since injection of botulinum toxin; each point was obtained from twenty normal and twenty abnormal neurones of one rat. ●, Normal rats, and those injected with toxin previously subjected to prolonged boiling; ○, rats injected with toxin. Values found between 5 weeks and 8 weeks after injection are significantly different from normal and control rats ($P<0.001$). No significant change is found in rats injected with boiled toxin.

the normally innervated left sternomastoid, and 70 days later the spinal accessory nerve innervating this muscle was transected and diverted[11], permitting the previously implanted hypoglossal nerve to innervate this "foreign" muscle; in the fifth, the procedure of the fourth series was followed, but the afferent nerves from the sternomastoid passing to the cervical plexus were transected at the same time as the spinal accessory.

Nerve injury (experimental series 1) caused a reversible decrease in dendritic spread (Fig. 2) closely resembling that found after botulinum toxin (experimental series 2) (Fig. 3). This suggests that the changes are not due to loss of axoplasm or to direct cell injury as such. The re-expansion of dendritic field found between the fifth and tenth weeks need not be due to re-establishment of functional contact between nerve and muscle but could be due to intrinsic late neuronal events; but, if regeneration is impeded by surrounding the transected nerve with normally innervated muscle (experimental series 3) no dendritic re-expansion occurs at this time (Fig. 4). The neurones of the implanted nerve remain quite capable of responding, however, for if this muscle is then denervated (experimental series 4), the dendritic fields grow out (Fig. 5). This late induced expansion is therefore independent of the initial loss of neuromuscular contact, but depends directly on its re-establishment and shows no specificity for the muscle originally innervated. Combined sensory and motor denervation of the recipient muscle (experimental series 5) did not influence the response obtained from motor denervation alone.

It therefore seems that dendritic retraction is due to loss of effective neuromuscular contact and that re-expansion is a direct consequence of re-establishment of such contact; failure of sensory denervation of the recipient muscle to influence the response obtained with muscle denervation alone does not show definitely that the effect is mediated to the perikaryon by the axon involved, for too many unaffected collateral sensory pathways exist which could convey information about sternomastoid contraction.

Preliminary results obtained from ultrastructural stereology indicate that the volume of hypoglossal dendrites changes in a manner closely corresponding to the dendritic field alterations reported here. It is therefore unlikely that the results indicate altered neuronal impregnation by the Golgi–Cox method in these circumstances.

The fate of preganglionic synaptic endings originally terminating on dendrites which are then caused to retract by axonal events, and the origin of synaptic endings appearing on re-expanded dendrites, are currently under investigation.

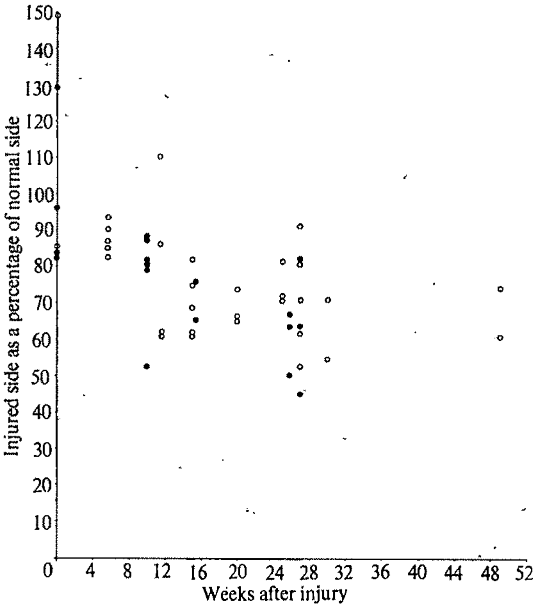


Fig. 4 Dendritic spread of hypoglossal neurones is expressed against the time since implanting the hypoglossal nerve into the normally innervated sternomastoid. ●, Transverse sections of medulla; ○, coronal sections of medulla. Note the failure of the dendritic tree to re-expand after the sixth week. The retraction is statistically significant ($P<0.02$; >0.01).

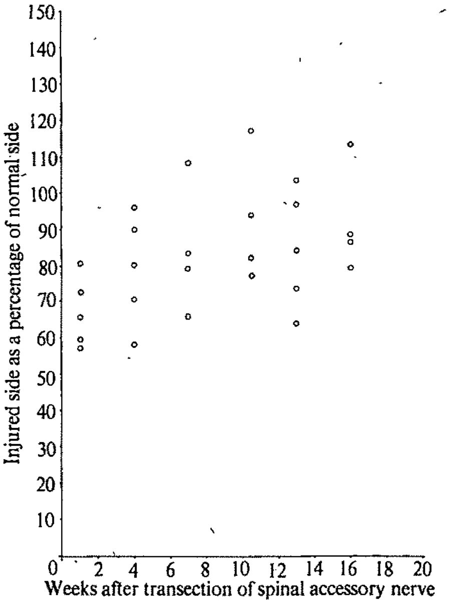


Fig. 5 Dendritic spread of hypoglossal neurones is expressed against the time since transection of the spinal accessory nerve 10 weeks after implanting the hypoglossal nerve in the sternomastoid. Zero time therefore corresponds to the 10 week point on Fig. 4. The dendritic field re-expands, and the points between 7 and 16 weeks after transection of the spinal accessory nerve are significantly different from those in the comparable period in Fig. 4, where the spinal accessory was not transected ($P<0.01$; >0.001).

We thank Mrs K. Grant and Mrs J. Anderson for technical assistance, and Dr Keppie for botulinum toxin; financial support was provided by the National Fund for Research into Poliomyelitis and other Crippling Diseases, and by the Medical Research Council.

B. E. H. SUMNER
W. E. WATSON

Department of Physiology,
University Medical School,
Teviot Place,
Edinburgh EH8 9AG

Received July 1, 1971.

[1] Cerf, J. A., and Chacko, L. W., *J. Comp. Neurol.*, **109**, 205 (1958).
[2] Eayrs, J. T., and Goodhead, B., *J. Anat.*, **93**, 385 (1959).
[3] Schadé, J. P., and van Groenigen, W. B., *Acta Anat.*, **47**, 74 (1961).
[4] Schapiro, S., and Vukovitch, K. A., *Science*, **167**, 292 (1970).
[5] Jones, W. H., and Thomas, D. B., *J. Anat.*, **96**, 375 (1962).
[6] Matthews, M. R., and Powell, T. P. S., *J. Anat.*, **96**, 89 (1962).
[7] Raisman, G., *Brain Res.*, **14**, 25 (1969).
[8] Sholl, D. A., *J. Anat.*, **87**, 387 (1953).
[9] Watson, W. E., *J. Physiol.*, **180**, 741 (1965).
[10] Watson, W. E., *J. Physiol.*, **202**, 611 (1969).
[11] Watson, W. E., *J. Physiol.*, **210**, 321 (1970).

Fluid Balance in the Sahara

IT has often been said that the inhabitants of arid regions seem to require far less fluid than non-indigenous visitors[1], but opinions vary as to whether this discrepancy is attributable to ethnic differences in the inherent physiological mechanisms which are concerned with sweating and water metabolism, or to cultural-adaptive factors such as diet, clothing and mode of activity[2-4]. In an attempt to elucidate this problem, I and a Toureg nomad undertook a 500 mile journey in the Central Sahara, and I present here the observations which were made of our respective fluid requirements during this trek.

To ensure that the physiological observations were directly comparable, I chose clothing that was similar in style and colour to that of my companion and I followed an identical régime of activity. We were alike in age and physique and shared the same food supply. In this way external factors which might have affected water requirements had the same influence in both of us. In the following account I refer to myself as subject 1 and to the nomad as subject 2.

Subject 1 was a member of the 1970 British Air Mountain Expedition which carried out a multidisciplinary research programme in the southern Sahara. At the start of the journey he had already been in the desert for more than 2 months and was therefore fully acclimatized. At this time he was 35 yr old, weighed 73.25 kg and was 1.79 m tall. Subject 2 was a 38 yr old Toureg nomad of the Kel Etedale clan. Although measurements were not obtained, he was similar in height, weight, physique and body fat to subject 1.

The journey began in March 1970 in the village of Iferouane (latitude 19° 20′ N; longitude 8° 10′ E) and ended in the oasis of Tamanrasset (latitude 22° 50′ N; longitude 5° 20′ E), in the Ahagger Massif of southern Algeria. Water and food were carried on two camels and two others were ridden intermittently. The 500 mile trek was completed in 20 days at an average rate of 25 miles per day.

Snacks were taken at dawn and noon, with a more substantial meal after sunset. Both subjects shared a mixed diet of millet, dried cheese and British Army "compo" rations. Their average 24 h intake was 2,200 calories, composed of 280 g of carbohydrate, 80 g of protein and 85 g of fat. Salt was added liberally to the food. Subject 2 preferred millet to meat, and it is likely therefore that his protein intake was somewhat less than that of his companion.

The water camel carried four 32 pint plastic jerry cans and a goat skin which held 42 pints. Wells were encountered intermittently throughout the journey and water was only once in short supply. On this occasion (day 12) an isolated well was found to be blocked by a dead camel. This emergency was resolved by a 4 day forced march to the next water point.

To facilitate the accurate measurement of fluid intake, all water for drinking was decanted into the subject's personal water bottle. A record was kept of the number of bottles of water which were consumed in each 24 h from sunrise to sunrise The quantity of drinking water remaining in a bottle at the end of the 24 h was measured, and because the total volume of the bottles was known, the individual's fluid intake could be calculated.

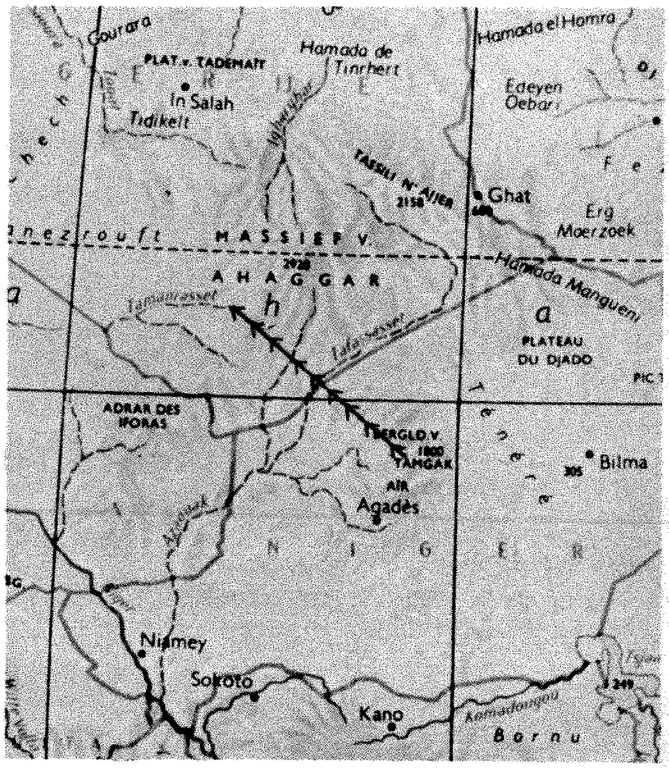


Fig. 1 The route that was followed from Iferouane in the Aïr to Tamanrasset in the Ahagger.

Apart from the 4 day period of water shortage, water was never rationed. The subjects were always able to drink their fill, according to the dictates of their thirst. It was noticeable that a considerable proportion of the water intake was drunk in the evenings when the day's march was over. The first morning specimen of urine was collected in a graduated plastic container and the volume and specific gravity were recorded. The Fantus test was carried out on this specimen to demonstrate the presence of urinary chloride. Because of the language barrier, attempts at collection of the total urine output met with incredulity and hilarity and this aspect of the investigation was therefore regretfully abandoned.

The daily fluid intakes are shown in Table 1, together with the early morning urine volumes and specific gravities. The distances covered and the noon air temperature and humidity, as measured by a whirling hygrometer, are also recorded.

The usual 24 h fluid requirements varied from 4 to 8 l. and were very similar in both subjects. The Toureg's intake was usually lower than that of subject 1, but only by a factor of a few per cent.

On day 9, which was largely spent in watering the camels and collecting fodder, the fluid intake was reduced. On days 13 14

Table 1 Daily Observations

Day	24 h fluid intake (l.)		Early morning urine Volume (ml.)		Early morning urine Specific gravity		Noon meteorological data			Estimated distance covered (km)
	Subject 1	Subject 2	Subject 1	Subject 2	Subject 1	Subject 2	Dry bulb °C	Wet bulb °C	Relative humidity	
1							32.4	18.1	23	22
2	4.5	4.1	520	490	1,012	1,008	31.8	16.7	19	58
3	5.3	5.0	410	500	1,008	1,010	32.0	16.9	24	62
4	4.6	4.8	670	610	1,020	1,008	32.0	16.9	24	58
5	6.1	5.6	610	670	1,012	1,012	32.3	17.2	19	68
6	6.2	5.9	470	520	1,014	1,012	32.9	17.0	29	57
7	6.1	5.8	250	670	1,022	1,008	33.4	17.1	16	66
8	8.0	7.2	480	610	1,008	1,010	37.0	17.3	9	56
9	3.1	3.0	600	420	1,008	1,012	36.1	16.8	9	26
10	7.9	7.6	560	890	1,010	1,004	37.0	17.1	9	61
11	7.7	7.8	610	390	1,014	1,012	39.1	19.4	12	58
12	7.9	7.5	490	410	1,012	1,008	40.2	19.4	14	66
13	4.0	4.0	470	610	1,012	1,008	40.9	19.8	11	62
14	2.2	2.2	210	190	1,028	1,030	39.8	19.4	11	87
15	—	—	180	160	1,038	1,036	39.7	19.2	11	83
16	13.6	15.1	—	—	—	—	35.9	17.0	10	20
17	9.1	7.8	340	700	1,016	1,012	36.2	17.1	16	56
18	7.7	7.0	640	540	1,008	1,010	35.1	17.1	12	59
19	6.4	6.4	510	610	1,012	1,012	32.8	17.2	18	62
20	4.5	4.1	570	620	1,008	1,014	32.2	17.2	19	47

and 15, because of the blocked well, the remaining water supplies were rationed and shared. The fluid intake on day 16, when the next water point was reached, was almost double that of the normal day. The subjects were probably still somewhat dehydrated on day 17, as their fluid intakes on this day remained high.

The urinary specific gravity observations and the morning urine volumes were within expected limits and indicated that the subjects were not in a state of chronic dehydration. Similarly, the tests for urinary chloride showed that they were never salt deficient.

Fig. 2 Toureg of the southern Sahara.

At an early stage of the journey it became obvious that the Toureg's mode of activity was optimal for life and survival in the desert. He avoided heat gain by spending the hottest part of the day in rest, by riding the camels during the moderately hot period and by walking during the relatively cool morning and evening. Similarly, his traditional dress was efficient in the prevention of heat gain from solar radiation and the facilitation of heat loss by convection.

The Toureg's diet was comparatively low in calories and moderately low in protein. A diet of this nature requires a relatively limited quantity of water for metabolism and it would prevent the accumulation of body fat and thus facilitate heat exchange. It must be stressed that this diet was eaten from choice rather than from any lack of food. In this context, it is of interest that the members of the Toureg race tend to be very tall, thin and angular in physique[5]. This somatic configuration facilitates heat loss from the body in a hot dry environment. (Compare the short fat Eskimo who is equally well adapted to the conservation of heat.)

Although water was frequently consumed during the heat of the day, a considerable proportion of the daily intake of fluid was drunk in the evening. This is in accordance with the observations of Adolph[6] and of Leithead and Pallister[7], who suggested that in a hot environment a water deficit of several litres could be accumulated, without noticeable effect on the thirst.

In my investigation the two subjects were alike in age, height, weight and physique. During the journey their pattern of activity, diet and clothing were almost identical. For this reason all the external factors which might have influenced their water metabolism were directly comparable. Their 24 h fluid intakes were very similar; that of the Toureg was consistently minimally lower than that of the Englishman.

These findings suggest that the fundamental water requirements of a Toureg nomad and an acclimatized European are the same. Discrepancies which have been observed previously have probably been caused by cultural–adaptive factors rather than by any difference in basic physiological mechanisms.

I thank Colonel J. Adam, RAMC, for his support during the planning of the expedition, and Dr K. Collins and Professor C. H. Wyndham for guidance during the preparation of the manuscript. This work was financed by a grant from the Medical Research Council.

PETER BEIGHTON *

St Thomas's Hospital,
London SE1

Received May 13; revised June 24, 1971.

* Present address: Epidemiology Unit, Department of Orthopaedic Surgery, University of the Witwatersrand, Johannesburg.

[1] Kirmiz, J. P., *Adaption to Desert Environments*, 88 (Butterworths, London, 1962).
[2] Kuno, Y., *Human Perspiration*, 416 (Thomas, Springfield, Illinois, 1956).
[3] Edholm, O. G., and Bacharach, A. L., *Exploration Medicine*, 211 (Wright, Bristol, 1965).
[4] Wyndham, C. H., *et al.*, *J. Appl. Physiol.*, **19**, 885 (1964).
[5] Lhote, H., *Le Saraha-désert Mystérieux*, 127 (Bourrelier, Paris, 1937).
[6] Adolph, E. F., in *Physiology of Heat Regulation*, 330 (W. B. Saunders, Philadelphia, 1949).
[7] Leithead, C. L., and Lind, A. R., *Heat Stress and Heat Disorders*, 143 (Cassell, London, 1964).

Experimental Study of Mimicry in Salamanders involving *Notophthalmus viridescens viridescens* and *Pseudotriton ruber schencki*

THE aposematically coloured eft stage of the red spotted newt, *Notophthalmus viridescens viridescens*, is both toxic and noxious to potential predators. The black chinned red salamander, *Pseudotriton ruber schencki*, is the most common red-orange salamander other than eft *N. v. viridescens* in the vicinity of the Highlands Biological Station in North Carolina. This study provides experimental evidence that *P. r. schencki* is a Batesian mimic of *N. v. viridescens*.

Because wild potential avian predators were found to be partially preconditioned to avoid both efts (model) and *P. r. schencki* (mimic), feeding experiments were carried out with domestic chickens to determine the behaviour of naive birds[1]. The chickens were maintained collectively in an outside enclosure but were individually isolated for testing. Each trial involved either a model or a mimic and a non-mimic salamander; this procedure is similar to that used by Brower[2]. Drab coloured, palatable salamanders of the genus *Desmognathus* (*D. monticola*, *D. quadramaculatus*) were used as controls (non-mimics). The feeding order of birds, the size of the salamanders, and the order of presentation of the controls were all randomized; two trials were made each day.

Seven chickens were divided into two experimental groups. The four chickens in group I were each offered ten models; after conditioning (consistent visual rejection of models), six mimics were offered to each chicken. The three chickens of group II were each offered six mimics before and after presentation of ten models. For both groups, the trials were discontinued for 7 days, after which mimics were again offered to each chicken. All the salamanders used in the trials were anaesthetized in separate solutions of 'Finquel' (tricaine methanesulfonate) to eliminate behavioural differences or similarities; they were offered to chickens dorsal side up in a shallow plastic dish. Each chicken was allowed 5 min to react to each salamander, and its response (characterized as: eaten, pecked but not eaten, and not touched) was measured to the nearest second.

The chickens in group I initially seized, but did not eat, the noxious model; rejection of models on sight, without contact, occurred after one to seven trials ($\bar{x}=5$). All the chickens in group I rejected all mimics after conditioning with efts (Table 1). Before conditioning with models the chickens in group II ate all mimics offered and seized, but did not eat, the first models presented; models were not touched after two to five trials ($\bar{x}=3.3$). After conditioning with ten efts, two chickens in group II rejected all mimics; one chicken ate all mimics (Table 1). The mean seizure times for the latter chicken were as follows: mimics before conditioning with models, 2.0 s; mimics after conditioning with models, 3.5 s; non-mimics, 1.3 s. When models were again offered to this chicken, one was pecked and five were not touched; when offered, mimics were again eaten. All non-mimics, *Desmognathus*, were eaten rapidly by all chickens, the mean seizure time ranging from 1.3 to 15.7 s for individual chickens in both groups. The mean seizure time for mimics eaten ranged from 2.0 to 22.2 s for group II. Analysis of variance revealed no significant differences in seizure times.

Table 1 Responses of Chickens to Model, Mimic and Non-mimic Salamanders

	Non-mimic	Model	Mimic before conditioning	Mimic after conditioning
Group I				
Eaten	83	1		0
Pecked, not eaten	0	13		1
Not touched	0	26		42
Group II				
Eaten	86	0	18	8*
Pecked, not eaten	0	11	0	1
Not touched	0	25	0	23

* All eaten by one chicken.

Statistically significant differences exist between number of mimics eaten after conditioning and number of non-mimics eaten during the same trials: group I, $\chi^2(3 \text{ d.f.})=43.0$, $P<0.005$; group II, $\chi^2(2 \text{ d.f.})=24.0$, $P<0.005$. There was no difference between the number of mimics and non-mimics eaten before conditioning ($\chi^2=0$). We conclude that the palatable mimic (*P. r. schencki*) derives a selective advantage over non-aposematically coloured salamanders due to their similarity in colour to the noxious model (*N. v. viridescens*), and that this relationship is clearly the result of Batesian mimicry.

We thank S. A. Gauthreaux, H. T. Coss, and M. Hare for their help. This work was supported by National Science Foundation grants to the Highland Biological Station, and a Clemson University FRC grant.

RONNIE R. HOWARD
EDMUND D. BRODIE, JUN.

Zoology Department,
Clemson University,
Clemson, South Carolina 29631

Received February 26; revised May 4, 1971.

[1] Howard, R. R., and Brodie, jun., E. D., *Amer. Zool.*, **10**, 475 (1970).
[2] Brower, J. V. Z., *Evolution*, **12**, 32 (1958).

Standing with a Bent Knee

I AM currently investigating knee forces by techniques similar to those already successfully used on the hip joint[1]. Although this work is not yet complete, it is difficult to reconcile Dr Roberts' article[2] with my observations and I should be glad of the opportunity to comment on this from an engineering viewpoint.

From the lever factors y/x in Fig. 3 of the article, the reader is given to understand that the equilibrium of the knee is produced by the force in vastus crureus (of the quadriceps group) acting directly about the joint centre. But this force acts on the patella which functions rather like a pulley attached to the femur and the equilibrium of the knee in the position shown must be due to the force in the patellar ligament. While the forces are similar in magnitude, they have different lever arms and the patellar ligament lever arm should surely replace y in the figure.

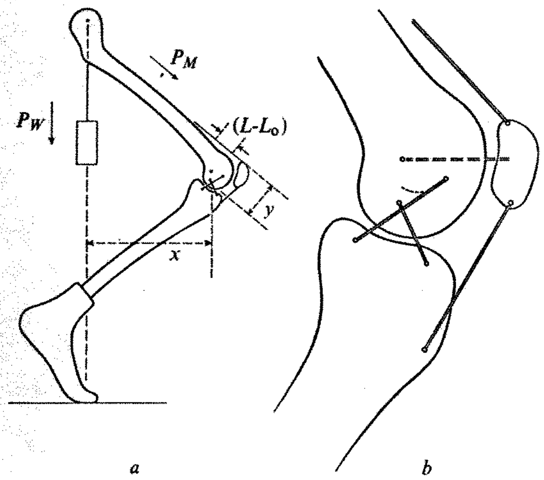


Fig. 1 *a*, Diagram of model leg to show how the offsets x and y were measured. *b*, Detail of the pivot points at the knee. The dotted line shows the bone profile round which the posterior ligament becomes wrapped in full flexion of the knee.

The model configuration shown in Fig. 2 (shown here as Fig. 1) is a most unusual position to adopt when standing on one leg. The superimposed body weight is shown acting through the hip so that the trunk must be approximately erect to bring the centre of gravity above the hip joint; the knee is flexed at right angles while standing on the tips of the toes. I have tried to assume this posture and invited several of my colleagues to do so with almost complete lack of success except among the young and athletic who, if they could do it, found the position most uncomfortable. The natural tendency when trying to assume this position on one leg is to incline the trunk forward, thereby moving the line of gravity toward the knee to reduce the force required from the knee extensors. A good example of this forward inclination of the trunk occurs in the common and similar activity of rising from a chair without assistance from the arms. Ciné film records, taken in the laboratories here, show that in extreme cases subjects bring their trunk practically horizontal when rising in this way.

Observation confirms that one-legged support is normally confined to smaller angles of knee flexion than that depicted. Yet, at very small angles of knee flexion, the line of gravity passes behind the joint, bringing the flexors into action and causing the conditions assumed by Dr Roberts to break down. It follows that the simple spring action postulated can only normally occur over quite a small range of knee angles. A further assumption implicit in the theory is that the centre of gravity will only move vertically but, in all normal activities, including balancing, horizontal movements also occur, since the centre of gravity is always free to move over a region equal to the support area of the foot. Such movements, which are likely to be as frequent as vertical movements, would require immediate compensation by the knee muscles, but would not of course affect the length of quadriceps so that spring action could be of no assistance.

In an activity such as walking, it has been known for many years that complex changes in the moment of the resultant force about the knee occur all the time[3]. The prop and paddle concept and the linear spring concept are surely an oversimplification of the real situation.

R. D. McLEISH

University of Manchester
Institute of Science and Technology,
PO Box 88,
Sackville Street,
*Manchester M*60 1*QD*

Received May 4, 1971.

[1] McLeish, R. D., and Charnley, J., *J. Biomech.*, **3**, 191, (1970).
[2] Roberts, J. D. M., *Nature*, **230**, 499 (1971).
[3] Bressler, B., and Frankel, J. P., *Trans. Amer. Soc. Mech. Eng.*, **72**, 27 (1950).

DR ROBERTS writes: In considering the lever factor, Dr McLeish looks at the force exerted by the muscle on the patella. I am looking at the forces on the femur. We are both neglecting the turning moment exerted by the compressive force between the patella and the end of the femur because the line of action of this force is offset by only a small distance from the axis of the joint. It is this force, however, that accounts for the difference between the actions of the muscle force above and below the patella.

The limb posture in Fig. 2 (shown here as Fig. 1) is less unusual than Dr McLeish maintains. The trunk does not have to be upright, if it is balanced by the free leg, neither does the line of the compressive force really have to be vertical, although in a model where a known force is to be applied at a point whose position is indeterminate it is convenient to use a hanging weight and thus to employ the vertical force of gravity.

Dr McLeish treats my article as though I had maintained that the resistance to compression is the only function to be served by the muscles acting at the knee. He thus takes no account of my treatment of the contribution of the two-joint muscles. Their action in exerting a turning moment at the hip is sufficient to account for all the situations that Dr McLeish adduces in refutation. My thesis, that it may be profitable for purposes of analysis to examine the prop and paddle actions separately, is not affected by his argument.

T. D. M. ROBERTS

Institute of Physiology,
The University,
*Glasgow W*2

Mechanism of Absorption of Phosphate from Soil by *Endogone* Mycorrhizas

ECTOTROPHIC mycorrhizas have often been shown to benefit the growth of their host plants, and similar results have also been found for endotrophic mycorrhizas formed by *Endogone* species[1,2] which are widespread on agricultural crops and are of considerable importance. Several workers have concluded that these growth responses are caused by an increased uptake of phosphate from the soils and possible mechanisms have been suggested[2] to account for this increased uptake. The purpose of our work was to distinguish between some of these possibilities, and we report preliminary results here.

A sandy loam soil of low phosphate status was labelled with ^{32}P and partially sterilized by γ-irradiation to eliminate indigenous fungi. Onions were grown in the soil in pots and half of these plants were inoculated with *Endogone mosseae* (yellow vacuolate spore type) by the method of Hayman and Mosse[4]. Onions were harvested on four occasions during a period of 4.5 weeks; at each time we measured dry and fresh weights and phosphorus (P) contents of root and shoot, count rate of plant tissue, total root length and the phosphate concentration in the soil solution. Infected plants were clearly larger after 2 weeks and had five times greater dry weight, and two times greater P percentage, than control plants at the final harvest. Infected plants were shown by microscopic examination to have endotrophic mycorrhizas and the hyphae extended into the soil around their roots.

The specific activities of P in the plants (corrected for P in the seed), were almost identical for mycorrhizal plants and controls at all harvests (Table 1). The specific activity of P in equilibrium with the soil solution was measured before, during and immediately after the end of the experiment. The mean of the three determinations, 35 c.p.m., is so close to the mean for the specific activity of P in the plant that it is likely that both groups of plants absorbed their phosphate from the soil solution or forms in rapid equilibrium with it. Neither roots nor hyphae seem therefore to solubilize non-labile inorganic phosphates or hydrolyse organic phosphates.

It is well known that phosphate ions have small diffusion coefficients in soils, and that there is likely to be a serious depletion of phosphate in solution immediately around absorbing roots[5,6]. The more actively the roots absorb, the greater this depletion will be. A root cannot, however, reduce the concentration of ions in solution at its surface below zero, and the rate of diffusion to such a cylindrical "zero sink" in the soil thus sets an upper limit to the uptake rate of a simple root. No process which occurs solely within the root can affect this.

Table 1 Specific Activity of Phosphate absorbed by Infected and Uninfected Onion Plants, Measured at Four Harvests

Plant age (days)	Infected plants	Uninfected plants
	c.p.m. × 10^8/mol P	
23	44	44
37	38	39
44	36	36
54	35	35

Table 2 Inflow of Phosphorus into Mycorrhizal (M) and Control (C) Onion Roots during Three Successive Growth Periods

Growth period	Duration (days)	Inflow mol/cm root/s × 10^{-14}	
		M	C
Harvest 1–2	14	17	5
Harvest 2–3	7	22	1.6
Harvest 3–4	10	13	4.2

The calculation of this maximum possible rate of uptake into a root system in a soil with defined properties is possible, but it is particularly difficult for phosphate. First, the diffusion coefficient for phosphate in the soil depends on concentration so that an average value has to be used[6]. Second, most phosphate diffusion occurs within a very short distance of the root, and it is uncertain whether the soil properties, such as water content and bulk density, are the same there as in the whole soil. Our estimate of the maximum inflow (uptake per unit length of root per second) is thus approximate, but is unlikely to be in error by more than a factor of 2. Using the simple model of Brewster and Tinker[7] with an average phosphate concentration in the soil solution of 5×10^{-6} M, an estimated mean diffusion coefficient of 3×10^{-9} cm^2 s^{-1} and an impedance factor of 0.3, we calculate it to be 3×10^{-14} mol/cm s^{-1}. The measured mean inflow to the control plant roots (Table 2) calculated by the formula in ref. 7, could thus be accounted for if all roots were active, but it is highly unlikely that the inflow to mycorrhizal roots could be transported by diffusion alone (Table 2). Because mass flow in the soil solution transported less than 1% of the absorbed phosphate, we conclude that entry into and subsequent transport through mycorrhizal hyphae occurred. Mechanisms which postulate a more active root cylinder cannot account for the results, because all the control plant roots were already functioning at near maximum efficiency. The hyphae may, of course, always produce extra absorbing surface, but the distribution of this surface with respect to the root is crucial—if it is immediately adjacent to the root surface, it merely competes with this for the diffusion-limited supply, but if it is dispersed away from the root, it is outside the root depletion zone, and its uptake is a net gain to the plant. This distinction may be particularly important in similar work on ectotrophic mycorrhizas, where the fungal sheath lies on the root surface.

These results thus support the simplest theory of the mycorrhizal effect, namely, that the hyphae form a better distributed surface for absorbing P from the soil solution than the root alone. If our results are confirmed with other crops and soils, they may be of value in deciding where *Endogone* infection can improve phosphate nutrition in crops. It is implicit in our explanation that this improvement should occur on soils where the maximum P inflow to a "zero sink" root is less than that required by the plant for maximum growth rate.

We thank Mr P. H. Nye, Professor J. H. Burnett, Professor J. L. Harley and Dr B. Mosse for discussion and advice, and Dr Mosse for samples of infected plant material. This work was financed by the Agricultural Research Council.

F. E. SANDERS
P. B. TINKER

Department of Agricultural Science,
University of Oxford,
Parks Road, Oxford OX1 3PF

Received June 20, 1971.

[1] Gerdemann, J. W., *Ann. Rev. Phytopathol.*, **6**, 397 (1968).
[2] Harley, J. L., *The Biology of Mycorrhiza* (Hill, London, 1969).
[3] Daft, M. J., and Nicolson, T. H., *New Phytol.*, **65**, 343 (1966).
[4] Hayman, D. S., and Mosse, B., *New Phytol.*, **70**, 19 (1971).
[5] Lewis, D. G., and Quirk, J. P., *Plant Soil*, **26**, 445 (1967).
[6] Drew, M. C., and Nye, P. H., *Plant Soil*, **33**, 545 (1970).
[7] Brewster, J. L., and Tinker, P. B., *Proc. Soil Sci. Soc. Amer.*, **34**, 421 (1970).

Rapid Leaf Closure of *Mimosa* in Response to Light

VARIOUS higher plants make rapid movements in response to stimuli[1–3], among them *Mimosa* which rapidly closes its leaves when touched. We wish to report a series of experiments demonstrating such fast closure in response to light alone. Usually, light causes opening of the leaves, although it is known that after a few hours in the dark, light (< 550 nm) will cause the petiole to fall rapidly[4,5].

Mimosa pudica was grown in soil from seed, raised in a greenhouse with a cycle of 16 h light and 8 h dark under natural and fluorescent lighting. When the plants were 3 months old, they were changed to a cycle of 14 h light and 10 h dark under incandescent lighting. Experiments were run when the plants were 4–9 months old. From each plant, leaves (with at least ten pinnule or leaflet pairs per leaf) were excised and floated in distilled water. This treatment produced results no different than those obtained for the entire plant. The leaves were illuminated for 30 min (to open the leaflets fully) with an incandescent (white) light source (45 mW/cm²/s) 35 cm from them. A water filter was used to absorb heat and in no case did the temperature of the air around the leaflets increase more than 0.1° C. Experiments were begun at least 3 h into, and finished more than 2 h before the end of, the photoperiod. After the 30 min opening period, a variety of light–dark presentations was used in an attempt to elicit a fast closing response of the leaflets. For each cycle of light and dark, twelve plants were chosen randomly as to the order they were to be run in each cycle. Two different leaves from each plant were used for each cycle tested. After the initial period of illumination the leaves were given dark periods from 1 to 20 min followed by a 10 min illumination from the same white light source. The treatments used and the results are presented in Table 1. A dark period of at least 5 min followed by light is necessary to produce rapid leaf closure, defined as movement of at least two of the pinnule pairs, initiated and completed within 3 s. The latency of closure is the time elapsed before the rapid movement begins. By Wilcoxon tests, there is no difference in latency among the D20, D10 and D7 treatments ($P > 0.05$, two tailed), but the D5 and D6 treatments have a significantly longer latency ($P < 0.01$, two tailed). This suggests that the response is becoming weaker as the dark period approaches D5; below this point, there seems to be no gradual increase in latency, but rather a sharp cutoff in the response. Because of variability in the sensitivity of leaflets, as Fondeville *et al.*[5] noticed, the exact point between closing and remaining open between D4 and D5 is difficult to determine. Those treatments that produced rapid closure could be repeated at least three times with rapid closure continuing and with no significant change in latency on the third presentation. But, when switched to D1, the leaves remained open during the light. Similarly, the D1 treatment followed by light would not produce closure even when repeated three times, but when switched to D10, rapid closure occurred during the light period.

Table 1 Response to White Light

Treatment (min) with dark (D)	Response (latency in s) to light of 10 min	
	First presentation	Third presentation
D20	Close (61)	Close (70)
D10	Close (54)	Close (49)
D7	Close (52)	Close (59)
D6	Close (79)	Close (77)
D5	Close (94)	Close (90)
D1, D2, D3, D4	Open	Open

Twenty-four leaves were used for each treatment.

To determine what wavelengths of light might be effective in producing this behaviour, interference filters were used with coloured filters and an incandescant light source to produce sharp peaks of light with known spectral characteristics. Two leaves from each of twelve plants were used again exactly as in the above experiment but only with a D10 treatment followed by light of 10 min duration. At an intensity of 0.3 mW/cm²/s, peak wavelengths of from 420–900 nm produced no closure. As the intensity (or energy level) was raised gradually to catch the peaks of the action spectrum, only certain wavelengths produced closure. At an intensity of 0.4 mW, peaks at 460–520 and 620–660 were the only wavelengths producing closure. Raising or lowering the intensity did not discriminate any further among these wavelengths.

We cannot say how these treatments produce rapid closure, which presumably involves changes in the permeability of the cell membrane of the motor cells in the pulvini with a subsequent change in turgor pressure[3]. It has been suggested, however, that pigments, phytochrome in particular, act, either directly by being bound to membranes or indirectly through some mediating substance, to control permeability change[6].

We thank Dr Ruth Satter for her assistance and encouragement.

PHILIP B. APPLEWHITE
FRANK T. GARDNER

Biology Department,
Yale University,
New Haven,
Connecticut 06520

Received February 28; revised April 28, 1971.

[1] Lloyd, F. E., *The Carnivorous Plants* (Ronald Press, New York, 1942).
[2] Ball, N. G., in *Physiology of Plant Growth and Development* (edit. by Wilkins, M.) (McGraw-Hill, New York, 1969).
[3] Sibaoka, T., *Ann. Rev. Plant Physiol.*, **20**, 165 (1969).
[4] Fondeville, J. C., Borthwick, H. A., and Hendricks, S. B., *Planta*, **69**, 357 (1966).
[5] Fondeville, J. C., Schneider, M. J., Borthwick, H. A., and Hendricks, S. B., *Planta*, **75**, 228 (1967).
[6] Galston, A., and Satter, R., *Rec. Adv. Phytochem.*, **4** (in the press).

A New Neandertal Child Mandible from an Upper Pleistocene Site in Southern Italy

A NEW mandible of a Neanderthal child was found by chance by Mr Adolfo Berdar on September 6, 1970, during the opening of a motorway at the Upper Pleistocene site at Archi, near Reggio Calabria, in Italy.

The geology of the site can be summarized as follows. The hill south-west of the town of Archi (Fig. 1), a remnant of one of the lowest terraces of the Aspromonte and cut by the valley of the River Torbido, consists of a sandy gravel series of Upper Pleistocene age. This terrace contains four complexes—*A*, *B*, *C* and *D* (see Fig. 2, I). Grey clays containing Pteropoda, *Dentalium*, *Nucula* and cyathoid corals from the higher Pliocene (*A*) are overlapped by a clinostratified sandy-gravel formation (*B*). The *B* formation which dips gently is younger than Calabrian and is probably Sicilian in age. It has abundant neritic malacological fauna (*Pectunculus*, *Modiola* cfr. *modiolus*, *Arctica islandica*, *Lucina glacialis*, *Cardium norvegicum*, and the like). A sharp unconformity separates this group from the overhanging fluvio-deltaic gravels and sands, the *C* series. Three subdivisions of *C* can be distinguished. First, horizontally layered coarser gravels from crystalline and metamorphic rocks (*C*-1), with iron oxidation on the cemented top. Second, "grey sands" (*C*-2), which consists of gently inclining lenses of sand and sandy gravels, some with ochraceous oxidation, with scattered marine shells (for example, *Spondilus gaederopus* and very rounded *Pectunculus* fragments, *Patella ferruginea*, *Anomya*, *Mytilus*). These may be remnants of reworked shells of the subjacent Sicilian, and of a destroyed Tyrrhenian (that is to say, *Strombus*) beach. In the higher levels there are frequent pumice fragments (*V*), which are evidence of volcanic activity in the Northern Sicilian area at that time. Third, after a crossed-bedding layer of fluvial origin, there is a layer of gravels and sand (*C*-3) 3–4 m deep, in which the human mandible was collected (*M*). Various other embedded bones of mammalian fauna were also found—*Paleoloxodon antiquus-italicus* of great size (for example, molar teeth, femur, vertebrae), *Dicerorhinus merckii*, *Bos* cfr. *primigenius*, *Cervus elaphus* of large size and *Megaceros* sp. Among the very scarce avifauna, a proximal extremity of tibia of the great auk, *Alca impennis* Linn. was found. Fossils of the great auk have also been discovered in the Mediterranean area at the Romanelli cave (Otranto)[1,2] and at Devil's Tower (Gibraltar)[3].

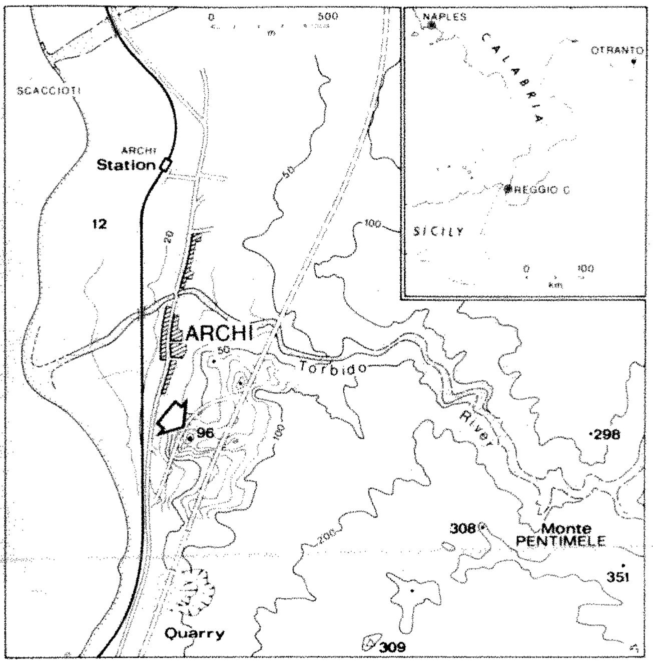


Fig. 1 The site near Reggio Calabria (height in m).

The Archi series continues with a very thin silty layer (*S*) containing freshwater and terrestrial molluscs (*Limnaea*, *Paludina*, *Bythinia*, *Anodonta* and *Buliminus*), and a sandstone with silicified wood and other badly conserved remnants of fossil flora. Next, there is a conglomerate which contains brackish oligohaline facies fauna (*P*), rich in *Tapes decussata* and *Cardium edule lamarki*, together with *Cardium papillosum*, *Solen*, *Venus* and Ostracoda (*Cyprideis*, *Xestoleberis*). The presence of this fauna suggests that there was a marine transgression which was followed by a rise of about 92 m in this part of the coast, so creating the terrace. The series ends with marls which contain traces of flora and gravels (*D*-1). An unconformity separates *D*-1 from the topmost "red gravel formation" (*D*-2), which also contains *Paleoloxodon antiquus-italicus*. A sizable cut and a deepening of the valleys resulted therefore from this considerable neotectonic rise, while an opposite movement of the same size took place on the side facing the sea.

This analysis of the Archi series has a bearing on the Pleistocene history of the Messina Strait area. A more recent origin must now be attributed to layers such as *C*, which have previously been regarded as probably Sicilian or even older[4-6]. Many radial valleys in the Aspromonte must now be considered to be very young. A considerable isostatic rise in the land must have occurred in the late Pleistocene with continuation in the Holocene and this must have measured more than 100 m in Calabria and north-east Sicily. As observed in Sicily[7], neotectonic movements in Southern Calabria were not uniform or contemporary. It seems that there were two important general changes in the palaeogeography of this region—between the Sicilian (marine, *B*) and the late Pleistocene (fluvial, deltaic and brackish facies, *C*), and between (*C*) and the uppermost continental (*D*).

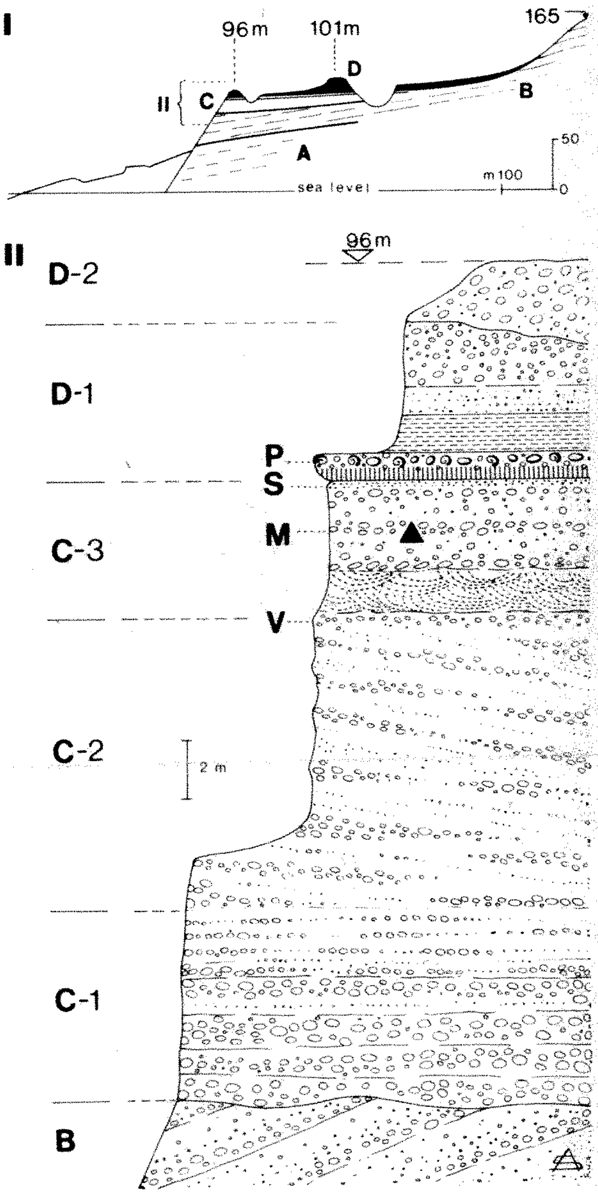


Fig. 2 Stratigraphy of the Archi Hill deposits—the 90–100 m terrace. I, Generalized section; II, the Upper Pleistocene series. Explanation in text.

The Neanderthal mandible reached our laboratories embedded in a hard concretion deposited by lime-impregnated water. When fully cleaned it was seen to be a highly mineralized and an incomplete child's mandible (Fig. 3). Both the ascending rami had been broken away long since by a fracture behind the alveolus containing the crown of the unerupted first permanent molar. All the deciduous incisors and the canine of the right side are missing, but the other deciduous teeth—the canine on the left side and all the molars—are in position. X-ray examination revealed the presence of the germs of all the permanent teeth from I_1 to M_1 which suggests that the mandible belonged to a child aged between 5 and 6.

The mandible is markedly massive and there is little doubt that it is male. The front of the jaw contains some interesting features (Fig. 3*b*). The chin definitely recedes, that is, the alveolar border projects and the basal margin falls back. But the slightly inward curve of the outline which corresponds to the alveolar part suggests an initial incurvatio mandibulae anterior. In the basal margin a low triangular elevation in the median line, the trigonum mentale, is visible and can be felt when the finger is passed over the bone. At the lower margin of the symphysis the trigonum continues into the interdigastric spine, so that it has a rhombic shape.

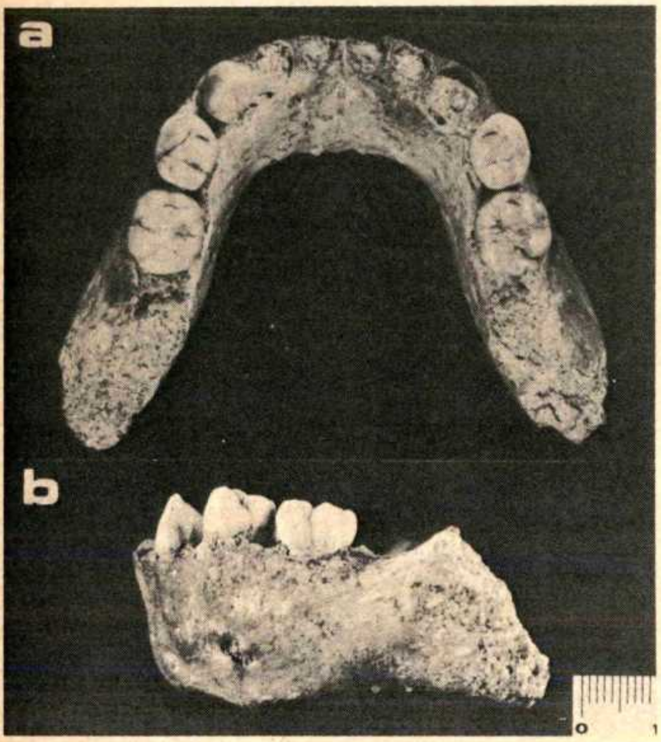


Fig. 3 *a*, Profile view (left side); *b*, occlusal view in alveolar plane orientation of the mandible.

The inner surface of the frontal part immediately below the alveolar border rises to a flat, smooth, diffuse swelling, the torus superior. Below this and without any break the muscular area is very clearly developed. It consists of a faint rough surface with two spine-like formations, one on each side. The spine on the left is long and narrow, whereas that on the right is very small. In the mid-line at the boundary between the torus superior and the muscular area there is one foramen supraspinosum.

The outline of both the inner and outer surface of the symphysis is given in Fig. 4*a*. The drawing was obtained using Sergi's diagraph and by making a cast of the jaw and cutting it at the required point. Measurements of the angle of symphysis (according to Weidenreich[8]), the angle of the chin (see McCown and Keith[9]) and the height and the index of the position of the foramen supraspinosum were taken from the drawing (Table 1).

On the lateral surface of the body of the mandible the foramen mentale is found opposite the first molar, about 10.2 mm to the right and 11.8 mm to the left of the alveolar border, and about 5.6 mm to the right and 6 mm to the left of the lower margin. The opening is of a long oval form, with an incipient septum between the front and back parts. The prominentia lateralis is well developed but, as would be expected in a child of between 5 and 6, it is found in the posterior alveolar part, that is to say, on the labial side of the place to be occupied by M_1.

On the inner surface of the body of the mandible there is no fossa sublingualis as a separate anterior part of the fossa subalveolaris beyond the linea mylohyoidea. The fossa subalveolaris itself ends at its lower boundary in an appreciable elevation which strengthens the inner surface of the lower margin of the jaw.

A drawing was made of the alveolar arch—the horseshoe-shaped area occupied by the alveoli—with the labial and lingual limits of the alveoli taken as boundaries. From this drawing the length and breadth of the arch and the mandibular index were measured (Table 1).

Table 1 Measurements of the Mandible (Linear Measurements in mm)

Height of the symphysis		22	
Height of the foramen supraspinosum		9	
Height of the body	20.7 (R)		20.9 (L)
Height of the body at M_2	18.4 (R)		19.2 (L)
Thickness of the body	11.8 (R)		12 (L)
Thickness of the body at M_2	13.4 (R)		13.7 (L)
Bimental width		40.6	
Width between buccal margins of M_2		50.1	
Width between lingual margins of M_2		34	
Length of the anterior alveolar arch		27.8	
Breadth of the anterior alveolar arch		44	
Index of robustness	57 (R)		57.4 (L)
Index of the foramen supraspinosum		40.9	
Index of the anterior alveolar arch		63.2	
Angle of the symphysis		65°.5	
Angle of the chin		81°	

R, right side; L, left side.

The crowns of the teeth are well preserved, and the cusps are only slightly abraded. The measurements are given in Table 2. The left canine has a strongly convex buccal surface bordered by a slight basal arch-like swelling which rises on each side. This basal structure can be considered a typical cingulum. The lingual surface is slightly concave and is bordered on all three sides by a thickened rim. The mesial side looks like that of an incisor. In the first milk molar the trigonid which has three cusps and both mesial and distal crests is markedly higher than the talonid. The talonid is an oval basin separated from the trigonid by the distal trigonid crest and the slope of the metaconid. The buccal surface is bordered at its base by a pronounced cingulum, but no definite tuberculum molare can be seen. In the second milk molar the trigonid is not higher than the talonid and is not retained in its entirety. In particular there is no longer any direct connexion between the protoconid and metaconid. The talonid is large and has some fine furrows in it which indicate the boundaries of the cusps and the presence of some wrinkles on the lingual side. X-rays of the teeth revealed a marked degree of taurodontism in the molars.

Table 2 Measurements (in mm) of the Crown of the Teeth

	C (L)	M_1 (R)	M_2 (R)	M_1 (L)	M_2 (L)
Mesio-distal length	7.2	9	10.6	9	10.4
Bucco-lingual breadth	6.6	7.7	9.3	7.4	9.2
Height	8.2	5.8	5.6	6	5.8
Robustness of the crowns	47.5	69.3	98.6	66.6	95.7

R, right side; L, left side.

A survey of all the anatomical features leads to the conclusion that this mandible possesses the primitive features found in Neandertals. A reliable comparison with other Neandertal specimens is difficult because so few are available, especially in this age group—there are only the mandibles of the Devil's Tower (Gibraltar) child[3] and of Skhūl I and Skhūl X[9].

The resemblance of this mandible with that of the Devil's Tower child is very striking in many respects—the angle of the symphysis (68°), the height of the symphysis (21 mm), the height of the body at M_2 (19 mm), the width between the buccal margins of M_2 (54 mm), the width between the lingual margins of M_2 (33 mm), and the length (27.5 mm), the breadth (46 mm) and the index of the anterior alveolar arch (59.8). The angle of the symphysis reported here for the Devil's Tower mandible was measured using the median section through the symphysis, according to Garrod *et al.*[3] (Fig. 4*b*). Moreover, it was assumed that this section was correctly oriented to the alveolar plane, and the position of the incision was deduced from the height of the symphysis (21 mm). The value obtained in this way does not correspond to that obtained by Weidenreich[8] (75.5°), who used a drawing of the section quite different from the original one. Finally, the angle of symphysis in our mandible is very close to that of the child of Ehringsdorf[10] (65°) according to Weidenreich[8].

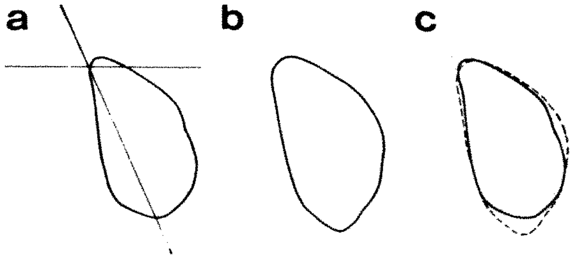


Fig. 4 Drawings in natural size of the section through the symphysis mentis in the mandible of Archi (*a*) and in that of Devil's Tower (*b*). Superimposition of the two drawings for comparison (*c*).

We thank Dr Laura Bonfiglio for assistance in fieldwork, and Mr Alessandro Benvenuti for technical assistance in cleaning the bone material. This work was supported by the National Research Council of Italy.

ANTONIO ASCENZI

Istituto di Anatomia Patologica,
University of Rome,
Ospedale Policlinico,
Rome

ALDO G. SEGRE

Istituto di Geologia,
University of Messina,
Messina

Received May 17, 1971.

[1] Blanc, G. A., *Arch. Antropol. Etnol.*, **50**, 1 (1920).
[2] Blanc, G. A., *Arch. Antropol. Etnol.*, **58**, 155, 365 (1928).
[3] Garrod, D. A. E., Dudley Buxton, L. H., Elliot Smith, G., and Bate, D. M. A., *J. Roy. Anthropol. Inst.*, **58**, 33 (1928).
[4] Gignoux, M., *Ann. Univ. Lyon*, NS, **36**, 77 (1913).
[5] Mantovani, P., *Boll. R. Com. Geol. Ital.*, **9**, 443 (1878).
[6] Malatesta, A., Motta, S., and Iacobacci, A., *Piano di Studi Sullo Stretto di Messina, 1: Ricerche Geologiche* (Regione Siciliana, Palermo, 1961).
[7] Trevisan, L., *Geol. Rdsch*, **43**, 207 (1955).
[8] Weidenreich, F., *Palaeont. Sin., Ser. D*, fasc. 3, 7, 1 (1936).
[9] McCown, T. D., and Keith, A., *The Stone Age of Mount Carmel: The Fossil Human Remains from the Levalloiso-mousterian*, **2** (Clarendon Press, Oxford, 1939).
[10] Virchow, H., *Die menschlichen Skelettreste aus dem Kämpfe'schen Bruch im Travertin von Ehringsdorf bei Weimar* (Fischer, Jena, 1920).

Submillimetre Wave Sensing of Nocturnal Moths

THE development of insects resistant to chemical pesticides has created a strong need for optical traps to control populations of economically important insects. But first it is necessary to understand their response to electromagnetic radiation. In 1968 preliminary tests indicated that pulsed coherent radiation of 337 µm wavelength might be attractive to insects. We have now made a behavioural and theoretical study of this phenomenon, using the fall army worm (*Spodoptera frugiperda*), the corn earworm (*Heliothis zea*), the Indian meal moth (*Plodia interpunctella*) and the codling moth (*Carpocapsa pomonella*).

Behavioural experiments were conducted using hydrogen cyanide and water vapour lasers providing signal at wavelengths of 28, 118, 311 and 337 µm and power levels between 16 and 50 mW to evaluate the frequency dependence of response. We used male *S. frugiperda* and *H. zea*, between 12 and 72 h old. All the tests were conducted during the period of peak nocturnal activity (8.00 p.m. to 1.00 a.m., EST). The experimental setup is illustrated in Fig. 1; the observation procedure has been described already[1]. The test chamber was a plastic cylinder of 4.5 inches in diameter and 80 inches long and most moths were at the end opposite the laser before the power was turned on. The number of moths attracted to the laser was counted as a function of time after the laser was switched on.

Precautions were taken to insure that the moths responded to the submillimetre wave radiation and not to any other stimulant. Experiments were conducted in a darkened room (21° C) and moths were counted using a red filtered flashlight. To reduce the visible and ultraviolet radiation emitted from the lasers, the ends of the test chamber were covered with black polyethylene with zero transmission in this region of the spectrum. The test cage was also covered with a black cloth to screen it from the visible radiation emitted from the laser plasma tube. To eliminate any doubt with regard to the test room bias the laser was detuned for 10 min and behaviour was observed before each experiment. (When the laser is detuned, the output power drops to zero while the visible and ultraviolet radiation remain at the same level.)

A strong attraction of both species to 337 and 311 µm radiation was observed, but, the response at 311 µm was less pronounced than at 337 µm (Fig. 2). *S. frugiperda* was not attracted to 28 or 118 µm radiation. The response of male *S. frugiperda* to an attractant light (7.5 W BL lamp) in similar environmental conditions is included for comparison. Chopping the radiation at a rate between 600 and 3,000 min^{-1} did not affect the response at 337 µm, but the attraction of *H. zea* to 118 µm increased slightly with a chopping rate of 2,400 min^{-1}. Further experiments indicate that the 337 µm radiation induces changes in the activity of the moths and reduces mating potential. Exposure of both species to 337 µm (50 mW) for 0.5 h inactivated them for the rest of the night although the experiment was conducted at the beginning of nocturnal activity period. The effect was more noticeable with *S. frugiperda*. Also, when fifteen virgin female *H. zea* were introduced in a cage containing

Table 1 Physical Dimensions and Propagation Characteristics of Nocturnal Moths

Moth species	D_{max} (µm)	D_{min} (µm)	D_{av} (µm)	*R*	λ_1 (µm)	λ_{op} (µm)
H. zea	216	109	167	0.705	266	328
S. frugiperda	210	116	160	0.8	220	347
C. pomonella	163	137	150	0.73	190	380
R. interpunctella	110	75	94	0.71	130	224

D_{max}, maximum outside diameter; D_{min}, minimum outside diameter; D_{av}, average diameter; *R*, ratio of inside to outside diameter; λ_1, cut-off wavelength of H_{01} mode; λ_{op}, optimum operating wavelength.

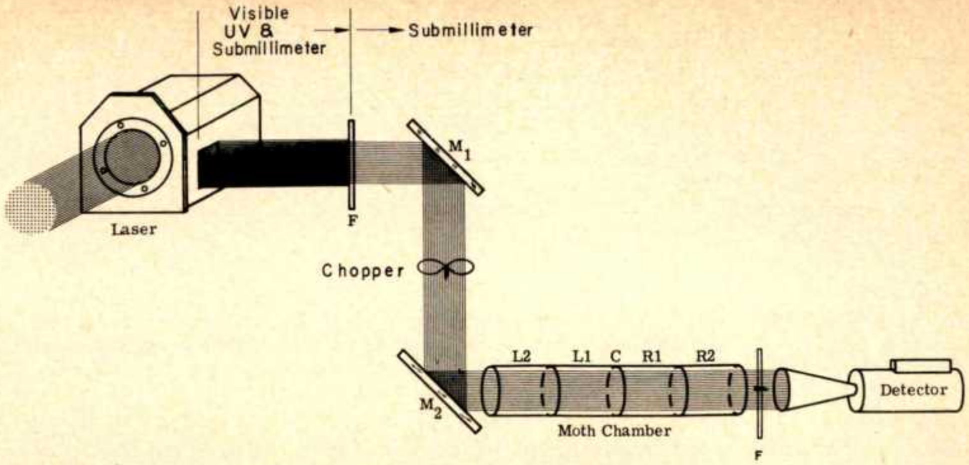


Fig. 1 Diagram showing laser and moth test chamber. M_1 and M_2 are mirrors and F is a polyethylene filter.

twenty-nine males, one each 5 min (10.30 p.m. to 12.30 a.m., EST) in the presence of 16 mW at 337 μm wavelength, fourteen out of them were attracted to the laser and no mating was observed. These effects may be similar to respiration changes induced by a BL attractant lamp[2].

Looking for a theoretical basis for these responses, we conducted experiments which show the failure of incoherent infrared sources[3], and coherent radiation from Klystrons (0.8 to 3.5 cm) and a Gallium Arsenide diode (0.85 μm) to elicit the response of the moths. This indicates the absence of electromagnetic thermal detectors in the moth system and suggests that moths respond to invisible radiation through tuned structures. Macroscopically the main spines of these moths' antennae are hollow thin walled tubes with tapered cross section and periodic variation in the outside diameter. This structure is suitable as a dielectric antenna coupling electromagnetic radiation between the moth and its environment.

An antenna would function properly if the radiation pattern was highly directive and the dielectric tube able to propagate electromagnetic waves over the useful bandwidth. Since pure E or H modes can be excited in dielectric tubes only in the case of perfect circular symmetry, it is reasonable to assume that only hybrid modes exist on moth antennae. Since all the modes except the HE_{11} mode are characterized by a cut-off wavelength[4] and the radiation pattern is highly directive when only one mode is excited; therefore dielectric tube antennae perform properly over the frequency band where only the HE_{11} mode can be propagated. (The subscript numbers indicate the number of half wavelengths which the interference pattern of the mode possesses in the plane of the tube cross section.)

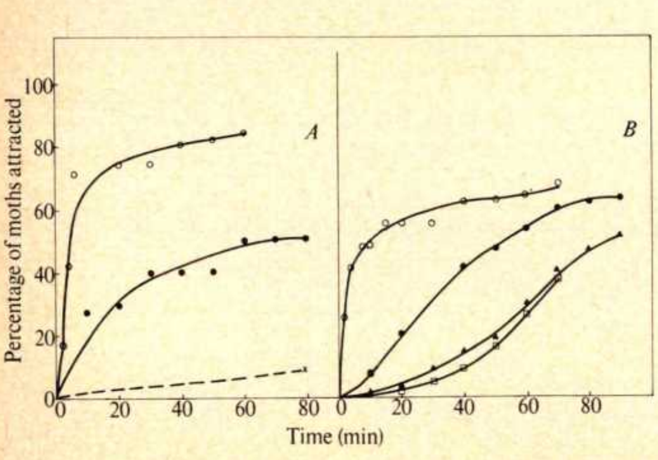


Fig. 2 Response of male *S. frugiperda* (*A*) and male *H. zea* (*B*) to electromagnetic radiation. The radiation wavelengths are 337 μm (○), 311 μm (●), 118 μm (▲) and 28 μm (□). The dashed line is the response to incoherent BL source. *S. frugiperda* did not respond at 118 and 28 μm.

The average of the pertinent physical dimensions of several moth antennae, together with their propagation characteristics, using a value of 2.5 for the relative dielectric constant[5], are listed in Table 1. The optimum wavelength λ_{op} is based on the criteria by Thourell[6], which compromise between antenna gain and directivity. Measured values of D_{min} were used to calculate the cut-off wavelength of H_{01} modes (lowest order cut-off mode). The previous discussion shows that electromagnetic radiation of wavelength λ_{op} could be efficiently coupled into the moths. As the wavelength deviates from λ_{op} the ability of the moths to respond to radiation drops gradually.

In conclusion, insects are strongly attracted to a submillimetre radiation source. Theoretical study on the moth antennae together with the frequency dependence of their response strongly suggests that the antennae may be the receptor through which insects are stimulated to respond. The response is very pronounced and has strong potential for optical traps to control the population of pest insects.

We thank Dr Kenneth Evenson and Mr Thomas Limperis for their help. This work was supported by US Department of Agriculture contract.

I. I. ELDUMIATI
W. C. LEVENGOOD

Biophysical Research Division,
Sensors Inc.,
303 *West Ann Street,*
Ann Arbor,
Michigan 48103

Received March 29; revised May 15, 1971.

[1] Levengood, W. C., *Mich. Academician*, **1**, 31 (1969).
[2] Levengood, W. C., *Z. Vergl. Physiol.*, **62**, 153 (1969).
[3] Levengood, W. C., and Eldumiati, I. I., *US Dept. Agric. Rep.* (1970).
[4] Kiely, D. G., *Dielectric Aerials*, 92 (Wiley, New York, 1953).
[5] Callahan, P. S., *Appl. Optics*, **7**, No. 8, 1425 (1968).
[6] Thourell, L., *The Antenna*, 176 (Chapman and Hall, London, 1960).

Lymphocyte Sensitization to Basic Protein of Brain in Malignant Neoplasia: Experiments with Serotonin and Related Compounds

IT has been found that blood lymphocytes from patients suffering from malignant neoplasia (even in an early stage) are sensitized to a basic protein[1] (encephalitogenic factor, EF) derived from human brain[2]. They react even more markedly (but to a strikingly similar degree) with a basic protein derived from a variety of human tumours[3]. During a visit to Dr Carnegie's laboratory some months ago one of us (E. J. F.) was shown the complementary structure of the serotonin molecule and the antigenic determinant (since published[4]) and realized that since the electrophoretic method[1] depends on interaction of EF with sensitized lymphocytes, structures complementary to the EF determinant might be able to mask it and so block the reaction.

In EF from human brain the main encephalitogenic determinant is associated with the sole tryptophan residue and the equivalent synthetic (eleven amino-acid) peptide[5] is known to be a potent inducer of experimental auto-immune encephalomyelitis[5,6]. Carnegie[4] further suggested that the encephalitogenic determinant might function as a receptor site for serotonin (5-hydroxytryptamine) and so be important in the pathogenesis of both experimental encephalitis and multiple sclerosis.

Here we examine the ability of serotonin and sterically related compounds, as well as other materials with well marked neuropharmacological activity, to block EF-lymphocyte interaction. The "goodness of fit" of these materials to the antigenic determinant has also been assessed.

From patients with cancer (lung, stomach, bowel, breast) 20 ml. of venous blood was removed and lymphocytes separated after preliminary defibrination as described elsewhere[7,8]. Sensitization of lymphocytes to EF was determined by the cytopherometric method and confirmed previous results. In principle the method depends on the interaction of sensitized lymphocytes with antigen (EF) resulting in the production of a material (dependent on protein synthesis by the lymphocytes[9]) with the property of slowing the electrophoretic migration of normal guinea-pig macrophages. The latter are thus used as a marker system for lymphocyte-antigen interaction. As before, the normal guinea-pig peritoneal exudate was subjected to 100 γ radiation to eliminate the potentiality of the 10–20% admixed guinea-pig lymphocytes to take part in a "mixed reaction" with the added human lymphocytes. Experimental details have been given elsewhere[7,8]. In brief, the lymphocytes to be tested were incubated together with the irradiated macrophages and antigen for 90 min and mobility of the macrophages measured (t_e). As control the mobility without antigen was estimated (t_c). When the blocking effect of a material was to be examined it was added to the cells and allowed to incubate at room temperature (20° C) for 30 min. The antigen was then added and after 90 min at room temperature the cytopherometric measurements on the macrophages were carried out. In the control experiments antigen was added first and after 1 h incubation the blocking agent added. After a further 30 min measurement of macrophage mobility was made.

Results were expressed as percentage increase in migration time. Thus if t_c = mean migration time of ten cells in each direction of the potential difference (twenty readings) over a given distance under control conditions (that is, lymphocytes + macrophages and no antigen), t_e = mean migration time with antigen added, then $\frac{t_e - t_c}{t_c} \times 100$ was calculated in each case. As in previous work[1,7,8] all specimens were numbered, scrambled and studied "blind". Results were later decoded.

In general EF produced about 15% reduction in migration rate of macrophages through interaction with lymphocytes from patients with malignant neoplasia[1]. A synthetic eleven amino-acid peptide of known encephalitogenic capacity[5,6] was also found to produce a similar reduction (Table 1). Thus the determinant on EF which reacts with lymphocytes from patients with malignant neoplasia seems to be antigenically similar to the encephalitogenic factor and should (if Carnegie's[4] speculation is justified) be susceptible to blocking by serotonin. While 10 μg/ml. of serotonin failed to influence the migration rate, 33 μg/ml. added before EF resulted in a 10% reduction (as compared with 17.2 when added after EF), and 18.2% when no serotonin at all was present. In later experiments 66 μg/ml. or 132 μg/ml. serotonin was used, the former producing 6.6% slowing and the latter 3.9%. A similar effect was produced by incubating 33 μg EF with 66 μg/ml. serotonin for 30 min at room temperature before adding to the cell mixture. In these conditions 3.4% reduction resulted. Because of the small scatter of the individual migration time readings, analysis showed that this was significantly different ($P < 0.001$) from the 6.6% obtained when serotonin was added at the beginning. In all subsequent experiments 132 μg/ml. serotonin was used and gave a reduction of below 4%, that is in the range obtained in normal (non-sensitized) subjects. Other neuropharmacologically-active or serotonin-related drugs were also tested. These included adrenaline, noradrenaline, acetylcholine, tryptamine, histamine, succinyl choline. None was found to be active in blocking EF-lymphocyte interaction in cancer patients. The protocol of typical experiments is shown in Table 2. It is apparent that serotonin alone has the capacity to block EF. Only D-lysergic acid diethylamide has a capacity to block partially—a result expected on grounds of steric arrangement of the molecule (as would be expected if we were dealing with a steric fit).

Table 1 Blocking Effect of Serotonin; Illustrative Protocols

	A	B	C
EF (*a*)	17.0	16.3	18.2
Serotonin + EF	3.9 (*b*)	1.1	10.0 (*c*); 18.6 (*d*)
EF + serotonin	17.3	16.4	17.2 (*c*); 18.6 (*d*)

	D		E
Synthetic peptide (3.3 μg/ml.)*	16.3	BP (33 μg/ml.)	16.1 ($P < 0.001$)
Serotonin + peptide	0	Serotonin + BP	10.4 ($P < 0.001$)
Peptide + serotonin	16.5	BP + serotonin	16.3

	F		G
PPD (33 μg/ml.)	—	BSA (33 μg/ml.)	13.6
Serotonin + PPD	14.0	Serotonin + BSA	13.8
PPD + serotonin	17.9 (*e*)	BSA + serotonin	13.1

	H
F1 (33 μg/ml.)	—
Serotonin + F1	17.4
F1 + serotonin	18.8

(*a*) 33 μg/ml. unless otherwise stated.
(*b*) 132 μg/ml. serotonin—the amount unless otherwise indicated.
(*c*) 33 μg/ml. serotonin instead of the normal 132 μg/ml.
(*d*) 10 μg/ml. serotonin instead of the usual 132 μg/ml.
(*e*) Both healthy and diseased subjects show PPD values between 15–20%.

Patient		Sex	Age	
A	B. A.	F	45	Carcinoma of the larynx
B	M. L.	F	62	Carcinoma of the breast
C	T. J. L.	M	59	Carcinoma of the bronchus
D	M. P. T.	F	50	Carcinoma of the bronchus
E	C. McA.	M	58	Carcinoma of the antrum
F	J. B.	M	29	Myasthenia gravis
G	W. F. A.	M	47	Sarcoidosis
H	P. A.	F	26	Normal

* Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg (SF 13a obtained through the courtesy of Dr F. C. Westall[4]).

Having established that serotonin could block the interaction of EF and sensitized lymphocytes in cancer we studied its blocking capacity with other antigens. To do this we took advantage of the fact that all subjects studied both in health and disease have lymphocytes sensitized to the F1 fraction (thyroglobulin) of thyroid gland[7] and that patients suffering from sarcoidosis are sensitized to a number of antigens including bovine serum albumin[10]. Attempts to block F1-lymphocytes inter-reaction by means of serotonin were

Table 2 Blocking of Antigens by Drugs other than Serotonin: Illustrative Protocols

	Patient E	Patient B	Patient D
EF	17.0	16.3	17.4; 16.3*
Acetylcholine	0.8	—	—
Acetylcholine+EF	16.0	16.1	16.9*
EF+acetylcholine	16.7	15.0	—
Histamine	0.4	—	—
Histamine+EF	16.3	—	16.3*
EF+histamine	16.9	—	—
Succinyl choline	0.0	—	—
Succinyl choline+EF	15.1	—	—
EF+succinyl choline	14.6	—	—
Noradrenaline	0.0	—	—
Noradrenaline+EF	18.2	15.7	—
EF+noradrenaline	17.8	15.1	—
Adrenaline	−0.2	—	—
Adrenaline+EF	17.6	—	—
EF+adrenaline	17.3	—	—
Tryptamine+EF	—	15.0	17.2*
EF+tryptamine	—	14.7	—
D-Lysergic acid diethylamide	—	—	7.8*

* Peptide (3.3 μg/ml.—see Table 1 note) substituted for EF (33 μg/ml.).

Patients E, B and D are those listed at foot of Table 1. All drugs were at a concentration of 132 μg/ml. except D-lysergic acid diethylamide 50 μg/ml.

unsuccessful. In a typical experiment serotonin (132 μg/ml.) followed by F1 gave 17.4%; and F1 followed by serotonin, 18.8%—results not significantly different. Similarly in a case of sarcoidosis BSA alone gave 13.6%; serotonin (132 μg/ml.) followed by BSA 13.8%; and BSA followed by serotonin 13.1%. It is thus apparent that serotonin has no capacity to block F1 or BSA interaction with lymphocytes. Its blocking effect is specific for the encephalitogenic determinant of EF.

Table 3 Illustrative Protocol of Blocking Experiment with Concanavalin

Patient	Antigen		Concanavalin* + antigen	Antigen + concanavalin
E.A. aged 10 erythema nodosum	EF	16.8	3.8	14.9
J.H. aged 57 sarcoidosis[9]	EF	17.9	—	—
	BP	16.4	1.3	16.5
	Muscle	14.1	2.1	—
	PPD	18.9	0.9	17.0
	Kveim reagent	13.5	1.0	—
	Ovalbumin	13.6	1.9	—
	BSA	13.7	1.9	—
	F1[6]† (thyroglobulin)	18.2	2.0	17.4

* 30 μg/ml.

† F1 values both in health and disease centre on 20%.

EF, BP, PPD, ovalbumin, bovine serum albumin and thyroglobulin were at a concentration of 33 μg/ml. Kveim reagent was from Public Health Labs, Colindale; 0.1 ml. was used in each test. A supernatant from centrifugation (250g, 10 min) of a 20% suspension of human muscle was prepared and 0.1 ml. used in each test.

Concanavalin was tested for blocking capacity. It was found to block all antigens both in neoplasia and sarcoidosis (Table 3) in the same way as antilymphocytic serum (ALS)[11]. This blocking activity is thus general and not specific. Both concanavalin and ALS differ from serotonin in that the blocking activity of the latter is limited to EF. It is known that a basic protein which may be extracted from human sciatic nerve (BP) has some antigenic determinant(s) in common with EF (made from human brain)[12–14]. Indeed in patients with malignant neoplasia (but with no evidence of either central or peripheral nervous system involvement) lymphocyte sensitization to BP is present though at a lower level than to EF[1]. On theoretical grounds it would be expected that serotonin might block BP less well than EF. This was found to be the case (Table 1). Whilst serotonin reduced the result with EF from 16.3% to 1.1%, with BP it was reduced from 16.3% to 10.4%. It may be supposed that the antigenic determinant centred on tryptophan may also be present in BP but modified sequence on either side may result in a less close fit.

Circulating antibody studies have indicated[15] that EF shared antigenic determinant(s) with purified protein derivative (PPD) of *M. tuberculosis*. Unpublished studies of lymphocytes from patients with severe active tuberculosis by the electrophoretic method have shown that these cells are sensitized to both PPD and to EF, confirming the original suggestion. The degree of sensitization to PPD (as shown by the percentage inhibition under the standard conditions of the cytopherometer test) always lay between 16 and 20% in all subjects examined. Serotonin was found to afford a partial block of PPD. In a typical experiment, for example, a subject giving 17.0% reduction with PPD, gave 17.9% if PPD was followed by serotonin (that is no change) but 14.0% if serotonin was added before PPD ($P<0.001$). There would thus appear to be a descending order of "goodness of fit"; serotonin blocking EF most effectively, BP less well and PPD least, whilst other unrelated antigens are not blocked at all. Both BP and PPD are blocked by ALS and by concanavalin, again indicating that the mode of action of these two materials is different from that of serotonin.

These experiments support the concept that the encephalitogenic determinant is a binding site for serotonin and open up possibilities of using molecules of known shape as potential blocking agents to study the conformation of receptive antigenic determinants. A further series of drugs related to serotonin is under test.

We thank Dr F. C. Westall for a sample of synthetic (eleven amino-acid) peptide, and our clinical colleagues for access to their patients. The cytopherometers used were purchased by grants from the North East Multiple Sclerosis Society and the Multiple Sclerosis Research Fund Limited. P. R. C. holds a research fellowship of the Multiple Sclerosis Society of Great Britain and Northern Ireland.

E. J. FIELD
E. A. CASPARY
P. R. CARNEGIE

MRC Demyelinating Diseases Unit,
Newcastle General Hospital,
Westgate Road,
Newcastle upon Tyne NE4 6BE

Received February 26, 1971.

[1] Field, E. J., and Caspary, E. A., *Lancet*, ii, 1337 (1970).
[2] Caspary, E. A., and Field, E. J., *Ann. NY Acad. Sci*, **122**, 182 (1965).
[3] Caspary, E. A., and Field, E. J., *Brit. Med. J.* (in the press).
[4] Carnegie, P. R., *Nature*, **229**, 25 (1971).
[5] Westall, F. C., Robinson, A. B., Caccam, J., Jackson, J., and Eylar, E. H., *Nature*, **229**, 22 (1971).
[6] Lennon, V. A., Wilks, A. V., and Carnegie, P. R., *J. Immunol.*, **105**, 1223 (1970).
[7] Field, E. J., Caspary, E. A., Hall, R., and Clark, F., *Lancet*, i, 1144 (1970).
[8] Field, E. J., and Caspary, E. A., *J. Clin. Pathol.*, **24**, 179 (1971).
[9] Caspary, E. A., *Nature*, **231**, 25 (1971).
[10] Caspary, E. A., and Field, E. J., *Brit. Med. J.*, **2**, 143 (1971).
[11] Caspary, E. A., Hughes, D., and Field, E. J., *Clin. Exp. Immunol.*, **7**, 395 (1970).
[12] Field, E. J., Ridley, A. R., and Caspary, E. A., *Brit. J. Exp. Pathol.*, **44**, 631 (1963).
[13] Caspary, E. A., and Field, E. J., *Europ. Neurol.*, **4**, 257 (1970).
[14] Bencina, B., Carnegie, P. R., McPherson, T. A., and Robson, G. S. M., *FEBS Lett.*, **4**, 9 (1969).
[15] Field, E. J., Caspary, E. A., and Ball, E. J., *Lancet*, ii, 11 (1963).

BOOK REVIEWS

Scientific Medicine

The Emergence of Scientific Medicine. By W. P. D. Wightman. (Contemporary Science Paperbacks No. 44.) Pp. 109. (Oliver and Boyd: Edinburgh, 1971.) 37½p.

ANYONE who has studied the reference literature of medical history will know how few books there are that really compel the reader to think beyond the commonplace. Dr Wightman's book belongs to this small group. Its text, based on lectures given over many years to medical undergraduates at Aberdeen, is dense and compressed with not a wasted word. In these days of verbosity it is to be welcomed for that reason alone.

More than anything, Dr Wightman dislikes the kind of prejudices that spring from perverted logic or ignorance of history. One of his particular hates is the fallacy that the Church impeded the progress of science and medicine in the Middle Ages and he quite obviously takes the greatest pleasure in setting the record straight, following the lead set by Dr Talbot in his *Medicine in Mediaeval England* (1967). He also delights in shaking us out of our arrogance, as for instance when he claims that a belief in the procedures of temple incubation is not *ipso facto* irrational "provided that . . . the opinion . . . as to right action . . . is based on a conscious relation of ideas".

His chief concern is with plotting the course of non-magical attitudes to the body, from Greek times to the seventeenth century. This he does by interposing his own comments between cleverly chosen extracts from the works of a dozen or so seminal writers. Here he explains their apparent contradictions; there he marks out the line that divides true originality from high competence. No hero worshipper, he gives credit to Aristotle for "imposing system upon the flux of experience" and yet recognizes, in respect of logic, the philosopher's debt to his teachers and predecessors; while Galen is praised not only for his experimental genius but in addition for the persuasive consistency of his humoral theory. In a sense, as Wightman points out, the latter can be said to prefigure the modern theory of endocrine balance.

As for Harvey, even he, in his Aristotelean view of the blood, is shown to be a product and disciple of tradition; whereas that sworn enemy of the orthodox, the "God-intoxicated ranter" Paracelsus, emerges almost as a blood brother of Francis Bacon, at least insofar as both men followed similar methodical lines of thought. Again, Fracastoro was a scientist in the modern mould, not so much because he formulated a contagion theory as because of the "critical manner in which he confronted theory with facts".

This short book should be read twice over, and not only by newcomers to the subject. It helps us to glimpse some of the conceptual difficulties that faced our forebears and it arouses admiration for the experimental methods evolved by men such as Richard Lower.

E. GASKELL

On Being Neurochemical

Methods in Neurochemistry. Edited by Rainer Fried. Pp. xi+374. (Marcel Dekker: New York, 1971.) n.p.

THIS book's preface claims neurochemistry as one of the fastest growing of the life sciences, and this is probably true: the *Journal of Neurochemistry* is now as large as was the *Biochemical Journal* of a previous generation. The contents of *Methods of Neurochemistry* suggest that one of the methods of growth of the subject is by capture. It is to cover "all important areas of the neurosciences, mainly in the fields of biochemistry and pharmacology". Of the book's six chapters, that by Donough O'Brien on biochemical screening in mental retardation could equally count as clinical biochemistry; while that of Krnjević on microiontophoresis embraces much physics and pharmacology. The neurochemical aspect of each contribution is nevertheless undoubted; the neurosciences by their nature encourage interdisciplinary work and thinking. Thus O'Brien's account, which is the largest in the book, gives both clinical and technical commentaries on some twenty metabolic abnormalities in man which are related to mental dysfunction. It lists briefly seventy to eighty such conditions and avoids discussion of those which are adequately recounted in books of reference; it also demonstrates well the need for expanded laboratory facilities in this field.

The stated editorial intentions regarding *Methods in Neurochemistry* are to present several distinct areas of research, rather than obey a unifying concept, and the book begins with accounts of the purification and properties of myelin isolated from neural systems (L. C. Mokrasch), and of the purification and properties of phospholipids (G. B. Ansell and S. Spanner). Methods of determining catecholamines and their metabolites are also described (D. F. Sharman); the three contributions include excellent experimental details.

Of very different character is the final and second largest contribution, which is by the editor and a co-worker and is a tabulation of "compounds of importance to neurochemistry". Errors in this section include the relatively harmless mis-spelling of names of substances, but also mistakes among less well known compounds. Saccharopine, an amino-acid derivative found as a urinary constituent in a form of mental disorder, is given the structural formula of a C_{10} compound and the molecular formula of a C_{11} compound. Three successive entries are: chlorpromazine, depicted with triply-bonded chlorine in a 6-membered ring; cholesterol with structure lacking angular-bonded methyl groups; and cocaine, with the molecular formula of a C_{17} compound and the structural formula of a C_{18} compound. Neurochemistry: important, expanding, should be able to command better service.

H. MCILWAIN

Blastocyst Biology

The Biology of the Blastocyst. Edited by R. J. Blandau. Pp. xi+560. (University of Chicago: Chicago and London, March 1971.) £12.40.

THE current publication explosion has produced torrents of books on almost every conceivable aspect of reproduction. Prospective readers are thus likely to scan new books very carefully for quality, price and comprehension before being tempted to invest their money. *The Biology of the Blastocyst* sets a high scientific standard, covers a comprehensive set of topics (perhaps too wide) and has a price that even by current standards is not cheap. It is obvious that this book will prove of considerable value to the rapidly increasing number of researchers engaged in reproductive physiology. It should serve as a valuable reference for data for some years before it is inevitably superseded by a later publication.

Reports of conferences seldom debate in detail a major advance in a particular topic; they tend to cover broad aspects of a subject, with chapters penned by authorities in these different areas. This book is no exception, for there are more than thirty authors involved in thirty-one chapters. In this area of biology the comprehensive description of the subject matter demands an interest in a wide variety of subjects such as endocrinology, differentiation, ultrastructure, tissue culture, immunological reactions between mother and foetus, teratology and uterine musculature. At implantation, as at certain other stages in pregnancy, many events occur simultaneously in the embryo and the mother. There can be no doubt that a full and fair treatment of these events is provided in these chapters, which are well written by authorities in each topic. Naming names would be tedious and unfair: let it suffice to state that almost every conceivable topic touching on the blastocyst is covered somewhere in the volume. Inevitably some authors have chosen to write a brief review of their topic while others have described their own experiments, and this inevitably leads to some unevenness in the book. Yet every chapter offers a wealth of detail or something interesting and stimulating, and it is a pleasure to browse through the pages, picking up ideas or comments. Discussions in the conference have been omitted, which is in many respects a relief from pet ideas or theories unsupported by critical material or analysis.

My major criticism concerns the repetition of subject matter in various chapters. This repetition obviously permitted more contributors to state their views in the symposium, but the combination of some chapters or the exclusion of others could have provided a more streamlined presentation. Some of the chapters contain material of peripheral interest, such as fertilization or the biochemistry of the cleaving embryo, and one chapter could have sufficed for these. Other chapters are somewhat superfluous because two or more contributors cover essentially the same subject matter, for example, there are three on immunology. More rigorous selection of speakers, or even joint chapters, would have led to a more concise and easily presented book.

The book is well produced in a handsome size, contains detailed references and is well illustrated. The editing has been done with care. It will prove a most valuable source of material.

R. G. EDWARDS

Bioelectromagnetism

Electromagnetic Fields and Life. By A. S. Presman. (Translated from the Russian by F. L. Sinclair.) Edited by Frank A. Brown, jun. Pp. xix+336. (Plenum: New York and London, 1970.) $25.

EVER since Franz Mesmer, the Austrian doctor and mystic, started some two hundred year ago to treat patients with iron magnets, controversy has continued over the question of whether magnetic fields can affect biological systems. In this book A. S. Presman, a biophysicist at Moscow University, sets out to review the experimental evidence for biological effects of electromagnetic fields with frequencies below 10^{12} Hz (thus excluding visible and shorter wavelengths, where quantum effects are important) and proposes a new hypothesis to explain them.

In the first part of the book the author describes the naturally occurring electromagnetic fields to which all organisms are continuously subjected. The electrical properties of living organisms are discussed briefly, and various physical mechanisms by which such fields could affect biological systems are listed.

In the second part the author reviews the experimental evidence for electromagnetic field interactions. Especially useful is the extensive bibliography, although references are only listed up to 1967. The principal effect is heating of tissue which leads to death if the heat cannot be quickly dissipated. Surprisingly, however, effects seem to occur at field intensities which are too low to cause appreciable heating. One reads, for example, of the good correlation between the incidence of cerebrospinal meningitis in New York and solar activity, and between magnetic storms and mortality from nervous and cardiovascular diseases in Copenhagen and Frankfurt. Planaria, bees, wingless termites and many flies can orientate themselves relative to the Earth's magnetic field. Seeds of corn and wheat sown with the radicle pointing south germinate one to three days earlier than those with the radicle pointing northwards, though the effect apparently depends on the phase of the Moon. Magnetic fields of greater intensity (120 kg) prove lethal to *Drosophila* if exposed for sixty minutes, while a combination of magnetic field (600 g) and microwaves is very effective in treating tumours in mice.

The fact is, of course, that no known physical theory can explain these low-intensity phenomena because the energy involved in the fields is far too small. There are thus two alternatives: either the experimental results are inconclusive, and the statistical correlations are misleading, or, alternatively, new processes are occurring about which nothing is yet known. Presman argues that the latter is the case, and thinks the evidence indicates a transfer of information from the environment to the organism which is independent of the amount of energy carried by the waves. The field may produce its effect by modifying the subtle neural interactions between the various parts of the organism, although there are no clues yet as to how this might be accomplished.

Finally, Presman speculates on the consequences of his hypothesis. Animals, for example, may be able to communicate using electromagnetic signals, and the simultaneous changes in the direction of motion of birds in flocks and fish in shoals could be initiated in this way. Eels may sink to great depths in the Sargasso Sea to protect their young from terrestrial electromagnetic waves, and it may prove necessary to protect hospital patients from such fields using suitable metal screens. Intriguingly, telepathy and other parapsychological phenomena may at last have a physical foundation.

It would be pointless to quibble over the many controversial statements made in any book as speculative and wide ranging as this. Two points should be mentioned, however. There is an error in the equivalent circuit of a typical cell (page 35) which leads the author to conclude, wrongly, that no electric current flows through a cell in a static electrical field. Second, the statement (page 85) that the minimum theoretical electric field strength which can affect a biological system is 10^6 V m^{-1}, is difficult to reconcile with the fact that such a field, established in normal pond water, would cause it to boil within 0.01 seconds, and would rapidly be detected by any biological system. Nevertheless, this is an interesting and readable book, and "the author's modest hope, that the ideas which he presents will stimulate further critical discussion and researches" should certainly be fulfilled.

ALUN M. ROBERTS

Living Together

Aspects of the Biology of Symbiosis. (Proceedings of a Symposium held in Boston.) Edited by Thomas C. Cheng. Pp. x+327. (University Park: Baltimore; Butterworth: London, July 1971.) £7.

THE title of this book might mislead some people into believing that this is a text-book, but it is not. It is a collection of twelve papers given at a symposium of the American Association for the Advancement of Science in December 1969.

There is no universally agreed definition of the term "symbiosis". Some restrict its use to clearly mutualistic associations, while others extend it to include many examples of parasitism and commensalism. This book tends to the latter use of the term, and its bias is strongly zoological, as exemplified by the selection of topics discussed: penetration stimuli for schistosome cercariae; schistosomules and mouse skin; communication between fishes in cleaning symbioses; nubibranch associations as symbioses; respiratory metabolism of a trematode metacercaria and its host; enhanced growth and shell deposition in parasitized molluscs; a new hypothesis on the goodness of parasitism; nutritional aspects of a symbiosis between a crab and a Hawaiian sea urchin; effect of external factors on host–symbiont interactions in green Hydra; blue-green algae in echiuroid worms; immunity in invertebrates and protection of anemone fishes from sea anemones.

The best aspect of this book is that all the articles are concerned with the experimental study of symbiosis, and mostly lack the rambling, vague speculation that has bedevilled some of the earlier writing about the subject. As in most books of this type, the articles are rather uneven in quality. For example, it is most surprising that the article on nudibranchs does not mention chloroplast symbiosis. The very long article by Lincicome on the experimental evidence for his hypothesis on the goodness or parasitism certainly presents a concept new and unfamiliar to most biologists concerned with symbiosis: "Parasitism, one of life's great phenomena, possesses a quality of goodness that has largely been overlooked because man has been so possessed with the disease aspects of this association". Kawaguti's very short but fascinating paper on blue-green algae in echiuroid worms states, in the discussion and conclusions, that *Ikedosoma* can consume ammonium and that its proboscis has a reduced oxygen consumption. Unfortunately, the experimental basis for such a statement is not mentioned in either the results or the materials and methods sections, both being exclusively concerned with microscopy. There are some articles, however, such as that by Lytle, Epp and Barthalmus on green Hydra, which manage to maintain a uniformly good standard of competence.

Most of the articles in this book are concerned chiefly with describing the results of research into rather narrow fields. I am therefore perplexed that the authors publish them in this book rather than in a journal. Books take much longer to be published than journals, and this can be important in fields which are rapidly advancing. Further, some of the articles (and hence, the reputation of their authors) would have been noticeably improved by the rigorous refereeing characteristic of the better journals, but lacking in most books of this type.

In spite of the flood of recent research into symbiosis, the really good book on the subject remains to be written: perhaps the subject has now grown so large that such a book has become impossible to write.

D. C. SMITH

Electrochemistry Now

Modern Electrochemistry: an Introduction to an Interdisciplinary Area, Vol. 1. By John O. M. Bockris and A. K. N. Reddy. Pp. xxxii+622. (Plenum: New York, 1970.) $19.50.

Modern Electrochemistry: an Introduction to an Interdisciplinary Area, Vol. 2. By John O. M. Bockris and A. K. N. Reddy. Pp. xxvii+623–1432. (Plenum: New York, 1970.) $19.50.

THE publication of another book by Professor Bockris is an event in the world of electrochemists and the present large two-volume effort, with Dr A. K. N. Reddy as co-author, certainly justifies the interest which it is commanding from that fraternity. The latter may have expected something rather different and it is important therefore to establish at the outset the purpose and philosophy of the book. Certainly it is not a research treatise, it is not a chemists' handbook to the theory and practice of electrochemistry, it is not even perhaps a chemistry students' text. What is it? The answer is contained in the sub-title and preface—"An Introduction to an Interdisciplinary Area" and ". . . a book which outlines the field at a level which can be understood by people entering it from different disciplines . . .". The authors see electrochemistry not as a branch of chemistry but as an emerging discipline groomed to the present age in which the solution to the problems of pollution and power supply together with so many appurtenances of modern civilization will be electrochemical. To this end "modern electrochemistry" emerges as a fusion of ideas at present scattered throughout the various branches of physical science, a fusion albeit at a comprehensive theoretical level. Thus the first volume deals essentially with theoretical considerations leading up to and relating to physical processes within the electrolyte phase. It includes structural and non-structural treatment of ion-solvent interactions, solvation numbers, dielectric constant of water and ionic solutions, ion-ion interactions including the Debye-Hückel equations and extensions, activity coefficients, ion-transport (including the laws of diffusion, and Laplace transforms), conduction and ion migration, and concludes with chapters on protons in solution and ionic liquids (fused salts, liquid oxides, and so on). The second volume has electrode phenomena ("electrodics") as its principal theme and covers the establishment, thermodynamics and structure of electrified interfaces, charge-transfer processes of simple and complex electrode reactions and electrode kinetics. The final two chapters deal with electrode reactions of special interest (for example, electro-catalysis, electro-crystallization) and those of technological importance (such as corrosion and electric power storage).

The electrochemist will undoubtedly appreciate having together in one publication so much of the fundamentals of his subject, but more so perhaps from the point of view of refurbishing his basic knowledge than in providing any detailed information on current research methods and practices. Thus there is no reference to a.c. impedance techniques and only brief reference to galvanostatic, potentiostatic and other experimental methods. Polarography is represented chiefly by a derivation of the Ilkovic equation, and present day amperometric and chronopotentiometric methods also receive scant attention. The absence of reference to practical procedures is a notable omission in a work of this importance, even accepting the authors' concentration on fundamentals, and we feel that a better balance would have resulted from such inclusion at the expense of many fringe topics. The inclusion of a section on batteries and fuel cells fits in with the authors' aims, but is too brief to do more than scratch the surface of one of those areas which is the *raison d'être* for the books. Even more surprising is the relegation of the section on galvanic cells to quite late in the second volume, where it receives rather limited selective treatment, a result no doubt of the constraint imposed by the authors' electrolyte-electrodic divison of the subject.

The bulkiness of the two volumes is in large measure a result of the praiseworthy efforts of the authors to provide the maximum of help to the reader, and this is exemplified by numerous informative diagrams, frequent essay-type summaries and a question/answer presentation. To the more advanced reader, however, much of this might well be regarded as seeking clarity at the expense of brevity and such readers may now, like us, hope one day to see a more succinct condensed version. This style of presentation is of course shared by other American writers of textbooks and is therefore more likely to be acceptable to readers from the United States. Certainly it increases the price to a level which must make possession by most undergraduates unlikely and possibly by many postgraduate workers too. Words like "electronation" (for cathodic reduction) irritate but neither this, nor our other criticisms, should mask the fact that the authors have produced the most comprehensive theoretical text so far, quite apart from their thesis that electrochemistry should be regarded as a new interdisciplinary subject. The two volumes are a must for any university/research library and those individuals who might be able to have their own copies should be agreeably satisfied with their purchase.

R. F. PHILLIPS
N. A. HAMPSON

Learning in Bed

Learning and Sleep. By F. Rubin. Pp. xi+138. (John Wright and Sons: Bristol, June 1971.) £1.75.

IF one mentions "sleep learning" to an academic psychologist one is likely to meet the same sort of defensive disbelief elicited by ESP. Fifteen years ago Simon and Emmons established that retention of material presented to a sleeping person is negligible unless he is in only very light sleep. Since then, outside the Soviet Union and Eastern Europe, the idea of sleep learning has been treated with scepticism, and criticism of studies has almost entirely been centred on electro-encephalographic evaluation of whether subjects were "really asleep". The baby has been thrown away with the bathwater: the idea that people might be able to learn in bed, at times when they are not soundly asleep, has been almost entirely ignored by professional psychologists.

This book therefore may serve an excellent purpose, bringing to the attention of those involved in teaching language courses, for example, the possibility of facilitating learning while we are in bed and not yet sound asleep. The opening chapters dispel misconceptions many people have about hypnopaedia (the term the author prefers). No wild claims of using all eight hours of sleep are made: learning is restricted to a period immediately after retiring and shortly before rising in the morning. The author lays great stress on the fact that here is no quick, easy short cut to knowledge. He discusses at length Soviet and Czech experiments (chiefly in language tuition), which present words or phrases, with their mother-tongue equivalents, to highly motivated students who simultaneously receive daytime tuition in the language. The crucial point is that the presentation of material during the drowsy state can facilitate learning, in properly structured courses. The experiments he describes have often not been rigorously designed: only three experiments with control groups are reported; nevertheless, the theory and evidence are sufficient to warrant practical tests of the scope and usefulness of hypnopaedia in the West.

A paperback aimed at the popular market would seem a good way of putting these points across: a pity therefore the book is priced so high. There are other annoying things, such as a pair of tables describing sleep stages which are blatently inconsistent (pages 19 and 21). This might not worry the casual reader, but there are more infuriating points: at times the author hardly makes sense (page 39, for example), and he is fond of quoting and requoting references, as if to impress the reader. The organization of the book is particularly frustrating. The fourth chapter is a study by the author which belongs with the other experiments discussed at the end of the book. Why does he proceed to practical details of which tape recorders to use and how to intone lessons in the fifth chapter only to return to the experimental evidence that sleep learning really works in the eighth chapter?

In spite of these serious faults I would still recommend this book to those not prone to irritability, who want a fresh look at a subject neglected by "serious" psychologists.

HUGH FIRTH

Underwater Acoustics

Underwater Acoustics. Edited by R. W. M. Stephens. Pp. vii+269. (Wiley Interscience: London and New York, December 1970.) £6.75.

THIS book comprises eight articles on various aspects of underwater acoustics, which are based on a series of lectures given at Imperial College, London, in 1967. The contributors are either physicists or engineers who are actively engaged in research in the different specialist topics presented in the book.

The purpose of the introductory chapter by the editor is to provide the reader with background information on the sea as an acoustic medium. In thus setting the scene for the specialist contributions that follow on topics such as sound velocity, sound attenuation, noise, reverberation and cavitation are briefly reviewed.

The first specialist chapter is a mathematical study of the propagation of acoustic waves underwater, when the effects of the medium boundaries and the medium's velocity profile are included. The limitations of solutions by ray theory methods in these circumstances are discussed. The approach favoured by the author is the method of normal modes which arise from the solution of the wave equation in cylindrical co-ordinates with the appropriate boundary conditions applied. The general complex solutions obtained are presented and discussed in some detail. Space is also devoted to the "not so spherical cow", that is the effects on the solutions when factors such as the "real" sea surface and bottom cause departure from the mathematical model analysed. The sea surface is in general nonspecular in its behaviour, and so the next chapter, which is an authoritative review of the back and specular scattering of acoustic waves from the sea surface, follows quite naturally. The discussion involves a fair degree of mathematical content, the scattering theory being developed from the Helmholtz–Kirchhoff integral. Frequent comparisons are made between theory and experimental results. A comprehensive reference list is also provided.

The fourth chapter is basically a review of the sonar equation and some pertinent aspects of signal processing in relation to acoustic exploration. In particular the discussion is based on the recognition differential and noise terms in the equation. Factors such as detection threshold, processing gain and visual integration are briefly examined. A fairly detailed review on background noise is presented which includes such aspects as reverberation, self noise, cavitation noise and ambient noise. The next chapter may be regarded as based on the target strength term in the sonar equation. It is essentially a detailed and lengthy review of experimental results associated with the echoes from simple geometrical objects and from more complex objects, in particular—fish. The information presented is extensive and the topics covered too numerous to detail here.

Non-linear acoustics form the subject of the sixth chapter. The emphasis is on the basic physical mechanism behind the non-linear effects within the range of validity of perturbation theory. A brief discussion of some of the applications of non-linear effects in underwater acoustics is also included. The next chapter is a review of underwater instrumentation. Subjects briefly discussed include transduction, telemetry, wave measurement and acoustic navigation systems. The final chapter is concerned with the audio communication between free divers. Physiological factors, such as hearing and word formation are discussed in addition to a number of the technical problems.

Although an attempt is made to retain a theme throughout the book, probably because of the diverse authorship, the book in general is a mixture. Consequently it is difficult to see for whom the book is intended. A number of the chapters are suitable for the more general reader but the remainder are specifically aimed at the research worker.

B. V. SMITH

CORRESPONDENCE

Elitism in Astronomy

SIR,—I have followed the various discussions about British astronomy, and have been surprised that the chief factor we would consider in the US has remained unmentioned in public.

I refer of course to a critical number of first rate scientists. Between the two Royal Observatories and Pretoria, as an outside observer I count six first rank astronomers. In Pasadena associated with the Hale Observatories, I count fourteen astronomers of distinctly higher average performance. The UK group is split between three locations and three establishments. In quantity of first rank people at one location, all three are operating at a sub-critical level.

It has been suggested that to set up a national centre with a critical number of high calibre astronomers is elitism. The question is not whether the UK wants an elite. It is whether it wants one place alive, or many half dead. Such a centre would not be a third centre. It would be a first centre. Its physical location should in the absence of special constraints be preferably chosen on logistical grounds. But the good reasons for avoiding existing institutions are that the petty inter-institutional squabbles and prior staff commitments of existing institutions might create a sickening home for a healthy baby organization.

Yours faithfully,

NEVILLE J. WOOLF

Director,
University of Minnesota Observatories,
Minneapolis, Minnesota 55455

Conquest of Cancer

SIR,—I would like to clarify some aspects of the Conquest of Cancer Bill, S.1828, recently passed by the Senate and now awaiting House action. As the director of a cancer hospital and research institute, I am vitally interested in the conquest of cancer, and as a member of the National Panel of Consultants appointed by the US Senate, I participated in the recommendations that led to S.1828.

Unfortunately, there is considerable confusion about the provisions of S.1828. This confusion, understandably, comes about because S.1828 evolved from several other bills and proposals with identical or similar names.

S.1828 provides for increased emphasis on cancer research within the framework of the National Institutes of Health. The National Cancer Institute will remain a part of NIH, but will have greater autonomy. It will not become a separate agency. Nor will its relationship to NIH be a "name only" affair as has been mistakenly stated by those who confuse it with other proposals. Under S.1828, the National Cancer Institute remains in its own buildings on the NIH campus. The NCI personnel will continue to interact with the other institutes within the NIH. The NCI will continue to use the Clinical Center of NIH for studies on patients. It will continue to use the NIH computer facilities and the NIH laboratory animal breeding and holding facilities just as it does today. The NCI will continue to use the NIH study sections for peer review of research grant applications, but will be able to supplement them with additional study sections concerned with specific aspects of cancer. For example, I hope to see a breast cancer study section added. The major change in the relationship of the Cancer Institute involves greater budgetary and planning freedom. Instead of the present arrangement that requires six (6) layers of officials above the Cancer Institute to study and approve all major plans and budgets, the Director of the Cancer Institute will report directly to the President. This administrative change will speed up the conquest of cancer considerably.

In every other respect the Cancer Institute will retain its present connexions with the other institutes and with NIH.

Furthermore, to be certain of good coordination with the NIH and other government agencies, S.1828 provides that the Advisory Council of the Cancer Institute must include, as ex-officio members, the director of NIH, the Secretary of Health, Education and Welfare, and the director of the Office of Science and Technology.

Some charge that S.1828 will break up the National Institutes of Health. But NIH is not so fragile that a change to increase efficiency and give greater autonomy to an operating institute will destroy it. Businesses and other government agencies are frequently reorganized for greater efficiency.

Another criticism is that other Institutes within NIH will want similar arrangements for themselves. Perhaps they will. Let's try it with one: the Cancer Institute. If cancer research moves ahead more rapidly under the new arrangement (as most of us who studied the situation believe it will), and if other institutes then wish to have a similar arrangement, why not give it to them? After all, we are primarily concerned with improving the people's health, not with maintaining any specific administrative pattern. If, on the other hand, cancer research does not progress more rapidly under the new arrangement, the other institutes would not want to copy it.

Some fear that the increased emphasis on cancer research implies a promise of a cure for cancer in a few years. No one associated with S.1828, the National Panel of Consultants, or the American Cancer Society has ever made or implied such a promise. We do believe that the time has come for an accelerated and intensified assault on cancer, and that the eventual conquest of this disease can be brought nearer.

Would passage of S.1828 result in neglect of basic research? Exactly the opposite is true! S.1828, and the Report of the Panel of Consultants both call for expanded basic research.

Finally, there is the point that the bill cannot guarantee a cure for cancer. There is no doubt about that and, indeed, no one would suggest there is a guarantee of a cure for cancer in any kind of legislation. There is the assurance in this bill that progress will be faster and that the cures will come sooner than they would without it. That is reason enough for its enactment and that is the reason my colleagues and I support it.

Yours faithfully,

SOLOMON GARB

American Medical Center at Denver,
Spivak, Colorado 80214

Research and Development

SIR,—Your review of Dr Duncan Reekie's survey for the Centre for the Study of Industrial Innovation of abandoned industrial R and D projects complains that it is "rich in detail and poor on analysis and generalization". We would be the first to admit that the survey is short on the kind of loose ill-defined and often unsupportable generalization from which the discussion of innovation greatly suffers. Your reviewer provides a number of new illustrations—for example, that "even successful research and development projects in Britain are understaffed". One major object of the centre is to study in detail what happens in firms so that general conclusions can rest on an adequate basis of knowledge. It is perhaps because firms realized the relevance of this approach (rather than because of "luck" as your review suggests) that they cooperated so fully in Dr Reekie's study.

Your reviewer writes as though the report suggests that projects should not be abandoned. On the contrary, it refers to the inevitability of this. But your review is altogether too complacent to claim that none of the cases Dr Reekie reported should cause surprise and that all of them merely represent "unavoidable ups and downs of commercial life". In no fewer than seven of the twenty firms visited development projects had been abandoned because they had been unwittingly duplicated within the same organization. In other cases, there had been several years of successful technical development before any market assessment had been undertaken. Surely no management could claim that such cases were unavoidable? In regard to the timing of abandonment your reviewer appears to agree with the centre—though with an odd twist he suggests that he is saying something different.

We appreciate your having paid so much attention to our first report. But we must advise those of your readers who wish to know what the report says to read it in the original.

Yours faithfully,

DUNCAN BURN

Chairman,
Centre for the Study
of Industrial Innovation,
162 Regent Street,
London W1R 6DD

Those who collect information but who neglect to analyse it must surely not be surprised if others draw lessons from it which may sometimes be unwelcome—Editor, *Nature*.

Erratum

Last week's News and Views editorial "Haemoglobin Synthesized in *Xenopus* Eggs" (233, 161; 1971), contained the erroneous statement that AUGpolyU is translated when injected into *Xenopus* eggs. Of course, as Gurdon *et al.* reported in their article on page 177 of that issue, this messenger does not promote the incorporation of phenylalanine.

British Diary

Tuesday, September 28

Centralized Control Systems (four-day conference) Institution of Electrical Engineers; and the Institution of Electronic and Radio Engineers, at Savoy Place, London WC2.

Schizophrenia: Biochemistry, Genetics and Therapy (two-day conference) Schizophrenia Association, at Church House, Dean's Yard, Westminster, London SW1.

Wednesday, September 29

Less Common Methods of Separation (9.30 a.m. symposium) Institution of Chemical Engineers, Midlands Branch, at the University of Birmingham.

Microbial Control in Pharmaceutical and Cosmetic Preparations (two-day symposium) Society of Cosmetic Chemists of Great Britain, jointly with the Pharmaceutical Society of Great Britain, at Imperial College, London SW7.

Thursday, September 30

Biochemical Engineering (2 p.m. symposium) Institution of Chemical Engineers, at the University of Technology, Claverton Down, Bath.

Friday, October 1

Autumn Meeting and Annual General Meeting (afternoon) Bone and Tooth Society, at the Royal Dental Hospital, London.

The Properties of Anticorrosive Pigments (10 a.m.) Mr J. Bowden, Oil and Colour Chemists' Association, Scottish-Student Group, at the St Enoch Hotel, Glasgow.

Saturday, October 2

Teaching of Mathematics in Schools in Relation to the Teaching of Physics (symposium) Institute of Mathematics and its Applications, jointly with the Institute of Physics, at Eton College, Windsor.

Monday, October 4

Practical Pigment Dispersion (7 p.m.) Mr J. R. Groom and Mr M. Baker, Oil and Colour Chemists' Association, at the Queens Hotel, George Street, Hull.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

An Index to the Systematic Collection of Minerals in the British Museum (Natural History). Twenty-ninth edition. Pp. 39. (London: British Museum (Natural History), 1971.) 20p. [96

University of Bristol, Department of Agriculture and Horticulture. Long Ashton Research Station—The National Fruit and Cider Institute. Report for 1970. Pp. xv+208. (Long Ashton, Bristol: Long Ashton Research Station, University of Bristol, 1971.) £1.25. [96

Plant Breeding Institute, Cambridge. Annual Report 1970. Pp. x+126. (Cambridge: Plant Breeding Institute, 1971.) 75p. [106

Agricultural Research Council. Meat Research Institute—Annual Report 1969–70. Pp. 80. (Langford, Bristol: Meat Research Institute, 1971. Obtainable from HMSO.) 89p net. [106

Myth, Magic and Archaeology. By Professor F. J Tritsch. (Inaugural Lectures delivered on 8 December 1970.) Pp. 20. (Birmingham: The University, 1971.) 15p. [106

Building Research Station Digest, No. 130: Drainage Pipelines—1. Pp. 8. (London: HMSO, 1971.) 5p. [116

University of Strathclyde Annual Report 1969/1970. Part 1: Pp. 1–130. Part 2: Pp. 131–193. (Glasgow: University of Strathclyde, 1971.) [116

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:
12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A. & Canada
Nature (Friday)	£14	$48
Nature + Nature Physical Science	£24	$83
Nature + Nature New Biology	£24	$83
All three editions	£29.50	$108
Annual Index	£1	$3

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 5431
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office
Copyright © Macmillan Journals Limited, September 24 1971

NATURE Volume 233 OCTOBER 1 1971

Public Money for University Research

UNIVERSITIES are notoriously proud of their independence, both individually and collectively, which is why it is such a great surprise that the Science Research Council has been able to keep its friends in the universities while persuading them to change their ways quite fundamentally. Within three years, the council has established the principle that the pattern of university research should be determined not by the statistical aggregation of the inclinations of university teachers but by a more deliberate process of planning in the course of which the council itself has assumed responsibility for picking out desirable directions for research. At the beginning, to be sure, a great many academics were up in arms, saying that academic freedom would be in jeopardy if the Science Research Council chose to head people off certain lines of enquiry or even to concentrate desirable work in selected universities. Some mathematicians are still complaining (see *Nature*, **233**, 225 ; 1971), but that protest has all the appearance of a rearguard action. Most university departments in Britain are resigned and even glad of the Science Research Council's initiative.

This is why one of the biggest dangers now facing the Science Research Council is that of complacency. The most recent annual report (see page 298) is a vigorous document, but there is a mild suspicion that the council may be more ready than is strictly necessary to compromise with unpleasant truths.

The chief of these is money. In the past year, it has been made known by the present government that the funds available for university research would grow less quickly than the numbers of students in higher education and therefore, by definition, that the proportion of university teachers able to recruit financial support from the research councils would diminish. This simple truth was spelled out clearly enough at the conference organized by the Science Research Council in Manchester last January. To everybody's surprise, the universities have raised no audible protest. By now the policy is accepted, and it is entirely appropriate that the Science Research Council, the principal source of grants for university research, should have decided that the time has now come to end the automatic but cumbersome procedure by means of which funds are transferred from the council to the University Grants Committee at the end of each university quinquennium (the next comes in July 1972) so as to keep in being research projects begun by the research councils. Under the new system, discrimination will be easier. Universities will be under less pressure to institutionalize research groups. To the extent that these effects are consonant with what the Science Research Council has been looking for, it is clear that the grand design can be implemented even more efficiently in the years ahead.

It is therefore proper to ask whether the assumptions on which the new policy is based are as appropriate as everybody supposes. One of the Science Research Council's objectives is to create a greater sense of liveliness in university research and so far, with some success, it has sought to do this by providing not merely research grants but also facilities—laboratories for high energy physics or for astronomy, contributions to organizations such as CERN and even arrangements for making sure that universities have access to computing machinery. Yet the Science Research Council remains only one of the four organizations responsible for channelling money towards science departments in universities. The three other research councils are smaller in aggregate but just as influential in their somewhat narrower fields. It is all very well for the Science Research Council to boast in its most recent report that all four of them have been able to put their names to a document in which four separate statements on pollution appear within the same cover, but that is a far cry from the coordinated policy on research which British universities now require. Why, after all, should it be the Science Research Council that makes grants for research on high pressure physics but the Natural Environment Research Council that gives out money for closely related topics in geology? Why should it be that the Science Research Council administers central facilities for high energy physics but that the Natural Environment Research Council looks after oceanography (with all its sea-going vessels)?

Another problem concerns the scale on which university research should be carried out. In past decades, the conduct of university research in Britain has been bedevilled by self-justifying prophecies about the capabilities of university departments to look after their own affairs. Large items of equipment have frequently been placed on extramural territory not merely because sharing among several universities is intended but also because it has been held that universities are unable to manage large installations (a notion easily disproved by the radio astronomy observatories which, by historical accidents, are in the pockets of university departments). As the policy of selectivity and concentration succeeds, however, there will be more and more arguments in favour of letting British universities do what American universities have done for the past three decades—to take managerial responsibility for large laboratory installations. When that time comes, the budget of the Science Research Council and its smaller associates should increasingly be spent within universities. That tendency is not merely something to be stomached—it is itself desirable.

A third class of problems stems from graduate education in British universities For years now, the Science Research Council has been saying that it wishes to encourage a different pattern of postgraduate teaching. Courses should be more relevant to the needs of industry, and students should be more alert to them, but at the same time there is a need somehow to broaden the base of postgraduate teaching so that graduates emerge as more flexible people. So far, these declarations have gone little further than wishful thinking. Yet the fact remains that British graduate education, good though it may be as an intellectual training for enquiring minds, is unsuited to the needs which taxpayers hope to meet by keeping universities in the state of grace to which, quite properly, they say they are entitled. What the Science Research Council should now do is to back up promises with performance.

Publish and be Damned a Second Time

Now that Mr Herman Kahn has made it respectable to think the unthinkable, it may be appropriate to ask what virtues still attach to the convention that reports of original scientific research should not be published more than once. Most scientific journals, *Nature* included, take a stern view of breaches of this convention and most scientists seem first of all to approve of the convention and then to do their best to live by it. The arguments for a rigid rule against multiple publication are of course familiar. The literature is full enough already, and if multiple publication became common practice, the speed with which libraries are forced to throw up their hands in despair would be enormously increased. But multiple publication can also cause bibliographical chaos. The problems of knowing whether two apparently similar titles by the same authors refer to substantially the same original article would turn every working scientist into a textual analyst. At the same time, it is only fair to recognize that there have grown up practices which consist of variations of the strict convention that those who publish the same article twice are guilty of dishonourable conduct. By now, for example, preliminary communications are widely recognized to be useful ways of making public important discoveries, but there are good reasons for complaint if the fuller version of the discoverer's report does not eventually materialize. More reprehensible, there has been a tendency in the past few years for reports of original research, once published, to pop up again in closely related forms as contributions to symposia, international conferences and other occasions on which professional scientists have been persuaded to travel. The equation between the seminar paper and the airline ticket is now uncomfortably precise.

In all these circumstances, it would of course be overzealous of a journal such as *Nature* to follow too rigid a policy, but there are some circumstances in which multiple publication is not merely an offence against accepted conventions but an assault on ordinary standards of decent behaviour. Earlier this year, there appeared in *Nature Physical Science* an article of some importance which was described by the principal author as "an extremely important breakthrough into the new field of . . .". With all despatch, the article was sent to a referee and published in the usual way. Only afterwards did it become known that the same article had been submitted to two other journals. By all accounts, it seems that the article was rejected by one journal but published, in a format substantially the same as that which appeared in *Nature*, by the second. Since then, the hapless authors have been assailed by several editors and the senior author has actually written to say "We were all very excited at the time and I would like you to think that my apparent breakdown in publication ethics was the result of overenthusiasm." With some regret, it has been decided not to publish the names of those concerned in this shabby business, but to scrutinize with immense care their claims to originality for other publications. In the long run, however, a public register of such offenders may turn out to be the best method of keeping authors on a straight and narrow path. No doubt such a record would have some of the attractions of a gossip column as well.

Another issue involving a form of alternative publication is much harder to resolve. In the past two decades, newspapers have become increasingly enterprising sources of scientific news. In the long run, of course, this is of public benefit, but there have been times when the editors of journals have been embarrassed to discover that the essence of some cherished article has been given to the daily press in such detail that full publication seems a mere formality. Thus there has grown up a running battle between some journals, with *Physical Review Letters* at their head, and those newspapers and magazines which have such a flair for seizing on important discoveries that they are, almost by definition, the best newspapers. It is only fair to say that the distinction between proper and improper practices is extremely hard to make in this connexion. Certainly there can be no rigid rule. It is also unreasonable to expect that the authors of an important discovery should keep this strictly to themselves for months on end while waiting for the solemn processes of formal publication to be completed. Not merely is this unnecessary but impractical as well, now that journalists habitually turn up at scientific meetings. And it would, of course, be altogether too toffee-nosed to ask that news of scientific discoveries should be kept even from the audiences at scientific meetings until the mills of the publication process have begun to grind. So it seems to follow that journals, *Nature* included, can ask authors to refrain from telling newspapers their good news only if the process of publication is reasonably swift. What matters is that the relationship between an author and the editors of the journal in which he seeks to publish should be open and courteous. And even then, there are bound to be occasions when news leaks out. In short, although journals are right to ask that their contributors will not call a press conference whenever a manuscript is accepted for publication, their best insurance against being scooped by the newspapers is to publish quickly.

---

100 Years Ago

THE First Commissioner of Works and Public Buildings announces that he intends again to distribute this autumn, among the working classes and the poor inhabitants of London, the surplus bedding-out plants in Battersea, Hyde, the Regent's, and Victoria Parks, and in the Royal Gardens, Kew. If the clergy, school committees, and others interested, will make application to the superintendents of the parks nearest to their respective parishes, or to the director of the Royal Gardens, Kew, in the cases of persons residing in that neighbourhood, they will receive early intimation of the number of plants that can be allotted to each applicant, and of the time and manner of their distribution.

From Nature, **4**, 453, *October* 5, 1871

OLD WORLD

EUROPEAN LABORATORY

ESRIN in Trouble

A PROPOSAL to close down the European Space Research Institute (ESRIN) is widely regarded as a setback to the cause of European collaboration. The laboratory is one of several established by the European Space Research Organization. It was originally intended as a centre for fundamental research relevant to ESRO's activities in space, and has been particularly concerned with plasma physics. If a resolution introduced at the ESRO council meeting in July should be adopted in November, the laboratory will be run down in 1972 and 1973.

The ESRIN laboratory is at Frascati, south of Rome. It has a permanent staff of about 70 and an annual budget of $2 million. In the past months, the institute has attracted a steady stream of visitors who add about a dozen to the professional complement. The director of the institute, Dr Nicola D'Angelo, succeeded Dr H. L. Jordan in 1970. He is thought to have received many expressions of support in the past few weeks, especially from university scientists, but there are fears that these may not be sufficient to distract ESRO from its present preoccupation with applications satellites.

Apart from purely budgetary considerations, it is possible that ESRIN has been threatened because the work it does might just as well be done in universities, although one of those involved says that the institute was originally set up to tackle problems beyond the reach of university departments, and much of its equipment reflects this intention. Evidently the peripheral role of ESRIN within ESRO has weakened its claim on public funds, but its supporters claim that it is one of the few examples of an international laboratory which has been an entire success.

Present and former members of ESRO complain that it will be a great waste if the laboratory is closed down just when it has been built up to full strength. Younger scientists who have recently left apparently secure positions in their own countries to work at an international laboratory are likely to be left with a great sense of frustration. Investment in ESRIN so far amounts to $10 million. The annual running cost is 2.5 per cent of ESRO's total budget of $80 million. Although it may be held that ESRIN was not strictly necessary, closing it down is certain to be widely regarded as a setback.

DETERGENTS

Phosphates to Stay?

COMFORTING remarks about the effects of detergents on the environment have become a feature of the annual reports of Procter and Gamble Ltd. In a special section in the report for 1970–71, the company says, for example, that the work carried out in Procter and Gamble's laboratories on enzyme washing powders shows that they are "safe for consumer use" and it quotes the British Government's Standing Technical Committee on Synthetic Detergents as support for its contention that enzyme detergents do not pose any problems in rivers or in sewage works (*Twelfth Progress Report of the Standing Technical Committee on Synthetic Detergents,* HMSO, 1971; £0.22½).

As far as phosphate residues are concerned, the Procter and Gamble report again quotes the Standing Technical Committee and says that it is wrong to assume that the eutrophication problems experienced in the United States are applicable in Britain. In the United States, much more sewage is discharged into lakes where there is little water movement and the largest single cause of eutrophication is thought to be compounds such as sodium tripolyphosphate which are included in detergents as water softeners and emulsifying agents. A recommendation by a United States congressional committee in April 1970 sent detergent manufacturers hurrying off to develop alternatives, but three government agencies last week declared in favour of phosphates because they seem to be less of a hazard than some of the substitutes, such as sodium nitrilotriacetate, about which there are still unresolved doubts on health grounds (*Nature,* **233,** 229; 1971).

According to the Water Pollution Research Laboratory at Stevenage, only 46 per cent of the phosphate content of sewage in Britain is in any case attributable to detergents, the remainder arising from human excreta and from industrial effluents (chiefly from metal finishing processes). Only about 70 per cent of the phosphate content of detergents could be replaced without seriously impairing the detergent function, so the advantage to be gained from the introduction of other compounds—whose properties are relatively unknown—would only be a decrease of about 30 per cent in the proportion of phosphate in sewage. It looks as though phosphate detergents are here to stay, at any rate until the alternatives have been proved to be safer.

The report of the Standing Technical Committee also says that even if phosphate-free detergents were manufactured in Britain, it is unlikely that the growth of algae would be very much inhibited.

INTELLIGENCE

Who Watches Whom?

from a Special Correspondent

THE first international conference on Communication with Extraterrestrial Intelligence on this planet was held at the Byurakan Observatory in Soviet Armenia from September 5 to 11. A joint project of the US and USSR Academies of Sciences, the conference was attended by fifty-six official delegates with one representative from each of Czechoslovakia, Hungary and the United Kingdom. There were present not only astronomers and communications experts, but biologists, historians, anthropologists, linguists and others, including two Nobel laureates, Charles Townes and Francis Crick. The subject, once treated as a bemusing speculation, is increasingly respectable.

At the symposium, much attention was given to the abundance of technical civilizations in space. Some old uncertainties were disposed of. In particular, the neurophysiologists painted a clear picture showing that one should expect everywhere the development of a complex central nervous system leading to an intelligent brain. The historians showed that this in turn leads to a highly systematized and organized society which inevitably develops technology. Ignorance in key areas, however, haunted the discussion. Although the first steps in the evolution of complex organic molecules from the simple molecules of the primitive planetary atmospheres have now been traced, these experiments have not come close to producing the key molecules of contemporary life such as DNA, and have not demonstrated that the development of such molecules is inevitable. The uncertainty prevents the transfer of the origins of life from the realm of miracles to that of statistics, to paraphrase Philip Morrison. We fall back on subjective probabilities of great uncertainty.

The abundance of detectable civilizations should be proportional to their longevity as power emitting entities. Fears have grown that civilizations only slightly ahead of those on this Earth will be so good at conserving energy that there is none wasted for us to detect. Indeed, it may be possible to detect only those that want to help us, and even those may be few; as Carl Sagan put it, they may have little interest in conversing with a species as antique, in their view, as we are. Compounding the probabilities, both statistical and subjective, the feeling developed that it was probably thousands of light years to the nearest detectable civilization.

It was noted that we might first detect other civilizations through evidence of astro-engineering activities such as the construction of devices to capture all the energy of a central star, this energy eventually appearing as thermal pollution in the far infrared.

But it was still quite generally agreed that the most likely route to detection of and communication with other civilizations is through electromagnetic radiation particularly at radio frequencies, where some existing systems could detect themselves across half the galaxy. There is still a search for syllogisms which would lead to optimum frequencies and places to search. Surprisingly, what had been a simple situation has become complicated. Where once there was one special radio wavelength on which to listen for other creatures, the 21-cm wavelength of hydrogen, there is now a multitude, including spectral lines of water, the stuff of life, more intense than any hydrogen line. There is no clear choice among them. Once there was a microwave frequency where the cost of transmission was minimal, with the cost increasing at lower frequencies due to cosmic noise, and at higher frequencies due to quantum noise. Now the existence of the universal 3° black body radiation has added a new noise source which spreads the most economical frequency from 1,200 MHz to 60,000 MHz. To make things worse, it no longer even makes much sense to examine the nearest stars first. It has been shown that if there is a range in the luminosities of civilizations in which only a small percentage are much more luminous than the rest, then the ones most visible to us will not be the nearest but the intrinsically brightest. These may lie in any direction, and so there is no guidance as to where to look. Such a situation is not unprecedented; the radio galaxies exhibit just this behaviour.

Despite these new uncertainties, the much discussed conclusion that success would lead to great scientific and social benefits, and the striking potentialities of the existing instruments, produced a remarkably strong consensus that major efforts should be made to search for extraterrestrial intelligent life. It was recommended that existing instruments make the valuable and promising searches they can make. Among other recommendations, it was also suggested that studies should be made of the design of giant instruments for searches: a radio telescope 1 km or larger, a 100 metre millimetre wave telescope, a thirty metre submillimetre wave telescope and a ten metre infrared telescope. The first of these, which has many other uses, could cost billions of dollars, a figure thought, after all, not out of keeping with the importance of the subject, and consistent with the sums spent on space and nuclear research. The Soviet workers at Gorky, under V. S. Troitsky, have in fact recently initiated a search for signals, so far testing a dozen stars, and watching for pulsed emissions from anywhere in the sky; the work continues.

The conference set up a committee to organize future conferences and meetings to coordinate research, if desirable. So far, such organizational activities and actual research have been confined entirely to the United States and the Soviet Union for no profound or good reason. It was expected that participation in the discussion and research in extraterrestrial intelligent life will spread elsewhere.

HUMAN ECOLOGY

Hopes Balance Fears

OPTIMISTS and pessimists were evenly matched at the annual symposium of the Eugenics Society in London last week. The subject was the now familiar population and pollution and Dr Eliot Slater of the Institute of Psychiatry, University of London, began with a warning that population growth, which meant that "a man does his neighbour more harm than good just by staying alive", was forcing society to adopt new standards of behaviour which were to him distasteful. Population pressure was undermining people's natural benevolence. The maintenance of social order requires more and more state intervention, with the result that human identity will be "increasingly translated into a set of digits on a computer". More crowding means more arousal—then drugs and even greater dangers, rage, despair and organized violence. The nuclear family has already been reduced and the pace of social change is such that "we are like a man racing down a mountainside whose legs cannot keep up with his centre of gravity". Does the encouragement of suicide follow the encouragement of abortion? Is it possible to avoid disaster if we regard man as a measure of all things?

The anthropologists took a longer view. People, even primitive people, have always damaged the environment. They are only worried about it now because they can no longer escape the results. The moors of Yorkshire and Wales, like the deserts of North Africa, are monuments to previous occasions on which people have been able to upset the delicate ecological balance. Pollution is not new, only the perception of it.

The environmentalists tended to the view that pollution is a problem in public administration. The urgent need is for collaboration at the level of local, national and international government. The herrings are disappearing from the North Sea, but DDT is probably not the explanation. So far as this symposium is concerned, the moral seems to be that the fight against pollution must and should begin at home.

The geneticists were more worried by chemicals than by radiation. Dr Mary Lyon, Girton College, Cambridge, argued that the radiation from nuclear fall-out is likely to be less mutagenic than the medical use of X-rays. Dr M. S. Legator of the United States Food and Drug Administration, on the other hand, took the view that respectable investigations of the mutagenic effects of certain chemicals had only just begun.

The sociologists opened with a new warning that it is affluence and not technology or over-population that spells pollution. In the maddening manner of their discipline, they argued that research tends to be concentrated not on the important problems but on those which can be solved, however trivially. To them, to be practical, zero or even negative population growth would be a worthwhile goal.

The capstone of the symposium was the Galton lecture by Dr C. L. Carter, an optimist. The new eugenics, he implied, is the amalgam of social work and genetics which makes it possible to avoid needless misery while allowing people freedom. Dr Carter argued that once family planning is effective the more intelligent parents will have the larger families, resulting in a higher average IQ. He looked for an even more rapid adaption in the future of "genetic endowment to fit society".

PATENTS

Inventions Incorporated

PLANS for the establishment of a European patent office are finally taking shape and the first European patents will probably be granted in 1973. The intergovernmental conference that has been sitting since 1969 has recently published a draft agreement and it is hoped that final recommendations will be made by June 1973.

The notion of a European patent was first put forward in 1955 by the Council of Europe, which agreed on a system of classification of patents that was eventually accepted in 1968. In 1962, the European Economic Community set up a working party to prepare a draft European patent but the plan was shelved until 1969, when the present conference of nineteen European countries was set up.

The European patent is being welcomed by the British Patent Office and Mr J. D. Fergusson, assistant comptroller, estimated this week that the setting up of a European patent office will decrease the number of annual applications from 60,000 to 155,000 or 20,000. He added, however, that the reported date of achieving the European patent was highly optimistic and that 1976 would be more realistic. The decrease in the number of applications to the British office would be reflected by a decrease in staff but it would not lead to redundancy as the European office would need qualified people. The site of the European office has not been decided although Mr Fergusson said that it must be within an EEC country.

The advantages of a European patents office are that it will avoid duplication of applications and work. Ideally, national patent offices should cease to exist when the European office is working, but Mr Fergusson said that it was not planned to close the British office and that a substantial number of applications would still be received as the cost of obtaining a European patent would be high. Most of the overseas applications, now about 60 per cent of the total, would go to the European office but a trickle of applications from the United States would still be received. The British Patent Office is planning to carry out a market research survey in 1972 to see how many of the British applications will be channelled through the European office.

The standards to be set by the European office are more stringent than those now in force in Britain and most European countries and comparable to those of West Germany. Agreement has already been reached by the nineteen countries presently negotiating that if the European office finds a patent invalid then it will also be declared invalid in the member countries. If an application is made simultaneously to the British and European offices, Mr Fergusson said that the British application would be suppressed until the result of the European patent is known.

The logical step to take after setting up a European office would be to obtain world-wide standardization of patents. Mr Fergusson said that this was not feasible because of the different concepts—now standardized in Europe—of the essential ingredients of a patentable idea. It seems, however, that this would be a laudable aim and the Patent Cooperation Treaty set up last year (see *Nature*, **226**, 888 ; 1971) goes a long way towards achieving it. This treaty eases the work on patent offices by sharing the exploratory work, but applications have still to be made to individual countries to register patents.

COMPUTERS

Profit the ERA Way

WITH declining sales and profits rapidly turning into losses in the computer service industry, it is surprising to find the Electrical Research Association launching a new company, Electra Computer Services. But this is a logical development of ERA's activity in the computer field over the past two years, and points the way for future profitable developments in the industry. The organization of the ERA is more like that of comparable American research institutions than of British research associations—more than 90 per cent of its activities are carried out under contract without the aid of government funds. There is no government support for the computer unit, which was established two years ago, chiefly for the benefit of members of the ERA.

Why has the ERA been successful when other computer service companies are falling by the wayside every week? The association says that it provides a personal service. The managing director of the new company is Mr Paul Addison who has headed the Association's computer activities since they started. He emphasized that customers are not really concerned with how a computer bureau solves their problems but only the accuracy of the solution. Whereas most bureaux fall into one of two categories—providing solutions to scientific and technical problems by the flexible use of their hardware, or selling off the peg packages to solve standard problems such as payroll calculations—the bureaux of the future must provide a complete data processing service to be credible. It is the aim of the ERA to provide such a service with Electra, solving unique problems or providing standard services as required, so that the customer has the same sort of close contact and versatility which he could expect from his own in-house computer.

The association claims that its customers have been persuaded by experience that it is often better to buy computer time rather than a machine. In one case, for example, it says that a customer was able to reduce his annual expenditure from £70,000 to £15,000 by forsaking his machine for the association's service. The association also claims its reputation with industry now allows it to give potential clients a feeling that the provision of computer services is not another way of robbing them of cash. For customers new to the use of electronic computers the ERA says that its services are a useful intermediate step to the purchase of a machine.

Appointments

MRS MARGARET THATCHER, Secretary of State for Education and Science, announced last week the appointment of new members to the Natural Environment Research Council, Medical Research Council, Computer Board for Universities and Research Councils and the Advisory Committee for Scientific and Technical Information.

Four new members are appointed to NERC: Professor P. Allen, University of Reading, Professor R. B. Clark, University of Newcastle upon Tyne, Professor K. M. Clayton, University of East Anglia, and Dr H. C. Pereira, East Malling Research Station. From October 1, 1971, NERC will be composed of the new members together with Professor F. H. Stewart as chairman, Professor Sir Edward Bullard, Professor R. W. Edwards, Lord Howick of Glendale, Rear Admiral Sir Edmund Irving, Dr C. E. Lucas, Mr T. A. L. Paton, Professor F. W. Shotton and Sir Owen Wansbrough-Jones.

The Medical Research Council also has four new members: Professor D. A. K. Black, University of Manchester and Manchester Royal Infirmary, J. P. Bull, MRC Industrial Injuries and Burns Unit, Birmingham Accident Hospital, D. G. Davey, Imperial Chemical Industries, and Professor R. B. Welbourn, University of London, Royal Postgraduate Medical School and Hammersmith Hospital. The non-retiring members of the council are: His Grace the Duke of Northumberland (chairman), Professor Sir Richard Doll, L. Pavitt, Professor W. S. Peart, Professor D. A. Pond, Professor R. R. Porter, Professor T. Symington, Professor P. M. B. Walker, Professor R. E. O. Williams and Dr J. A. B. Gray.

The Advisory Committee for Scientific and Technical Information is increased to twelve members by the appointment of Professor G. Black, University of Manchester, Professor L. Maunder, University of Newcastle upon Tyne, and Dr R. Weck of the Welding Institute to the committee. The other members are: Dr L. Rotherham, chairman, Dr J. W. Barrett, Mr J. G. N. Brown, Dr G. M. Dyson, Mr. H. J. Habakkuk, Professor J. B. Jepson, Mr N. Mackenzie, Mr D. T. Richnell and Professor G. D. Sims.

Professor G. A. Barnard, University of Essex, and Professor J. C. Gunn, University of Glasgow, are appointed to the Computer Board for Universities and Research Councils. The other members of the board are: Professor D. J. Finney, chairman, Mr A. H. Duncan, Dr J. Howlett, Dr K. W. Morton. Professor D. J. Newell and Professor M. H. Rogers.

Gesture of Independence

THE Science Research Council has given the government a clear hint in its report for the year ending on March 31 (HMSO, £0.65) that the present review of arrangements for spending public money on scientific research should not be completed without full consultation. In particular, the council says that it "looks forward to the publication of the Dainton report and the opportunity to discuss its findings before any decisions are taken on the reorganization of civil science". The Dainton report, commissioned by the Council for Scientific Policy, was completed in May this year, and has since been regarded as a part of the material with which the Rothschild review should be provided.

The Science Research Council is also as open about its wishes as any official body might dare. The report says that the council reaffirms "its belief in the present role of the research councils and stresses the need for close collaboration and cooperation between all the bodies contributing to the support of civil science". It also asks for closer links between "those in industry and government who are concerned with the role of science in national life". Although the council can boast only of the somewhat lukewarm collaborative document on pollution research as evidence of the way in which the research councils might collaborate, it promises more studies of other common interests.

In its own defence, the Science Research Council says that autonomous organizations are best able to judge the "intrinsic merit" of proposals for expenditure in education and research. It draws attention to the way in which its position outside the government proper enables it to call on the voluntary services of people "with the experience and qualifications to advise how public funds can best be used", pats itself on the back for its policy of selectivity and concentration and promises that it will be increasingly vigilant in trying to match the directions in which research and education are supported with what are called national needs.

The council says that the diminished pace of growth of expenditure will begin to restrict the council's activity in the coming financial year. The report says that "beyond March 1972, it is certain that the funds available will be such that it will not be possible for the council to carry out in full the programme which could be justified on educational scientific grounds". The council is worried that the university population will be growing more quickly than its own resources and it says that there will be an even greater need for the determination of priorities in different fields of the council's work. The council is particularly anxious about long-term capital projects now urgently needed in Britain.

On strategy for research, the report says that it will continue to give priority to engineering and the applied sciences as well as to areas of research which have been picked out for their promise of intellectual and practical benefit. The council says that it will do what it can for astronomy and in particular help to build an observatory in the northern hemisphere, but that the cost of participating in the project to build a 300 GeV European accelerator will have to be accommodated, as every high energy physicist has known for more than a year, by reducing the cost of the domestic programme. On relationships with the universities, the council says it wishes no change in the present division of responsibility for government support of university research between itself and the University Grants Committee, but that in future the practice will be abandoned of transferring funds from the Science Research Council to the University Grants Committee for the support of research projects begun under the council's auspices.

There is also to be a relaxation of the rules which have so far restricted the numbers of people employed as research assistants with the help of research grants, although the council says that the duration of these research posts will be limited so as to persuade both the universities and the people concerned that more permanent arrangements would be in their best interests. In spite of the council's previous attempts to divert research students from PhD courses to other courses of advanced training, possibly as preliminaries, the number of new research studentships increased by seven per cent to 2,158 in 1970 and the number of awards for advanced courses by two per cent to 1,568.

The report says that for the first time the council had to turn away qualified applicants in engineering departments. In 1970, 32 per cent of its research studentships were awarded to people in applied science departments, but since 1969 the pace of growth has been faster in the pure-science departments (10 per cent) than in the applied science departments (5 per cent). There has, however, been a substantial increase (from 180 to 200) of the cooperative awards in pure science by means of which people working in industry are enabled to carry out research at a university. The council is also pushing hard for different kinds of PhD courses, preferably with a broader base. The Engineering Board of the Science Research Council seems to have been especially active in the past year in assessing what it calls the "total technology" needs of young engineers.

Expenditure by the SRC in 1970–71 amounted to £51 million, an increase of £4 million on the previous year. The Rutherford Laboratory remains the largest of the council's domestic pensioners (£8 million in 1970–71), the Daresbury Laboratory with its electron accelerator comes next (£4.5 million) and the Atlas Laboratory and the Radio and Space Research Station cost £1.16 and £1.63 million respectively. Grants to universities cost a total of £9.65 million, with engineering the most generously treated (£3.89 million), nuclear physics still keeping its head above water with £1.56 million and the Astronomy, Space and Radio Board generously increasing its expenditure by more than 20 per cent to £1.12 million.

The report welcomes the international collaboration which allows British scientists to use facilities abroad and enabled 220 foreign scientists to spend up to a year in Britain last year.

The report is also enthusiastic about collaborative programmes, drawing particular attention to the UK interest in the Institut des Hautes Etudes Scientifiques, the proposed use of the Franco–German High Flux Beam Reactor at Grenoble and the Radio and Space Research Station link with the Italian satellite project SIRIO, and the SRC is particularly delighted that the government—at the SRC's consistent recommendation—has agreed to UK participation in the CERN 300 GeV project.

Disappointment is expressed that the discussions on the future of the European space programme have so far been unproductive, no agreement being reached on either a future European programme or on European involvement in NASA's post-Apollo programme. But the SRC is encouraged by Britain's individual involvement with NASA, citing the presence of American experiments in the payloads of the UK-4 and UK-5 satellites, the provision of experiments for the American programme by British scientists and the continuing analysis of lunar samples in Britain. The SRC also welcomes the possible future involvement in the ultraviolet astronomy satellite SAS-D.

Pure Che stry Library
SCIENCE COLLEGE
92 Upper Circular Road
CALCUTTA

NEW WORLD

DDT Nailed Again

by our Washington Correspondent

A SCIENTIFIC advisory committee appointed by William D. Ruckelshaus, Administrator of the Environmental Protection Agency, has added its voice to the chorus of opposition to the use of DDT in the United States. Five top level scientific committees in almost as many years have recommended that the pesticide be phased out of domestic use as soon as possible, but in spite of the armoury now available to pesticide critics, the battle of words over the safety of DDT is far from finished.

So far this year, DDT has been the subject of two decisions of the US Court of Appeals, its use in the United States has been cancelled by the Environmental Protection Agency (EPA), that decision has been appealed against by chemical manufacturers, a lengthy public hearing has been started and a scientific advisory committee has issued a report. These skirmishes are, however, the result of a skilful rearguard action being fought by pesticide manufacturers to delay a ban on DDT, and there seems little likelihood of anything other than final victory for the anti-pesticide forces.

The first indication that the tide had turned against DDT came in November 1969 when the Department of Agriculture cancelled four major uses of the pesticide in the United States—on tobacco, shade trees, over water and in the home. Spurred on by the taste of blood, environmental groups led by the Environmental Defense Fund initiated a sequence of court actions which culminated in a decision of the Court of Appeals in January this year directing the EPA to cancel all remaining uses of the pesticide, and to consider suspending its registration (immediate suspension would prevent DDT from being marketed while the cancellation process takes place).

The EPA obediently cancelled all uses of DDT in the United States but concluded that suspension is not warranted, thereby setting in train a public hearing, another court action and a study by an advisory committee. The public hearing and the setting up of the advisory committee were requested by manufacturers exercising their rights under the Federal Insecticide, Fungicide and Rodenticide Act to appeal against a cancellation order, and the court action was brought by the Environmental Defense Fund in protest against the EPA's decision not to suspend registration of DDT. The upshot of the bureaucratic manoeuvring so far is that the advisory committee has recommended rapid phasing out of all uses of DDT, but that the pesticide should continue to be made available to the public health service for "control of disease-bearing insect vectors until satisfactory alternatives are developed", the appeals court has instructed Ruckelshaus to reconsider his decision not to suspend registration of DDT and to make a final decision by November 1, and the public hearing is likely to drag on until next year.

Ruckelshaus has ninety days in which to respond to the advisory committee report, and with two court decisions to back him up, he is unlikely to revoke the cancellation order. But if he is looking to the report for inspiration to help decide the vexed problem of suspension he will be disappointed, for on the imminence of the hazards associated with use of DDT, the advisory committee is skilfully ambiguous. It suggests that present use of DDT "does not present an imminent hazard to human health, in terms of bodily functions and safety", but continued use of DDT is "an imminent hazard to human welfare in terms of maintaining healthy desirable flora and fauna in man's environment". Even if all present uses of DDT were outlawed, however, the committee points out that because of the present levels of the pesticide and its persistence in the environment, a rapid decrease in the use of DDT will probably achieve the same result as suspension.

The committee bases those observations on the finding that in spite of the heat generated by the battle of words over DDT, there is little evidence that it is acutely toxic to man, no evidence that it is teratogenic and, on the basis of present intake of DDT and its metabolites, "the probability of tumorigenesis and carcinogenesis is low". The case against DDT therefore rests essentially on its effect on non-target species and on the ecosystem itself, and here the committee is able to cite a sheaf of undesirable consequences of widespread use.

By far the most damaging behaviour of DDT is that it can remain in the environment for several years in its original form or as the metabolites DDD and DDE. The result is that in spite of a rapid decline in the use of DDT in the United States during the 1960s (from about 70 million tons a year in 1962 to about 30 million in 1969), there has been no parallel decrease in the contamination of the environment. Levels of DDT in the soils of south-western, southern and

Table 1 Predicted Concentration of DDT and DDE in Human Adipose Tissue with Various Assumptions about DDT Usage

Year	Continued reduction of DDT usage at present rate	Zero future usage of DDT	DDT usage maintained at 5×10^6 lbs./year	DDT usage maintained at 1966 levels
1970	5.14	5.14	5.14	5.30
1974	4.18	4.13	4.20	5.08
1978	3.41	3.35	3.53	5.32
1982	2.78	2.73	3.02	5.60
1986	2.27	2.23	2.60	5.84
1990	1.86	1.82	2.25	6.03
1994	1.52	1.49	1.98	6.20
1998	1.24	1.21	1.75	6.32
2002	1.01	0.99	1.56	6.43
2006	0.82	0.81	1.41	6.52
2010	0.67	0.66	1.29	6.59
2014	0.55	0.54	1.18	6.65
2018	0.45	0.44	1.10	6.70
2022	0.37	0.36	1.03	6.73

eastern seaboard states still stand at between 0.10 and 0.56 parts per million, although in orchard soils they have been found to reach 52 ppm, and DDT is still found in rivers and lakes at concentrations approaching the limits of its solubility.

What seems to be happening is that run-off from agricultural soils has pumped DDT into the rivers to the extent that it has settled in bottom sediments. This creates a pool of DDT which is stirred up and released into the atmosphere by storms, and the rivers are continually being recharged by run-off from the soil. The upshot is that there is no prospect of marked decrease in water contamination until levels in soils and sediments are decreased—a process that will take years even if no more DDT is pumped into the ecosystem of the United States.

The chief cause of concern over the continuing contamination of the marine environment by DDT is that its high solubility in body fats causes it to accumulate in the tissues of animals in the higher levels of the food chain, sometimes with devastating consequences for animal populations. Evidence that the decline in populations of the peregrine falcon and bald eagle is linked to the build-up of DDT or its metabolites in their tissues is already well documented, and a committee of the National Academy of Sciences concluded earlier this year that marine fish are almost universally contaminated by chlorinated hydrocarbon residues. What happens is that phytoplankton accumulate DDT from water, and the pesticide then finds its way into the tissues of fish, and so on up the food chain. An example of how the levels of DDT become magnified in living tissue is the fact that half of the fish caught in Lake Michigan in 1966, for example, had at least five parts per million of DDT in their tissues, compared with 0.0012 parts per million in the water.

Although that sequence of contamination has been drummed into the public through the protestations of environmentalists for the best part of a decade, the committee nevertheless complains of a lack of hard evidence on which to base predictions of the body burden of DDT in future years. Present evidence indicates that a man consumes about 0.01 mg of DDT or its metabolites a day, and has about 6 ppm of DDT and DDE in his adipose tissue, but while DDT intake has been more than halved since 1966, tissue concentrations have declined by less than 25 per cent.

The committee believes that levels of DDT in food are now in equilibrium with levels in the environment, and since the environmental burden is unlikely to decline very rapidly, even if no more DDT is used in the United States, food levels will probably remain fairly constant over the next few years. Moreover, since average concentrations of DDT in human tissue have not yet reached equilibrium with the concentrations in food, they will continue to remain high for some time to come. These predictions are, however, necessarily hand waving extrapolations from incomplete data, and the committee decided to explore the possibility of devising a simple systems model to predict the level of DDT metabolites in man up to the end of the decade.

A study carried out by R. V. O'Neill and O. W. Burke of the Oak Ridge National Laboratory concluded that even if no more DDT is used in the United States, the adipose tissue of Americans would still contain 0.36 ppm by the year 2022. Moreover, tissue levels of DDT will decline at almost identical rates if DDT usage continues to decline at its present rate, or if the pesticide is removed completely from the US market. A small use of DDT will, however, have a marked effect on tissue concentrations at the end of the century (see Table 1).

Is the alarm engendered by the build up of DDT in human tissue matched by evidence of its chronic toxicity? The advisory committee believes not, and it bases its *sang froid* on the fact that there is more information on the toxicity of DDT than on any other pesticide, and yet there have been no well documented reports of fatal uncomplicated DDT poisoning and there is little evidence of carcinogenicity at the levels encountered by man—although the committee is quick to point out that firm conclusions cannot be drawn.

The committee cites studies carried out in 1956 which showed that rats fed DDT in doses of 15 ppm produced hypertrophy, inclusion bodies and cytoplasmic granulation in the liver, but that these changes were reversible after withdrawal of the diet. A later study by the Bionetics Research Institute, and studies carried out under the auspices of the International Agency for Research on Cancer, both showed a statistically significant increase in the incidence of hepatomas, and a study carried out in 1967 produced evidence of hepatic cell tumour in trout fed relatively low doses of DDT. These results have led the advisory committee to conclude that "the evidence to date clearly shows that DDT induces hepatomas and suggests it may be carcinogenic", but studies of workers in DDT factories, where average daily intake of the pesticide probably reaches about 18 mg, have produced "no clinical or laboratory effects attributable to exposure to DDT". Nevertheless, the committee suggests that long-term studies of occupational exposure to DDT are urgently needed, and that in spite of the evidence that there is little cause for alarm from health hazards, the possibility of carcinogenicity cannot be dismissed.

As for toxicity to animals and birds, however, the committee points out that data are incomplete and further research is needed to support definite conclusions. Nevertheless, there is sufficient circumstantial evidence to link build up of DDT levels in some species with their decline in numbers, and "there is sufficient toxicological information on DDT in aquatic species to indicate that reduction and prevention of contamination of water sources is a problem of major concern".

Materials Regrouped

by our Washington Correspondent

REPERCUSSIONS of the so-called Mansfield Amendment, directing the Department of Defense to keep its nose out of research not directly related to weapons development, are still being felt in the National Science Foundation. Apart from being given an extra $40 million in its budget to pick up projects dropped by the Defense Department, the NSF has inherited responsibility for so much materials science that it has decided to regroup its support programmes. A new division of materials science has been set up to look after the twelve interdisciplinary research laboratories acquired from the Advanced Research Projects Agency—a defence department agency designed to get advanced projects moving (it was at one time responsible for the Arecibo telescope)—and the National Magnets Laboratory that was once the responsibility of the Air Force. The new division will also take charge of the materials science activities that were funded by the other divisions of the NSF. The foundation last year distributed grants worth about $30 million for materials science, and its stake in the field is expected to double this year.

The director of the new division is Dr Harold W. Paxton, recruited to the NSF from the metallurgy department of Carnegie-Mellon University, and the deputy director is Dr Howard Etzel, formerly director of the NSF's Solid State and Low Temperature Physics Program. The chief problem facing the division at present is that although it is already formally constituted, it has run into staffing troubles because of restrictions imposed on government agencies by the wages and prices freeze.

These conclusions have led the committee to recommend that the use of DDT be phased out, but that it remains available for the control of disease bearing insects at least until satisfactory alternatives have been developed — a conclusion that has already been interpreted by the court of appeals as calling for suspension of the pesticide, and by some manufacturers as an acceptance of their thesis that DDT is the safest pesticide to use in most applications. Although the days for DDT in the United States are clearly numbered, there is no sign as yet that it will be allowed to pass peacefully.

MANPOWER

A Modest Plan

by our Washington Correspondent

THE Department of Labor has put up $3 million to help find work for highly qualified scientists and engineers who are at present swelling the ranks of the unemployed. The money will be used to establish internships in federally funded laboratories for some 420 young unemployed scientists and engineers who hold advanced degrees. A brainchild of the Office of Science and Technology and the Department of Labor, the scheme is being administered by the National Science Foundation, and it is expected to swing into action in the near future.

The idea was sketched out in a memorandum addressed to all directors of federally funded laboratories with more than 100 professional staff on their payroll, sent out last week by Raymond L. Bisplinghoff, acting director of the NSF. It explained that the foundation will provide $7,000 to pay the salary of each intern on a one year non-renewable basis, provided that the laboratory at least matches that sum to supplement the salary and to meet overhead costs. Laboratory directors have been asked to furnish the NSF with an estimate of the number of internships which can fruitfully be used to the mutual advantage of the laboratory and the intern, and the foundation will parcel out 420 internships on a first-come first-served basis. Interns will then be recruited by the laboratory with preference being given to veterans and those from areas of particularly high unemployment, which in general means areas in which defence and aerospace industries are concentrated.

Edward E. David, the President's science adviser, said last week that he hoped that the interns would be employed in positions "where they can best benefit the nation and themselves" —projects concerned with pollution, rubbish disposal, nuclear energy and the like. But the NSF memorandum made no mention of any constraints on the type of project except that care should be taken "in assigning interns to tasks which will improve their potential for later job placement".

The scheme is an offshoot of a $42 million programme launched by the Department of Labor in April to help find work for scientists, engineers and technicians thrown out of work by cutbacks in the defence and aerospace industries. The thrust of the programme is to provide retraining for about 10,000 people who have a strong possibility of being employed by specific employers, relocation grants for scientists and engineers who find employment out of their home area, and to set up a national registry to help match jobless engineers with job openings. These facilities are limited to applicants from fourteen areas particularly hard hit by unemployment, unlike the internships scheme, which is limited neither by the area in which the applicant is living, nor by his previous employment. One reason for the difference between the schemes may be that the NSF has been able to dictate the terms of the internship project.

The new initiative by the Administration in trying to find employment for some of the jobless scientists and engineers is probably a measure of the political embarrassment in having expensively produced graduates in the dole queues during election year. But the internship scheme is hardly likely to be a decisive influence on the problem, for according to a survey conducted by the NSF, some 4,000 scientists and engineers holding a master's degree or a doctorate were out of work in the spring of this year.

Short Notes

FAS on the Attack

THE Federation of American Scientists has launched a stinging attack on the F-14 carrier-based aircraft programme. A statement issued by the FAS describes the programme as "a purposeless, enormously expensive, gold-plated procurement scandal dependent on a missile that may not work; its only hope is to become a *fait accompli*". The idea of the programme is to provide about 300 aircraft to serve on carriers in "high threat areas", in an effort to protect them from enemy action, but the FAS—which has in the past been a consistent critic of federal arms policy—suggests that carrier-launched attacks are unlikely to be effective in Soviet wars, and that land-based aircraft are unquestionably superior in terms of both capability and cost.

The military appropriations request for 1972 is for $1,034 million for 48 aircraft—a figure which the FAS points out would result in a cost for 300 aircraft of $6,000 million, allowing for research and development on the first batch. "It is a self-evident distortion of priorities to give carriers marginally improved abilities to do a largely unnecessary and hopeless job," the FAS tartly states. The federation also calls the procurement of the F-14 a scandal because the House of Representatives has had no chance to debate the programme. It charges that the chairman of the Armed Services Committee held back the procurement request pending a review by Mr Packard, Deputy Secretary of Defense, of the number of aircraft to be bought, but long before the conference committee report is debated, the Administration will sign a contract for production of 48 aircraft.

Davis lashes OMB

JOHN W. DAVIS, chairman of the House Subcommittee on Research and Development, has lashed the Office of Management and Budget's decision to impound $30 million of the National Science Foundation's funds for support of science education. The OMB's action, which has already led to the abrupt resignation of Lloyd Humphries, an assistant director of the NSF, was cited by Davis in a letter to President Nixon as likely to endanger the "long run health of American science". Davis asked Mr Nixon to take immediate steps to remedy the OMB's action. The subcommittee on Research and Development specifically increased the NSF's budget authorization for science education, and it is naturally chary of having its work negated by a decision of the Office of Management and Budget, taken behind closed doors.

Aggressive Bees

A COMMITTEE of the National Research Council has been set up to plan methods to cope with the possible intrusion into Central and North America of a particularly aggressive strain of honey bee. The bee was accidentally introduced into South America 15 years ago during experiments designed to breed into the ordinary domestic bee the extraordinary work capacity of the African honey bee *Apis mellifera adansonii*. Unfortunately, however, the African bee, which has a painful sting and will sometimes attack when unprovoked, escaped and spread rapidly over South America. Beekeepers in North America fear that if the African bee spreads to North America, its viciousness might lead to demands for eradication of all bees near urban communities.

NEWS AND VIEWS

Cancer and Immunity

It would be hard to find a cancer researcher who would these days deny that the state of an animal's immune defence system crucially affects its response to carcinogens and transplanted cancer cells or that the growth of cancer cells into a tumour is correlated with at least a partial failure of the immune system. Indeed it has been seriously argued that the immune system first evolved as a defence mechanism against autochthonous tumour cells and only secondarily became adapted as a defence against infecting microorganisms and other foreign antigenic materials arriving from outside the individual. But if one of the chief functions of the immune system is to provide a surveillance mechanism which can recognize and destroy cancer cells because they carry tumour antigens on their surfaces which are not present on normal cells, how do tumours ever develop? In other words, by what mechanism is the immune system undermined to such an extent that tumours can grow and metastasize?

In an attempt to shed some new light on this crucially important question, Ambrose, Anderson and Coggin at the Oak Ridge National Laboratory have analysed the role of circulating antibodies during the development of tumours in hamsters injected with Simian Virus 40. And as they report on page 321 of this issue of *Nature*, they believe that cytostatic antibodies play an important part in tumorigenesis, during the early stages of which they seem to prevent the rapid proliferation of the initially arising tumour cells. Once the tumour, in the face of these antibodies, reaches some critical mass, however, tumour antigen becomes locally in excess, swamps the available antibody and cell mediated immunity and uncontrolled growth ensues.

In spite of its limitations, Ambrose and her colleagues chose to use as their model system Simian Virus 40 as a carcinogen for baby hamsters for three chief reasons; first, the virus reproducibly induces tumours; second, all SV40 induced tumours bear the same transplantation antigens; and third, and most importantly, it is possible to block the induction of tumours by immunization with either SV40 virus or SV40 tumour cells killed by irradiation.

The first problem encountered by Ambrose *et al.* was to devise ways of distinguishing the effects on tumorigenesis of humoral or antibody immunity responses from those of cellular immunity mediated by sensitized lymphocytes. That cellular immunity plays an important part in the defence against cancer is, of course, indisputable—non-immune animals, for example, can be protected against tumour cells by injections of lymphocytes from immune animals—but the role of antibody immunity is much more obscure and is something Ambrose *et al.* were intent on elucidating. To test whether antibodies as well as lymphocytes in animals immune to SV40 tumours can kill or at least prevent the proliferation of SV40 tumour cells, they placed such cells in plastic chambers permeable to antibodies in the peritoneal cavities of immune and non-immune hamsters. Circulating antibodies in the immune animals, but not those in the controls, retarded or prevented the proliferation of the cells.

The Oak Ridge group then exploited this technique to follow the course of appearance of these cytostatic, if not cytotoxic, antibodies during the latent period between the injection of SV40 virus into newborn hamsters and the appearance of tumours months later. During the first ten weeks of life the amount of circulating cytostatic antibody increases and in those animals which do not develop tumours, even after 500 days, antibody persists, but in those animals fated to develop tumours the amount of this antibody declines precipitously after about ten weeks. These findings suggest that during the first ten weeks after injection with SV40, transformed tumour cells are present but latent and indeed such cells can be detected by histopathology. Furthermore, by incubating SV40 tumour cells with sera from normal hamsters, immune hamsters and tumour bearing animals, Ambrose and her colleagues proved that sera of immune animals but not tumour bearing animals prevent the multiplication of tumour cells *in vitro*.

There can be no doubt that circulating antibody from immune animals is cytostatic, but sera from animals with tumours lack this material. A further set of experiments established three important facts about this antibody: first, it seems to be invariably produced before tumours appear; second, it retards tumour growth; and third, it is always present in immune animals. And if the transient appearance of the antibody precedes the appearance of tumours, what happens when tumours are removed by surgery; does the antibody reappear? As might be anticipated, it reappears in at least some animals.

What is the simplest interpretation of all these data? Ambrose and her colleagues, who firmly believe as a result of their own experiments as well as those of others, that the so-called tumour specific transplantation antigens are probably normal early embryonic antigens caused to be re-expressed by transformation, draw an analogy between the tolerance of a foetus and the survival of a tumour. They believe that immediately after exposure to a carcinogen, a small tumour mass is established and may for a few hours grow without restriction. But as cytostatic antibodies are produced the rate of proliferation of surviving tumour cells is greatly reduced and during the latent period the tumour mass increases only gradually. When, however, a critical mass is reached the cytostatic antibodies are locally mopped up by an excess of tumour antigen. The tumour cells can then grow rapidly producing more antigen and as this excess builds up circulating antibody becomes undetectable and amounts of antigen always outweigh amounts of antibody, unless of course the tumours are removed by surgery. And once antigen is in excess, antigen–antibody complexes may block cellular immunity and even cause immune enhancement.

This scheme clearly contains many elements of speculation but it is nonetheless attractive and suggests that assays for antitumour antibodies, be they cytostatic or cytotoxic, might provide an early warning system for human cancers still in their latent period and an indication of when treatment of established tumours is being successful.

NUCLEIC ACIDS

Genes Made Manifest

from our Molecular Biology Correspondent

ONE of the prettiest techniques to bubble up from the cauldron of molecular biology in recent years is Inman's denaturation mapping of DNA. The DNA is brought to a precisely defined temperature, or *p*H, at some point within its narrow melting range. Formaldehyde is introduced to prop open the melted parts of the chain, and a population of molecules so treated is examined in the electron microscope. The denatured regions are then indexed along the chain for a sufficient number of molecules on the grid, and correspond to tracts of sequence relatively lower in (G+C) than the rest. An interesting new application for this approach has now been found by Wensink and Brown (*J. Mol. Biol.*, **60**, 235; 1971), namely the identification of a gene, using its characteristic denaturation map as a fingerprint.

It is known that the DNA corresponding in eukaryotic cells to the ribosomal RNA is present in the form of many clustered copies, apparently interspersed by spacer segments, much richer in (G+C). Moreover this ribosomal DNA is separately amplified, and occurs as an extra-chromosomal component, differing apparently only in that none of its cytosine residues is methylated. Such a DNA, when subjected to the denaturation mapping technique, displays in the electron micrographs an immediately apparent periodicity, repeating up to fifteen times in one molecule. To ensure, however, that this effect is no trick of the eye or mind, Wensink and Brown have devised a means of testing for the presence of a periodicity and determining its precision and spacing. The measured distribution is matched against a Fourier function, the wavelength of which is varied until a correlation appears and is maximized. A sharp peak comes up at a periodicity of $5.4 \pm 0.4\mu$, whereas a denaturation map of T2 phage DNA shows only a flat background. From a calibration of the length measurement against T7 DNA, as a molecular weight standard, the repeating unit turns out to correspond to a molecular weight of some 8.7×10^6 which is the same as the molecular weight determined by Dawid and his colleagues of 9×10^6.

The repeating unit consists of a rather readily denaturable region, and another, somewhat shorter and much more resistant to melting, which may therefore be surmised to be richer in (G+C). This is exactly what would be expected from the size and composition assigned to the spacer region between successive copies of the ribosomal RNA gene. A comparison with Inman's results on λ phage DNA, which can be divided into two halves differing by 20 per cent in their (G+C) content, indicates that there is a similar difference between the ribosomal RNA tract and the spacer.

This is a very satisfactory result, but there is still more information in the denaturation maps, for the map of the low-melting segment contains a highly reproducible fine structure, which persists over a wide range of melting. Dividing the repeating unit into 100 elements, a histogram of the fraction of chains in which each element is melted shows a relatively broad region of melting, followed by a trough, corresponding to a much more resistant sequence, then another, narrower peak, followed in turn by a narrow and extremely resistant minimum, and then a mirror image of the first pair of separated peaks. The pattern is thus symmetrical about a short, highly (G+C)-rich segment on which the patterns are most readily indexed. This whole pattern corresponds to the 5×10^6 molecular weight of the 40S ribosomal RNA precursor, which contains the 28S and 18S chains of the large and the small subunit. In conversion of the precursor to these products a small portion of the chain is lost. This portion is believed to be rather rich in (G+C), and may be presumptively identified with one of the two shallower troughs in the denaturation map. This latter must therefore separate the 28S and 18S parts of the chain. The short very stable region can also be accounted for, because partial enzymatic degradation of 28S ribosomal RNA from other animal cells has led to the isolation of a sizable fragment (200,000 molecular weight) containing no less than 80 per cent (G+C). Wensink and Brown have compared finally the denaturation maps of the chromosomal and amplified ribosomal RNA, and found them to be identical.

Lord Kelvin's criterion that only that which can be measured and expressed in figures is science, can also be applied to another interesting essay in electron microscopy. Freifelder (*ibid.*, 401) has given experimental proof to the proposition that intercalative binding in DNA causes a change in the pitch of the double helix with a consequent increase in length. Using monodisperse phage DNA he has measured the length distribution of the molecules as a function of the concentration of added ethidium bromide. The standard deviation of measured lengths in each binding state is only 3 per cent, and the increase in length with ligand concentration reaches a sharp saturation level. At this point the chain has stretched by some 27 per cent. Moreover, the molecules look quite different: instead of a rather crinkly thread, broadly curved filaments are seen. Circular DNA–dye complexes have the appearance of smooth ellipses. It seems then that the hydrodynamic stiffness of the molecule must change on intercalation, so that direct inferences about the length from viscosity or sedimentation measurements are not in fact justified.

Locating a Quasar

OUTSIDE the circle of observational astronomy it sometimes comes as a surprise to learn that the exact positions of celestial objects are not always known, and in particular that there is considerable difficulty in relating positions measured by optical and radio techniques. A little thought, however, makes it seem more remarkable that identifications at optical and radio frequencies can be compared at all in some cases, at least to the accuracy required before theories can be built upon the observations. Even optical positions can only be measured relative to certain reference stars, and at radio frequencies sources can only be pinned down by a technique such as occultation, or, most commonly, by finding an optical counterpart to the source. The embarrassing aspect of the second technique is that it is less than adequate when the object of the exercise is to determine whether an optical source and a radio object are one and the same.

A classic example of the success of the occultation technique was provided by the identification of the source 3C 273, the first known quasar, by timing the eclipse of the radio source by the Moon. But it has proved impossible, until now, to state unequivocally that the small component 3C 273B really is coincident with the associated QSO, because of a difference of 0.7 arcsec between the best optical and radio positions published. Dr C. Hazard and his colleagues at the University of Cambridge and the Royal Greenwich Observatory have rectified this situation, and publish in next week's *Nature Physical Science* positions which confirm the association of the radio and optical objects to within 0.3 arcsec. Unlike the earlier recorded difference (0.7 arcsec) this figure is well within the limits of experimental error. Indeed, the best of the lunar occultation observations gives a radio source position exactly at the mean of the optical positions. Because of the differing reference systems, however, such good agreement is not to be taken at face value. There is no reason to prefer either the radio position or the optical position, so that the best position now available for 3C 273B should be taken as the average of the two positions determined by this work.

COSMIC RAYS

Hobart Conference

from a Correspondent

THE twelfth international conference on cosmic rays was held for the first time in the southern hemisphere at the University of Tasmania, Hobart, from August 16 to 25, 1971. In spite of the large distances involved for most participants delegates from twenty-two countries, not including Australia, attended.

In the high energy sessions the delegates heard of further experiments that had been carried out in the search for quarks and other particles. The contributions did not advance the position, however, and no conclusive new evidence for the existence of these particles was presented. The Sydney quark experiment has not been run significantly since the Budapest conference and no new events were reported. The experiment is currently being refurbished by the inclusion of five high pressure cloud chambers.

At the Calgary cosmic ray conference in 1967, the University of Utah group reported that their underground measurements on the variation of cosmic-ray muon intensity with zenith angle, θ, in the TeV range, showed no "sec θ" enhancement as predicted by theories involving pion parentage. It was suggested that this could be explained in terms of muon parents of very short lifetime, subsequently referred to as X-particles by the Utah group. The observations of the Utah group have continued and have been refined but the onset of the effect seems to have receded to somewhat higher energies and reaches a maximum, only to disappear again at even higher energies. Other experimenters, notably the group at the Tata Institute working in the Kolar goldfields, continue to find no evidence for a departure from the sec θ enhancement. It would seem that perhaps a third independent experiment at a depth of about 1,500 hg cm^{-2} should be carried out to try to resolve these apparently contradictory results.

Details of several large solid iron muon spectrographs being built or recently completed in order to study this effect directly were given at the conference. But the problems associated with the measurement of muon energies of about 3 to 5 TeV, coupled with the low flux of particles at these energies, mean that some time must elapse before these instruments will be able to contribute to an elucidation of this problem.

Although extensive air showers have been studied for several years, the origin and nature of the primary particles are not yet well understood. At the Jaipur cosmic ray conference in 1963 the results of the MIT (Volcano Ranch) group suggested that the slope of the integral primary energy spectrum changed from 1.6 (below 10^{16} eV) to 2.2 (between 10^{16} and 10^{18} eV) and back to 1.6 at energies greater than 10^{18} eV. These "ankles" were tentatively interpreted as reflecting a change in the mass composition of the primary cosmic-ray particles. The part of the spectrum beyond the second ankle was thought to be attributable to primary protons of extragalactic origin.

Perhaps the most significant change at the Tasmanian meeting was the report from the University of Leeds group working at Haverah Park in Yorkshire. These results showed no evidence for the existence of the second ankle. Furthermore, it was shown that by applying the same method of analysis to the MIT Volcano Ranch data, the evidence for the second ankle disappeared.

Preliminary data presented at the conference from the Sydney University Giant Air Shower Recorder (SUGAR) in the form of muon size spectra also showed no evidence for the second ankle at 10^{18} eV. Should these results be substantiated they will constitute an important observation which could lead to queries about the existence of extragalactic radiation. Measurements of the arrival directions of the most energetic showers yield no evidence of any anisotropy even when the galactic centre is overhead.

Several speakers described attempts to link the observed shower properties at sea level with the mass of the primary particles. Some progress in this field was reported by the Imperial College and University of Nottingham groups. It would seem that studies of the electron and muon component coupled with the observations of the radio emissions from showers might well be able to give more definitive answers.

Direct studies of the mass composition of the low energy part of the primary spectrum have continued. By using new techniques including superconducting magnets, knowledge of the light/medium element ratio (L/M) has been extended up to 10,000 MeV per nucleon. It has been found that the value is approximately 0.2, and that the composition at the higher energies is equivalent to that at the lower energies.

Studies of elements with $Z>26$ have shown that the abundance of uranium is greater than expected. The disagreement between the values of Z obtained from nuclear emulsion and plastic detectors has been resolved since the Budapest conference with the exception of one event. Studies of the light and heavy nuclei suggest that the age of the cosmic radiation is between 10^6 and 10^7 yr.

Arabinose Operon *in vitro*

IN *Nature New Biology* next week two groups, Zubay, Gielow and Englesberg and Greenblatt and Schlief, report the successful reconstruction *in vitro* of the regulatory system which controls the expression of the arabinose operon of *Escherichia coli*. Their experiments are notable, for this is the first time that a positive regulator protein which causes the expression of a specific operon has been made to function *in vitro*. Furthermore, these *in vitro* experiments confirm the picture of the regulation of the arabinose operon which has emerged from studies of intact *E. coli* and they provide a method for assaying for the elusive arabinose regulator protein.

Zubay and his colleagues used the cell free system developed by his group to support the regulated expression of the *lac* operon to define the elements required for the synthesis *in vitro* of L-ribulokinase when the system is programmed with phage *θ80dara* DNA which includes the arabinose operon. They find that in addition to cyclic AMP, the activator protein which binds cyclic AMP and ppGpp, cell extracts containing the protein specified by the *ara*C gene are required together with arabinose. Genetic analyses had indicated that the *ara*C gene protein can act both as a repressor and an activator of the arabinose operon and these *in vitro* experiments confirm the latter function of this protein.

Greenblatt and Schlief have, like Zubay and his colleagues, exploited mutants of the *ara*C gene to prove that the *ara*C protein is an activator *in vitro* of the arabinose operon. Moreover, they have shown that under certain conditions the *ara*C protein can act *in vitro* as a repressor of the arabinose operon. These findings fully confirm the idea that the *ara*C protein can exist in two functional states, repressor and activator, in equilibrium and that an inducer such as arabinose favours the activator form. Greenblatt and Schlief have also reached the interesting conclusion that the protein specified by constitutive mutants of the *ara*C gene can activate the arabinose operon *in vivo* in the absence of arabinose not because it is "frozen" in the activator state but because it can respond not only to arabinose but also to some as yet unidentified metabolite always present in growing cells.

Attempts to purify the *ara*C protein by virtue of its ability to bind to arabinose or arabinose operon DNA have all failed, but now that the cell free system for ribulokinase synthesis provides an assay for this protein, its purification can be anticipated and that will open the way to a detailed analysis of a regulatory molecule.

MINERAL RESOURCES

New Prospects

from a Correspondent

TYNAGH, Gortdrum and Silvermines are household words in metal mining today. All are mines in the Republic of Ireland which have been brought into production within the past decade and are likely to be followed by others in the near future. Great Britain can point to a major new tin development, the Wheal Jane mine in Cornwall, and hopes are high for potash, copper and nickel elsewhere in the country. Both Ireland and Britain are being explored for metalliferous minerals on a scale and in ways never seen before. Their geologists and mining engineers came together in Edinburgh on September 10 to describe the new findings and the methods of exploration at a symposium arranged by the Institution of Mining and Metallurgy as one of a group of earth science meetings held to celebrate the centenary of the University of Edinburgh Geology Department.

With so much ore already proved, the geologists working in Ireland inevitably had much to say. Dr R. W. Schultz (Northgate Exploration Ltd, Dublin) gave high marks to geochemical soil and drainage surveys in his analysis of exploration practice. Although reserving judgment on the origin of mineralization in the Lower Carboniferous of central Ireland, Dr C. J. Morrissey (Irish Base Metals Ltd, Dublin), Professor G. R. Davis (Imperial College, London) and Mr G. M. Steed (Imperial College, London) made a lucid comparison of the characteristics of these valuable lead, zinc and copper deposits. In England, the Carboniferous is receiving most attention for fluorite, and Dr T. D. Ford (University of Leicester) and Dr P. R. Ineson's (University of Sheffield) comprehensive account of the Derbyshire orefield will simplify the task of future exploration in that area. The Lower Palaeozoic rocks of Wales, eastern Ireland, the Lake District and southern Scotland together form a major exploration target and the recognition by Mr C. J. V. Wheatley (Imperial College) of an axial belt of copper deposits associated with acid volcanic rocks and bordered by lead–zinc mineralization provides a highly stimulating basis for considering the controls of metallization within them.

The rapid re-development of the Wheal Jane mine into a major tin producer has surprised many people. There is clearly more mineral wealth in Cornwall than is contained in narrow tin lodes and the account of the structural environment of the deposit by Mr B. D. Rayment (Consolidated Gold Fields Ltd, London), Professor G. R. Davis and Mr J. D. Willson (Consolidated Gold Fields Ltd) will help to stimulate further exploration in south-west England. In the Scottish Highlands, reconnaissance investigations by the Institute of Geological Sciences have revealed new evidence of uranium, lead and molybdenum in the Old Red Sandstone sediments and Caledonian granites.

ENTOMOLOGY

Insect/Plant Relations

from a Correspondent

IN contrast to other recent contributions to the subject of insect/plant relations, the symposium held in London on September 16 and 17 by the Royal Entomological Society covered the field in breadth in a way which made it possible to appreciate the overall relevance and interrelations of disciplines ranging from biochemistry to ecology.

In introducing the symposium, Professor T. R. E. Southwood (Imperial College, London) considered relationships in terms of shelter, food and transport. He mentioned in particular the evolutionary hurdle which is seemingly presented by seed plants as food, because in spite of the great abundance of these plants they have been exploited by relatively few insect taxa. Within these taxa, however, the insect/plant systems have co-evolved from predation by the insect on the plant through parasitism to symbiosis.

Biochemical relationships were well exemplified by Dr D. Osborne's (ARC Unit of Developmental Botany, Cambridge) contribution on mutual regulation of growth and development. Secondary substances in the plant may thus control development of the insect or even be needed to induce the female to emit its sex pheromone. Conversely, the insect may affect plant growth, not only by causing malformations but also by stimulating or delaying normal processes of leaf growth and senescence.

The Hon. Miriam Rothschild (Elsfield Manor, Oxford) discussed her work on warningly coloured insect species which sequester and store toxic substance from plants. This was a fascinating account of mechanisms which underlie forms of mimicry defined a century ago but, until now, inadequately examined biochemically. Miss Rothschild described how some tiger moths select only those host plants which retain certain alkaloids toxic to bird and mammal predators; the processes involved in such selectivity were examined by several other speakers. Dr L. M. Schoonhoven (Laboratorium voor Entomologie, Wageningen) described the chemoreceptors of a lepidopterous larva, which respond separately to a wide range of nutritional and secondary plant substances. He suggested that the basis for host plant specificity is more than mere recognition of particular plant substances and is dependent on the insect's ability to recognize the complex of many different substances associated with its own host plant and its nutritional requirements. Professor D. L. Wood (University of California, Berkeley) also described his research on

New Polynucleotide Structures?

BRAM has recently reported some unexpected findings on the dependence of DNA structure in solution on the base composition, and in next Wednesday's *Nature New Biology* he gives results of X-ray scattering studies on a set of two-stranded synthetic polynucleotides in solution. Leaving aside large differences in hydration or binding of counterions, differences in the low angle X-ray scattering envelopes from polynucleotides in solution should mean that they have differences in structure. The double-helical complex, poly dI.poly dC, shows a wide-angle scattering pattern which is different from that of A or B-form DNA. The axial radius of gyration, as derived from low-angle scattering, seems to be larger than that of B DNA and is interpreted to signify that the centre of gravity of the base pairs lies off the helix axis. The same is also found in poly dG.poly dC.

For the alternating double-helical polymer, poly d (I-C) the axial radius of gyration is similar to that of DNA and the alternating poly d (A-T). The corresponding helix cross-section agrees with that found in fibres. Bram finds, however, that the wide angle scattering patterns of both this polymer and of poly G.poly C are different from any others found in polynucleotides or natural nucleic acid.

The double-stranded RNA analogue, poly rI.poly rC, gives scattering patterns which are compatible with the A-DNA-like structure (A′-form) of this polymer, found in fibres. Bram regards this agreement as support for the validity of experiment and the theoretical analysis. The generalization with the data so far available is that in double-stranded polynucleotides with purines and pyrimidines on opposite strands, the radius of gyration is larger than in alternating polymers, and the base pairs lie off the helix axis. Altogether Bram now identifies six different structures for DNA-type molecules. He suggests that the unique configuration of the poly G.poly C raises the possibility that parts of a natural DNA rich in these nucleotides could have a different structure from the rest of the chain.

a bark beetle which kills its host tree following the attraction of massive numbers of beetles to particular volatile substances in frass and resin produced by the activity of perhaps no more than one initial beetle colonist.

The behavioural relationships of an insect to its host plant were emphasized in Professor J. S. Kennedy's and Mr I. H. M. Fosbrooke's (Imperial College, London) discussion on the plant in the life of an aphid. They suggested that the migratory aphid's flight behaviour continues to be influenced importantly by light reflected from plants irrespective of the height at which the insect is flying.

In discussing the plant in the population dynamics of insects, Dr H. F. van Emden (University of Reading) and Professor M. J. Way (Imperial College, London) pointed out that even "chemosensority susceptible" host plants often cause surprisingly large direct mortalities to populations of insects established on them. They also emphasized the role of the plant in providing the framework within which subtle density dependent effects of intraspecific competition operate.

The role of the host plant in the population dynamics of phytophagous insects is still very inadequately understood—and vice versa—as was shown by Dr P. Harris (Canada Department of Agriculture, Belleville), who used examples of successful biological control of weeds to show how introduced phytophagous insects have severely limited the distribution and abundance of a few introduced plant weeds. In general, however, the insect's effect on population dynamics of indigenous plant populations still seems to be largely a matter for speculation except in circumstances where the plant has become dependent for pollination on certain insects.

PACIFIC SCIENCE

Island Conservation

from a Correspondent

THE occasion of the twelfth Pacific Science Congress, held at the Australian National University from August 18 to 27, was taken to celebrate the fiftieth anniversary of the Pacific Science Association which held its first meeting at Honolulu in 1920, under the presidency of its founder, Professor H. E. Gregory.

The congress was addressed by its president, Sir Macfarlane Burnet, after which the Gregory Medal was presented to Dr F. Raymond Fosberg (Smithsonian Institution), the Shinkishi Hatai Medal to Dr Carl L. Hubbs (Scripps Institution for Oceanography) and honorary life memberships were conferred on Dr Sarwono Prawirohardjo (Indonesia) and Sir Maurice Yonge (Great Britain).

Much of the programme of the congress had relevance to the International Biological Programme with its emphasis on conservation. The resolutions submitted by the council at the final meeting reflected the general concern about the environment which, even in remote Pacific islands, is being increasingly degraded by the effects of pesticides, of mining, of the cutting down of primaeval forests and, by no means least, the effect of tourism with its impact on the dignity of native peoples.

Such problems had been the particular concern of a regional symposium on the conservation of reefs and lagoons held before the congress, at Noumea, New Caledonia, under the auspices of the South Pacific Commission in collaboration with the International Union for the Conservation of Nature and Natural Resources (IUCN). A resolution coming from this symposium included reference to the urgent need for appropriate environmental and eological surveys being conducted before any "developmental" project. It also urged intergovernmental action on the imaginative "islands for science" proposal whereby a representative number of islands, largely uninhabited, could be set aside as permanent reserves unaffected by environmental changes induced by man.

It was also suggested that periodic surveys might be carried out on coral reef areas to record long-term fluctuations in the comparison of reef ecosystems. The importance of such knowledge is emphasized by the present widespread population explosion of the coral destroying starfish, *Acanthaster planci*. In this connexion, also, the Pacific Science Council established a new Committee on the Scientific Study of Coral Reefs for the better coordination of work on reefs which, for a variety of uncertain reasons, are now widely endangered.

New Normal Geomagnetic Event

DETERMINATION of the detailed geomagnetic polarity-time scale from continental igneous rocks of the past four million years or so has proved a comparatively simple task, partly because the polarities of young fresh rocks unaffected by appreciable continental drift are determined easily and unambiguously, partly because radiometric dating of such rocks is less likely to be affected by such factors as mineral alteration, and partly because within this period the absolute errors of the potassium-argon dating process are small enough to enable polarity intervals of as short as a few tens of thousands of years to be resolved. For older rocks these advantages are lost; and so it seems unlikely that the detailed pattern of magnetic reversals will ever be delineated for tens or hundreds of millions of years, lest it be on the basis of ocean magnetic anomalies. The essential problem is that even if continental rocks ideal for radiometric dating and magnetic measurement are found, the inherent dating errors will always be larger than all but the longest geomagnetic polarity intervals.

But as Creer *et al.* point out in next Monday's *Nature Physical Science*, there is one geological period within which comparatively short magnetic intervals may be, if not resolved, at least located with a fair degree of precision. This is the period of about 60 million years within the upper Carboniferous and Permian during which the geomagnetic field polarity was apparently almost always reversed. Until recently no normal intervals at all were known within this Kiaman reversed period (about 235 to 290 million years). McElhinny (*Spec. Publs Geol. Soc. Austral.*, **2**, 61 ; 1969), however, has reported a normal event at about 280 million years, which he named the Oak Creek event, and which Burek (*Bull. Amer. Assoc. Petrol. Geol.*, **54**, 1120 ; 1970) later renamed the Graham event. Less well documented is a second normal event present in Italian mid-Permian sediments as reported by Guicherit (*Geologica Ultraiectina*, **14**, 1 ; 1964). But the point is that normal events within the Kiaman reversed interval seem to be so rare that a few which may remain to be discovered are likely to be easily distinguished from each other and from those already found.

And so it has proved in at least one case, for Creer and his colleagues have discovered a new normal event well separated in time from the Graham event. The rocks in question are basalts, andesites and rhyolites of the Porphyritic Series exposed in Mendoza Province, Argentina. All samples were magnetically cleaned ; and so there can be no doubt that normal polarities exist within this series. Moreover, the normal palaeomagnetic directions are consistent with the Permian field of South America as determined from the more common reversed rocks within the Kiaman interval. The stratigraphic age of the samples lies between about 270 and 210 million years ; but, more importantly, the potassium-argon ages of two of the normal samples are 262±6 and 264±6 million years, respectively. The best age for the new normal event within the Kiaman is thus 263±5 million years, which is distinguishable from the Oak Creek/Graham event.

PLANT ROOTS

Water Transport

from a Correspondent

A MOST useful exchange of information and ideas between plant scientists from both sides of the Iron Curtain took place from September 7 to 10 at Tatranská Lomnica, a mountain resort in the High Tatras National Park, during a symposium on the structure and function of primary root tissues. Unseasonably cool weather and showers lessened the lure of the mountains and the botanists were able to give their undivided attention to the matter in hand. The symposium was organized by the Institute of Botany, Slovak Academy of Sciences, and among the topics discussed were the organization and development of the root apex. Dr John Torrey (Harvard University) described some features of the quiescent centre—a group of non-dividing cells characteristically found in the middle of root apices—and speculated about its possible role in hormone biosynthesis.

Other sessions were devoted to consideration of the metabolic and enzyme basis of tissue differentiation and to the functioning of roots in the absorption and transport of water and mineral salts. One of the most stimulating and controversial contributions to the symposium programme concerned the absorption of water by root hairs. Dr Marcel Cailloux (University of Montreal) described an elegant technique which has enabled him to measure absorption of water by different parts of a single hair measuring less than 50 μm in diameter. Cailloux has observed that water is taken up much more readily by the growing tip of the hair than by the maturer regions and he associates this with an aggregation of cytoplasm at the tip. As growth of a root hair declines, so does its ability to absorb water and older hairs were found to excrete water rather than absorb it. These observations cast doubt on the commonly held view that root hairs serve to increase the area of absorbing surface in a root. They also raise the question of the mechanism of water absorption.

According to the classical view, the absorption of water by root hairs occurs by diffusion along a gradient of water potential between the soil solution and cell sap, which is maintained by accumulation of solutes. Cailloux disputes this because he finds that altering the relative humidity of the air surrounding the root hair from 99 per cent to 96 per cent, which is equivalent to an increase in the water potential gradient of about 44 bars, does not alter the absorption rate. Furthermore, metabolic inhibitors, such as malonate fluoride and cyanide, reduce absorption. It is concluded that water uptake by root hairs is controlled by some process other than diffusion which is closely linked with metabolism.

The idea that water is "pumped" metabolically into plant cells is not a new one, but no such convincing evidence has been presented before. A major objection to the concept of active water transport has been the deduction that the resistance of cell membranes to flow of water by diffusion in either direction is so low that a water pump would be grossly inefficient and make impossibly high demands on the energy supplies of the cell. It remains to be seen whether the cell membranes of root hairs, and perhaps other specialized water absorbing cells in plants, have unique properties which enable these cells to pump water efficiently.

STEREOLOGY

Analysis of Structure

from a Correspondent

BERNE proved an excellent choice of venue for this year's meeting of the International Society for Stereology. The president of the society, Dr E. Weibel (Berne University), chaired the congress organization committee and the whole meeting, which took place between August 26 and 31, was run with Swiss precision.

The word "stereology" was coined a little more than ten years ago to cover the discipline of obtaining three-dimensional information from two-dimensional sections or their projections on a surface. There is now a wide interest in stereology, particularly among microscopists, and members attending this meeting—the third international congress—were drawn from such diverse fields as anatomy, materials science and mathematics.

The basics of stereology are obviously the mathematical relationships between the object and the way it is imaged or sectioned. Professor G. Mathéron and his colleagues (Bureau de Recherches Géologiques et Minières, Paris) surveyed their work on the application of the theory of sets to this problem. A particularly interesting contribution from Dr R. Miles (Australian National University) derived one simple multi-dimensional formula from which all the standard formulae of stereology could be derived. Dr E. Underwood (Lockheed Georgia Research Laboratory, Atlanta), who is president-elect of the society, derived the formulae for the stereology of projected images and his approach was reinforced by Professor J. E. Hilliard (Northwestern University, Evanston) in a talk covering quantitative measurements on scanning electron micrographs.

Dr H. Elias (Chicago Medical School), the founder and first president of the society, illustrated the many pitfalls that could occur from a non-stereological interpretation of sections. Professor R. DeHoff (University of Florida) illustrated the use of serial sectioning in determining the topography of grains in metals and derived the basic equations of connectivity.

A major portion of the meeting was given over to the use of computers in image analysis with a survey from Dr G. A. Moore (National Bureau of Standards, Washington). It became apparent that the advent of cheaper computers of various types and interface software had already made stereology considerably easier for a much wider spectrum of scientists.

The generally held view was that this meeting was both excellently organized and scientifically worthwhile. It is quite clear that the International Society for Stereology will continue to grow rapidly and it is hoped that it will continue to maintain its interdisciplinary role.

Viscosity of the Earth's Core

IN next week's *Nature Physical Science*, Dr R. Hide of the Meteorological Office, Bracknell, argues that the kinematical viscosity of the Earth's core is not greater than 10^6 cm^2 s^{-1}. Previous estimates of this parameter have varied between 10^{-3} and 10^9 cm^2 s^{-1} and Hide's new upper limit is a clear step towards pinpointing the viscosity more accurately.

Hide bases his arguments on the discovery of a statistically significant correlation between gross features of the Earth's gravitational and magnetic fields (see Hide and Malin, *Nature*, **225**, 605; 1970), and on variations in the length of the day, both of which suggest that the core–mantle boundary is not smooth but is characterized by bumps which would seem to have horizontal dimensions of as much as a few thousand km and rather small heights of only a few km.

The height of a bump, Hide suggests, must be greater than the thickness of the viscous boundary layer at the core–mantle interface by at least a quantity determined by the horizontal dimensions of the bump, the Alfvén speed, the angular speed of the Earth's rotation and the radius of the core. Using a value of 1 km for the height of the bumps (as suggested by measurements of gravitational distortions) Hide deduces that the thickness of the viscous boundary layer must be $\lesssim 1$ km and, therefore, that the core viscosity is $\lesssim 10^6$ cm^2 s^{-1}.

CANCER CELLS

Chemical Vaccines

from our Cell Biology Correspondent

CANCER cells and cells transformed *in vitro* by chemicals or tumour viruses are about ten times more susceptible to agglutination by lectins such as concanavalin A or wheat germ agglutinin than are normal, untransformed cells. That much is generally agreed by those workers who have used lectins to investigate the surfaces of cancer cells. Why cancer cells are more agglutinable is of course still a matter of dispute; one camp argues that transformation leads to the exposure of previously cryptic lectin-binding sites whereas the other, which currently seems to have the upper hand, contends that it is the distribution of these sites rather than their number which differentiates the two classes of cells, the cancerous and the normal. But in any event there is a difference and it is common to all cancer cells, a fact which Shier has exploited in a remarkable set of experiments published in the current issue of the *Proceedings of the US National Academy of Sciences* (**68**, 2078; 1971).

Shier argues that because cancer cells are more readily agglutinable by wheat germ agglutinin than are normal cells (and he was assuming that this difference reflects a difference in the number of receptor sites exposed), it might be possible to immunize mice against tumours by raising an immune response against the wheat germ agglutinin receptor site. Exploiting Burger's discovery that this receptor is a soluble glycoprotein containing N-acetylglucosamine, he performed what seems to be an almost ridiculously simple and over-optimistic experiment, but the long shot has paid off handsomely.

Shier prepared a synthetic wheat germ agglutinin receptor site by condensing dimeric N-acetylglucosamine residues onto the sodium salt of polyaspartic acid and as a control he crossed linked polypeptide backbones without adding the N-acetylglucosamine residues. Having shown that wheat germ agglutinin has a much greater affinity for the synthetic receptor than for the control, the crosslinked polypeptide, Shier complexed the receptor and the control to methylated bovine serum albumin and used the two complexes (antigens A and B) to immunize mice in the presence of Freund's adjuvant. Two sets of mice, one immunized with the receptor-albumin complex and the other with the control antigen B, were then challenged with various numbers of cells of two BALB/c myeloma cell lines. Mice immunized with the synthetic receptor antigen rejected at least five-fold more tumour cells than those immunized with the control antigen B. Furthermore, the time taken for palpable tumours to develop after exposure to a large dose of the chemical carcinogen 3-methyl cholanthrene was significantly greater after immunization with the receptor antigen than after immunization with the control. Mice which had been immunized with the receptor antigen grew normally and their cellular and humoral immune responses were not impaired although wound healing, as judged by liver regeneration, was slightly impaired compared with that of mice immunized with the control antigen.

In short, Shier seems to have raised an immune response not only against transplanted tumour cells but also against the progression of cells transformed *in vivo* by a chemical carcinogen by immunizing against the wheat germ agglutinin receptor sites. No doubt hosts of laboratories will now be urgently mobilizing their chemists to prepare the antigens. Shier has used and numerous variations on his theme, in attempts to confirm and extend his fascinating observations, which, although they defy simple interpretation, provide a most novel and promising lead to the pipe-dream of immunotherapy for cancer. It can only be hoped they are not too good to be true.

Atlantic Islands and Continental Drift

EVIDENCE has been accumulating that the Eastern Canary Islands (Fuerteventura, Lanzarote and Concepcion Bank) may play an important part in the fit of Africa and North America before continental drift. The most recent of such fits is by Dietz and Sproll (*Nature*, **226**, 1043 ; 1970), who suggest that the most prominent gap (the Ioni Gap) is well matched by the East Canaries block. A small strike-slip translation of the East Canaries would essentially fill this gap. Deitz and Sproll's proposal that the East Canaries constitute a sialic continental fragment (a microcontinent) detached from the African margin is therefore attractive to geophysicists.

Before this idea is accepted, however, it is essential to take into account other evidence which might point to an origin for the Canary Islands (perhaps recent oceanic volcanism) fatal to the hypothesis. The bathymetry of this general region (from US Naval Oceanographic Charts) reveals that Fuerteventura, Lanzarote and Concepcion Bank together form a single elongate and largely submerged plateau lying subparallel to the African coast, with a submarine morphology not at all suggestive of a volcanic plateau. In contrast, the Western Canary Islands are revealed as rudely circular volcanic piles with much more steeply sloping sides than the East Canaries plateau. Seismic refraction and reflexion profiling, combined with gravity data (see, for example, Bosshard and Macfarlane, *J. Geophys. Res.*, **75**, 4901 ; 1970), suggests strongly that the four most westerly Canary Islands do not form part of the African continent but are independent volcanic edifices lying on essentially oceanic crust. The geophysical work shows Gran Canaria to lie in a transitional area between oceanic and continental crust, but is at present inadequate for defining the crustal structure around Fuerteventura and Lanzarote, although it suggests that it is essentially continental.

The purely geological evidence bearing on this question is the subject of much controversy. The occurrence of carbonatites on the Eastern Canaries (but not on the Western Canaries) might be thought to constitute further evidence in favour of their continental nature, because recent surveys of carbonatite occurrences (*Carbonatites*, edited by Tuttle and Gittins, Interscience, 1966) show that they are typically found in fracture or rift zones in old shield areas.

Carbonatites were in fact reported from Fuerteventura by Fuster *et al.* in 1968, and from the Cape Verde Islands (which also have a bearing on this question) in 1965 by Assuncao *et al.* But the distinction between hydrothermal carbonate rich rocks and carbonatites is sometimes difficult on petrological grounds alone. In next Monday's *Nature Physical Science*, Allegre *et al.* report the results of carbon, oxygen and strontium isotopic ratio studies of rocks from Fuerteventura and the Cape Verde Islands which show them unequivocally to be true carbonatites. Portuguese geologists (Assuncao *et al.* have already suggested that the occurrence of carbonatites in the Cape Verde Islands could be explained if these islands were situated in the Mesozoic Mid-Atlantic Rift Valley when the North American and African continents started to drift apart. This suggestion can now be applied also to the Canaries.

It is worth noting that further support for this idea can be derived from the pre-drift fit of Dietz and Sproll, which results in the Fuerteventura carbonatite occurrence being located in fairly close opposition to the Canadian occurrences in the provinces of Ontario and Quebec. Nevertheless, problems remain for the petrologist in that the host rock of the carbonatites in Fuerteventura is a mafic and ultramafic plutonic formation similar in most of its features to the basements occurring in the truly oceanic islands of La Palma and Gomera.

BOTANY

Endemism in Manchester

from a Correspondent

A CONFERENCE on the taxonomy and phytogeography of higher plants in relation to evolution, held at the University of Manchester from September 9 to 11, was organized for the Botanical Society of the British Isles, the Linnean Society and the International Organization of Plant Biosystematists by Professor D. H. Valentine (University of Manchester), who gave a short opening address.

Endemism was perhaps the most dominant theme which emerged, and several speakers related this phenomenon to past geological and climatic history. In the Aegean area, endemism is clearly a consequence of fragmentation of the land mass, and this was described in some detail by Dr W. Greuter (Jardin Botanique, Geneva) for Crete and Dr A. Strid (Lund University) for the Cyclades. In older islands such as the Canaries (Dr D. Bramwell, University of Reading) the proportion of endemics is higher and good examples are found at all taxonomic levels. Professor C. Favarger (University of Neuchâtel) provided an analysis which showed higher proportions of endemic species in the southern European mountain ranges than in the central mass of the Alps, as a result of the greater isolation in the former. Dr S. M. Walters (University of Cambridge) outlined the special case of the almost totally apomictic *Alchemilla*, many of whose agamospecies are narrow endemics yet show some ecotypic differentiation. Professor H. Lewis (University of California, Los Angeles) distinguished between recent endemics of California which have arisen as a result of hybridization and those which have resulted from other sorts of isolating barriers. In the West Indies (Dr B. Morley, National Botanic Gardens, Dublin) the pattern of endemism in *Columnea* varies between the Greater and Lesser Antilles because of differences in geological history and the distribution of bird pollinators between the two areas.

As might be expected, differences of opinion were expressed concerning the mechanisms which have given rise to disjunct distributions, although the diversity of ideas is no doubt to be partly explained by one of the examples chosen. These ranged from bipolar (Dr D. M. Moore, University of Reading) to amphi-Pacific (Professor H. Hara, Tokyo University) distributions. Professor J. K. Morton (University of Waterloo, Ontario) considered that the scattered distribution of montane species in West Africa can be explained by cool periods during which the montane species extended to lowland areas and migrated freely. Professor O. T. Solbrig (Harvard University) described semi-desert areas of great overall similarity in Mexico and Argentina, and outlined the aims and methods of an international team who are attempting to shed light on such situations by defining as precisely as possible the characteristics and relationships of the taxa and conditions involved. Dr F. Rose (King's College, London) presented evidence to show that the territory immediately north of the Somme Valley of northern France has been a greater barrier to the northern migration of species into Britain than has the English Channel, and that a number of continental species in southern England probably arrived from the former area direct and not by way of the Pas de Calais.

Hybridization is playing a major part in the modern rapid evolution of Hawaiian taxa. Dr G. W. Gillett (University of California, Riverside) pointed out that in many cases hybridization does not seem to be related to recent human activity, because it occurs in wind as well as in insect-pollinated genera (and is hence not related to human effects on pollinators) and the open communities favoured by hybrid progenies and segregates are provided by natural phenomena such as lava flows. Professor H. G. Baker (University of California, Berkeley) expounded his ideas on weed evolution with reference to California and described many different methods by which plants had become successful weeds there. Native species are most likely to become weeds in man-made situations which mimic natural ones, whereas alien weeds more often colonize new sorts of habitats.

The migration of taxa was the theme of several other contributions. Professor G. L. Stebbins (University of California, Davis) discussed the notion that angiosperms had originated in mesic conditions and subsequently migrated to areas showing extremes of temperature and water availability. Such a theory is based on the belief that the major characteristics of taxa and their evolution are similar now to when the angiosperms arose. Professor C. van Steenis (Rijksherbarium, Leiden) described the "hour-glass" type of world distribution in which there are wide temperate ranges linked by a narrow tract through Malaysia. Such situations are often accompanied by vicariousness at the family, genus or species level, for example, *Fagus* and *Nothofagus*. *Nothofagus* is now southern temperate, but probably arose further north and migrated to the south by way of Malaysia. A similar route was postulated for *Epilobium* by Professor P. H. Raven (Missouri Botanic Garden), because the genus is in many ways more stereotyped in Australasia than in the northern hemisphere even though there are more species compared with the area covered. Professor T. W. Böcher (University of Copenhagen), on the other hand, discussed east–west migrations in the Arctic region.

Inter-continental similarities and differences have often been investigated by anatomical means, and two contributions exemplified different facets of this sort of study. Dr D. F. Cutler (Royal Botanic Gardens, Kew) showed how a superficially similar stem anatomy in the Restionaceae is accomplished by quite different developments, and Dr A. W. Exell (British Museum (Natural History)) and Dr C. A. Stace (University of Manchester) described situations in the Combretaceae where certain apparently non-adaptive features were limited to one continent or another throughout a wide taxonomic spectrum.

MYCOLOGY

International Liaison

from a Correspondent

AT the First International Mycological Congress held at the University of Exeter from September 8 to 14, an International Mycological Association was established for the encouragement of mycology in all its branches, particularly international aspects, by promoting international mycological congresses and by liaison with other relevant international and national bodies. Membership of the association is open both to international and national societies or groups having mycological interests and to individual mycologists in countries lacking a mycological society.

According to the *International Mycological Directory* (published for the congress by the Commonwealth Mycological Institute, Kew, Surrey, and available at £0.50, post free), there are already a dozen series of international meetings devoted to special branches of mycology, and there are at least an equal number of recurring international congresses or meetings which include some mycology. An important task of the new association will therefore be to attempt to rationalize these events.

The first officers of the association are: president, Professor C. J. Alexopoulos (USA); treasurer, Dr J. A. von Arx (Netherlands); secretary, Dr C. Booth (Commonwealth Mycological Institute, Kew, Surrey, England).

Immunoglobulin E (Reagin) and Allergy

D. R. STANWORTH
Department of Experimental Pathology, University of Birmingham

A tissue-sensitizing factor found in the sera of allergic subjects has been identified as a unique immunoglobulin, IgE. Investigations of its mode of action could lead to the development of new forms of treatment.

FIFTY years ago Prausnitz showed that it was possible to sensitize sites on his forearms by the intradermal injection of serum from one of his allergic patients (Küstner) who was hypersensitive to fish[1]. The injection of fish extract into the same sites 24 h later evoked immediate weal and erythema reactions similar to those shown by the patient himself (and by other fish-sensitive individuals) in response to the direct intradermal injection of fish extract.

This observation was a historic "landmark" in a field which has not always been noted for objectivity. Besides demonstrating that human hypersensitivity of this immediate-type could be transferred to normal tissue, outside the influence of the allergic individual, it meant that a passively induced reaction could be restricted to a localized area of the skin with minimal discomfort to the non-allergic recipient. Of even greater significance, however, was the inference that immediate hypersensitivity (unlike that of the delayed type, of which tuberculin sensitivity is an example) was mediated by a humoral factor.

There soon followed similar demonstrations of the local transfer of human hypersensitivity to many common inhalents (such as grass pollens, animal danders and house dusts); the Prausnitz–Küstner (P–K) test, as it is known, being used as an alternative to direct skin testing in the diagnosis of hay fever and allergic asthma. Its principal application, however, has been in the experimental study of immediate-type hypersensitivity; where, until a few years ago, it offered the only satisfactory means of assay. Consequently attempts to characterize the skin sensitizing factor more fully have been seriously handicapped.

Early investigations showed this serum constituent to resemble antibodies which appear in the circulation of humans and animals in response to conventional immunization procedures. But it seemed to lack certain properties commonly associated with immune antibodies, such as the capacity to form a precipitate and to fix complement when combined with specific antigens *in vitro*. For these reasons, some investigators[2] preferred to reserve judgment on its antibody status by terming it "atopic reagin" ("atopy" denoting a form of human hypersensitivity in which there was evidence of a hereditary predisposition, the sensitizing substances being referred to as "atopen"). The factor demonstrable in the serum of individuals with immediate hypersensitivity of the asthma–hay fever–urticaria type, has since been termed "reagin" and it has been customary to refer to the provoking agents (in inhalents, foods and so on) as "allergens".

P–K testing revealed that reagins become firmly "fixed" in normal isologous human skin where they are detectable (by subsequent allergen challenge) several weeks after the transfer of the allergic serum. In contrast, antibodies produced by the immunization of rabbits with allergenic substances (such as pollen extracts and egg protein) failed to fix to human skin; although unlike reagins, they could passively sensitize the skin of heterologous guinea-pigs for relatively short times. Human antibodies (γG type) against diphtheria toxoid likewise disappear rapidly when injected into isologous human skin (with a half-life of 12 h)[3], whereas reagins transferred isologously have been estimated[4] to have a "half-life" of 15 days.

Quantitative P–K testing[5] based on the accurate measurement of the areas of weals produced on the backs of normal recipients showed that a maximal skin response was achieved with a time interval of about 50 h between transfer of allergic serum and challenge with specific allergen. Once this was effected, however, a weal and erythema reaction developed rapidly with the weal approximately doubling in area during 10–20 min after introduction of the allergen. The susceptibility of reagins to relatively mild heat treatment was also confirmed[5]. Similar conditions are used in the destruction of the complement activity of immune sera, but the addition of fresh normal human serum to heated allergic serum fails to restore P–K activity[6] and other evidence indicates that reagins do not depend on the complement system for their activity.

Reagins were also found to differ from immune (7S type) antibodies in their inability to cross the placenta. This situation is obviously fortunate for the offspring of allergic women, as is the apparent absence of reagins from human colostrum. It has been attributed by some investigators to the macroglobulin (19S) antibody nature of the tissue sensitizing factor, which was thought to be of sufficiently large molecular size to be retained by the human placenta's supposed sieving mechanism. But extensive studies by Brambell and his associates[7], and others, have indicated that the placental transmission of immunoglobulins is a highly selective process (controlled by sites located within their Fc regions).

The biological properties of reagins revealed by these early investigations, based principally on P–K testing, are summarized in Table 1. This was the position around 1940, when techniques of free-solution electrophoresis and ultracentrifugation were first applied to the fractionation of plasma proteins. Although such physico-chemical procedures offered better means of separating complex protein mixtures than had been previously possible, by salt or low temperature-ethanol precipitation, they were essentially analytical tools. Nevertheless, electrophoretic analyses of the sera of animals before and after immunization provided the first evidence of the γ globulin nature of precipitating antibodies[8]; while corresponding ultracentrifugal studies indicated a size heterogeneity (antibody activity being associated with both 7S and 19S components).

It was naturally hoped that a similar approach would provide more convincing evidence of the antibody nature of reagins; but this was thwarted by practical difficulties. It was established, however, that reagins were electrophoretically faster than immune γ globulins, moving in the β region (where

Table 1 Main Biological and Physico-chemical Properties of Reagins (γE-Antibodies)

(1) Bind firmly to isologous, and closely related heterologous, tissues
(2) Heat labile (destroyed by 56° C for 1 h)
(3) Not dependent on complement
(4) Fail to cross the placenta
(5) Move in the "fast γ" region on electrophoresis
(6) Sediment near to 8 S

19S type immune antibodies were also found). But this conclusion was very much an extrapolation, and the minute amount of skin sensitizing factor present in allergic sera could have been moving in the electric field in combination with a major constituent. More refined preparative fractionation procedures were required.

Physico-chemical Characteristics

As in the characterization of many other minor active protein constituents of biological fluids, it was the advent of zone-fractionation procedures which gave the first definitive indication of the physico-chemical characteristics of the skin sensitizing factor. Thus, zone electrophoresis of allergic sera in starch blocks[9] confirmed that reagins moved in the "fast γ" (slow β) region; whilst zone-centrifugation in buffered sucrose gradients[10] showed clearly that they sedimented in the 7S region (and were not macroglobulins, as had been suggested to explain their failure to cross the placenta). There were, of course, limitations to this type of correlative approach because each of the major serum electrophoretic fractions, including the γ fraction, comprised several components. It was at this stage, however, that the application of highly-specific and sensitive immunological techniques began to compensate for the shortcomings of the physico-chemical procedures. Studies on the monoclonal γ globulins, isolated in large quantities from the sera of patients with multiple myelomatosis and Waldenström macroglobulinaemia, led to the production of specific antisera which distinguished three major immunoglobulin classes[11]: γG or IgG (previously referred to as 7S γ globulin): γM or IgM (previously referred to as 19S macroglobulin) and γA or IgA (a newly discovered third class[12], which when isolated from normal human serum by a zinc precipitation procedure was found to comprise a major 7S component, with minor amounts of polymerized material, and which migrated in the fast γ region on electrophoresis). It was established that the three major immunoglobulin classes then known possessed common light polypeptide chains, but distinctive heavy chains (Fig. 1).

The problem then was to show that skin sensitizing activity was a property of one (or perhaps more) of these antigenically distinguishable immunoglobulins. Antigenic and physico-chemical analysis of fractions of allergic human serum separated by chromatography on DEAE-cellulose (which provided a high degree of resolution of the various classes of immunoglobulin) revealed maximal P–K activity in a fraction in which

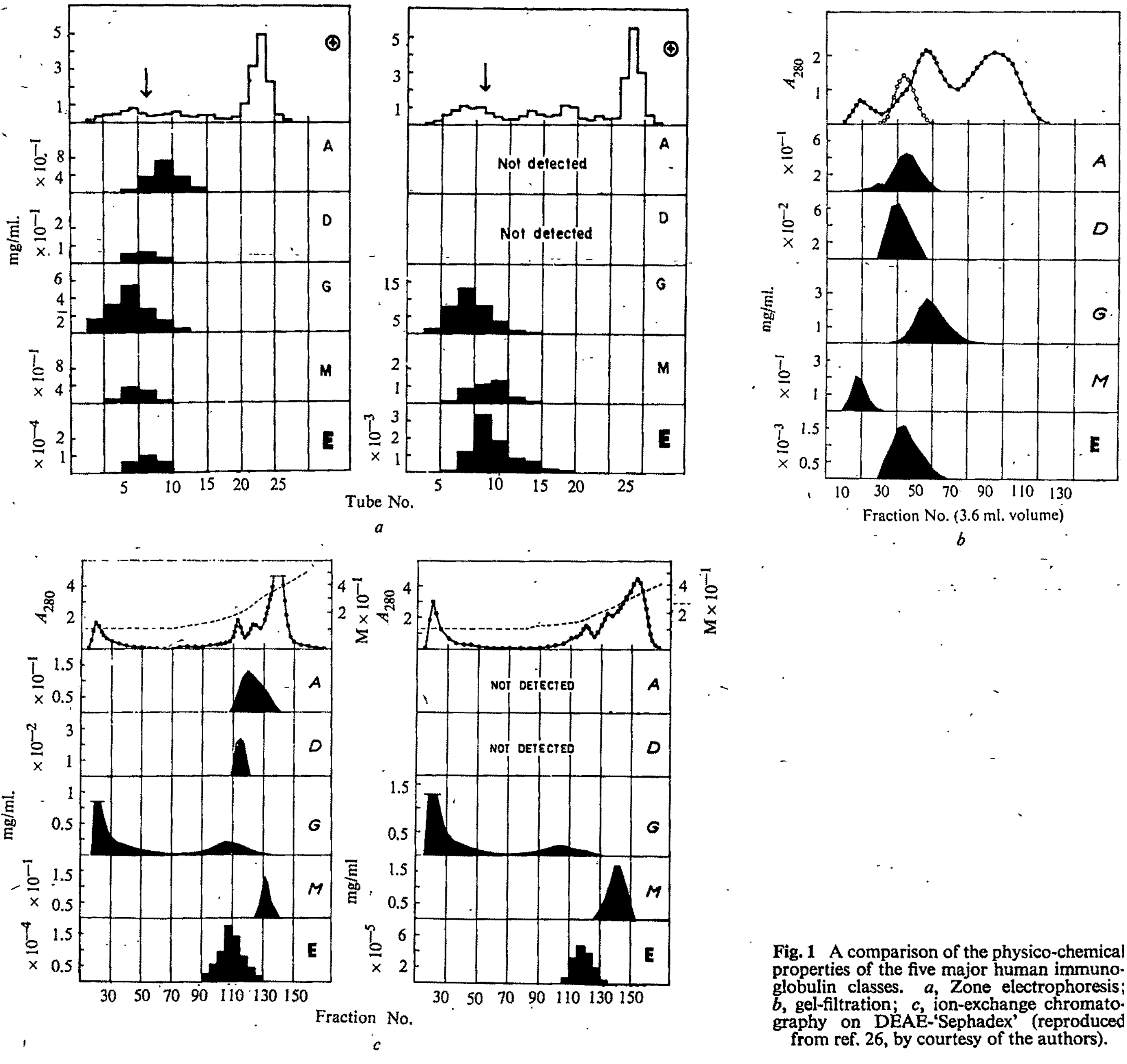


Fig. 1 A comparison of the physico-chemical properties of the five major human immunoglobulin classes. *a*, Zone electrophoresis; *b*, gel-filtration; *c*, ion-exchange chromatography on DEAE-'Sephadex' (reproduced from ref. 26, by courtesy of the authors).

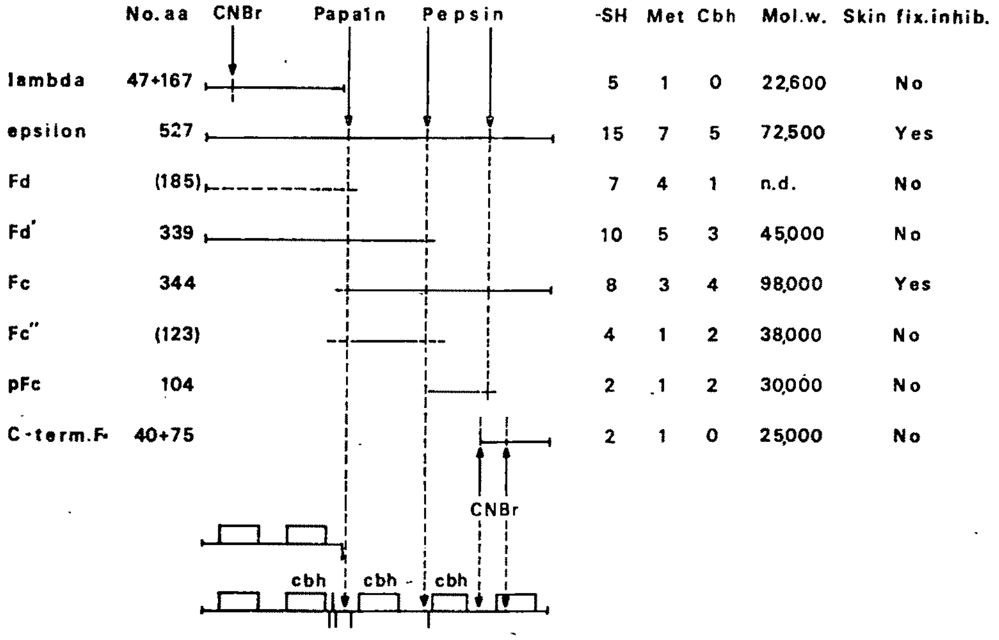


Fig. 2 Working model for molecular structure of IgE indicating the disposition of antigenically and biologically active fragments, and carbohydrate (cbh) prosthetic groups. (Reproduced from ref. 62, by permission of the authors.)

only electrophoretically fast 7S γG globulin could be detected. As Fig. 2 shows, this was in an elution position well ahead of any detectable γM globulin; and even ahead of any γA globulin detectable by immunodiffusion analysis using specific antisera[13].

This latter observation was particularly significant, at a time when many claims were being made that reagins were γA globulins. These were based on such evidence as the recovery of P–K activity in highly purified γA globulin preparations[14], the removal of activity from allergic sera by absorption with specific sheep anti-human γA globulin antiserum[15] and the blockage of P–K reactions by normal human γA globulin or its isolated heavy chains[16]. They were of obvious teleological appeal as γA globulin had been found in high levels in nasal and other secretions, where it supposedly acted as a "front-line" defensive agent. But a lack of correlation was observed between reagin activity and γA globulin distribution in DEAE cellulose chromatographic fractions of allergic sera[13]; and other studies[17] suggested that P–K activity was associated with a minor component of the sera of hypersensitive individuals, which had appeared as a contaminant in the supposedly pure γA globulin fractions investigated previously.

Were reagins a unique type of immunoglobulin (a prospect on which I had speculated in 1963 (ref. 18))? The discovery of a new human immunoglobulin class (IgD or γD)[19], which resembled γA globulin electrophoretically, seemed a possibility; but several independent investigations suggested this immunoglobulin did not possess skin sensitizing activity.

Reagins were found, however, to have unusual physicochemical characteristics as well as distinctive biological properties (for antibodies). For example, the gel filtration of sera of people sensitive to ragweed (on 'Sephadex G200'[20]) led to the recovery of maximal P–K activity slightly ahead of the 7S peak containing the γG and γA globulins, but well behind the 19S peak containing the γM globulin. Furthermore, reagins in such sera were found by density gradient ultracentrifugation[21] to possess a sedimentation coefficient of 7.7S (7.4–7.9S range)—significantly greater than human γG and γA globulins. It also seemed significant that the reagin-like antibodies, occurring naturally in some animals (such as the dog) and induced artificially in others (for example, rats and rabbits), had sedimentation coefficients greater than 7S but much less than 19S (Table 2).

Table 2 Sedimentation Characteristics of Reagin-type Antibodies formed in Various Mammalian Species (as determined by Density Gradient Ultracentrifugation)

Species	Mode of antibody formation	Sed. coeff. (S°)
Human	Spontaneously	>7.4 S–7.9 S
Dog	Spontaneously	6.8 S–10.1 S
Rat	Experimentally	>7 S, ⩽19 S
Rabbit	Experimentally	>7 S, ⩽19 S

Further evidence that reagins were probably a unique class of immunoglobulins was obtained by Ishizaka and his associates[22], who isolated active preparations by application of a multi-step procedure to the fractionation of pools of sera from individuals showing marked hypersensitivity to ragweed pollen and who developed highly sensitive radioimmune-diffusion assays as *in vitro* alternatives to the P–K test for the measurement of the reagin in the isolated fractions. Reagin preparations thus obtained were used to produce anti-reagin antisera, which were rendered specific by absorption with the major classes of immunoglobulin and which were used in radioimmunoelectrophoresis to provide evidence that a radiolabelled ragweed allergen (E) preparation combined with the reagin isolated from ragweed-sensitive individuals' sera.

Ishizaka and associates[23] suggested that reagin activity was carried by a unique immunoglobulin which they tentatively termed "γE" (in view of its specific binding capacity for ragweed allergen E). Despite the evidence presented in support of this idea, however, some investigators felt that such claims were perhaps a little premature until chemical evidence was available to suggest the existence of a unique type of polypeptide chain.

Myeloma Protein

The final evidence that reagins were a unique class of immunoglobulins came with the isolation of an atypical myeloma protein (originally designated "IgND"), from the serum of a patient ("ND") with myelomatosis and Bence Jones proteinuria[24], which lacked the antigenic determinants characteristic of the heavy polypeptide chains of the then known immunoglobulin classes (γG, γM, γA and γD) while possessing similar light chain determinants (of sub-type L). Like myeloma γA and γD globulins, this atypical protein moved in the "fast γ" region on zone electrophoresis (Fig. 1); but it was eluted slightly ahead of these classes of immunoglobulin during chromatography on DEAE-'Sephadex'. Of greater significance, however, was its elution position during gel-filtration on 'Sephadex G100', where it emerged after the γA globulin but just ahead of the γG globulin. Furthermore, free solution ultracentrifugal analysis[25] showed the atypical myeloma protein (IgND) to possess a sedimentation coefficient (S°20, W) of 7.92S (and molecular weight 196,000 from Archibald analysis); a value very similar to that which had been assigned to the reagins in sera of ragweed-sensitive individuals on the basis of the observed rate of sedimentation of the P–K activity in buffered sucrose gradients.

This similarity in sedimentation coefficient between the new type of myeloma protein and reagin could have been fortuitous, but it seemed significant that the myeloma protein isolated from the serum of patient "ND" was the first known type of immunoglobulin to have similar size characteristics to the skin sensitizing factor. Other observations were beginning to suggest a relationship between the new class of immunoglobulin and reagin. For example, by means of a radio-immunosorbent technique based on the use of 'Sephadex'-coupled antibody directed specifically against the new myeloma protein[24], a significantly elevated concentration (5,900 ng/ml.) of an antigenically similar protein was detected in the serum of an individual with allergy to dog dander; in comparison with the much smaller amounts (100–700 ng/ml.) found in normal sera. Later comparative studies[26] of the antigenic characteristics of the new myeloma globulin and the reagin-rich fraction (designated γE) isolated from sera of ragweed-sensitive individuals were to provide evidence of a close structural relationship between the two proteins.

The most convincing evidence that the myeloma protein (IgND) was a pathological counterpart of reagin was obtained, however, from inhibition-P–K testing in a normal human recipient[27]. The intradermal injection of the myeloma protein mixed with, or 24 h before, the sensitizing serum (at a dosage of 5–50 times in excess of the level of reagin in the serum) completely inhibited a weal and erythema response on subsequent challenge with the specific allergen (isolated from horse dandruff). Thus not only was the myeloma protein (IgND) antigenically similar to reagin, it also seemed to have its striking affinity for isologous tissues. There was no evidence, however, that the myeloma protein could bind antigen (that is allergen). Thus it seemed to be a pathological counterpart of a new immunoglobulin class of which reagin was a normal representative, just as an over-production of monoclonal forms of the major immunoglobulin classes has often been seen in plasmocytic and lymphocytic neoplastic disorders.

Immunological and chemical studies[25] indicated that the myeloma protein IgND was a new class of immunoglobulin, with similar light chains to those in the other four classes, but distinctive heavy polypeptide chains somewhat larger (75,500 molecular weight) than those in the other immunoglobulins. Consequently at a meeting initiated by the World Health Organization in Lausanne in 1968 (ref. 28) it was decided that there was sufficient evidence to conclude that reagin was representative of this new class of immunoglobulin, which it was proposed should be designated "IgE" or "γE".

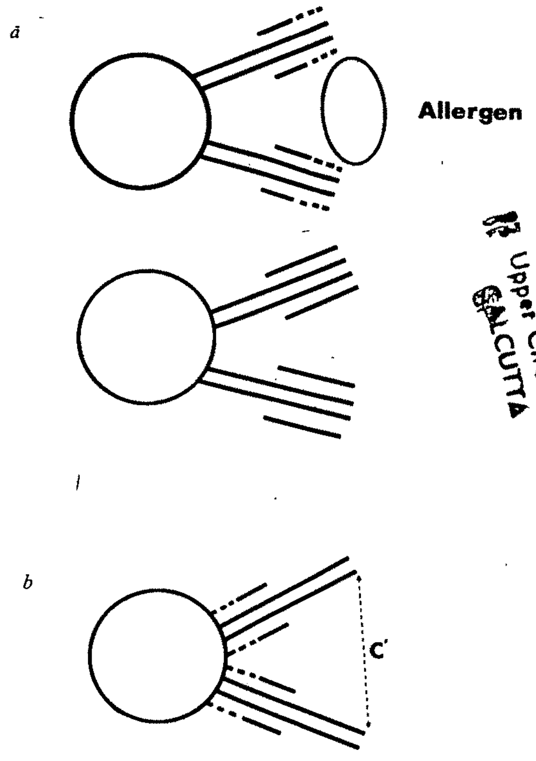
The availability of the myeloma protein offers a means of chemically characterizing reagins, and ultimately of explaining their unusual biological properties in structural terms. It has been calculated that the chance of finding a case of monoclonal production of γ globulin of the E type is about one in 50,000 that of finding a more common case of myelomatosis; so the protein isolated from the serum of the Swedish case has proved particularly valuable and highly sought after. Fortunately, however, a second case has been reported[29] in the United States, who has clinical manifestations similar to those seen in the case originally reported by Johansson and Bennich in Sweden.

Already the two γE myeloma proteins are providing important information about the role of the antibody (reagin) in immediate hypersensitivity reactions. For example, the myeloma IgE has been degraded into different types of polypeptide fragment by means of proteolytic and chemical cleavage procedures, and tested the ability of these to inhibit skin sensitization by the reagins (γE antibodies) in the sera of individuals sensitive to horse dandruff and grass pollen[30,31]. Of the various types of fragments tested (originating from the different parts of the IgE molecule as illustrated schematically in Fig. 2) only those incorporating the Fc region had the inhibitory activity of the parent molecule. Similar findings have resulted from inhibition studies in sub-human primates (for example, rhesus monkeys and baboons) which, like non-allergic humans, have proved receptive to sensitization by human γE antibodies but which (for obvious reasons) are now preferred as test recipients.

Other inhibition sensitization tests in baboons[32], have involved myeloma IgE preparations which have been reduced to various extents with 2-mercaptoethanol, to ascertain the importance of the relatively large number of disulphide bridges in maintaining the conformational integrity of that part of the IgE molecule involved in tissue binding.

Binding of IgE

Inhibition tests with proteolytic cleavage fragments of myeloma IgE have led to important conclusions about the binding of antibody IgE to isologous (or closely related heterologous) tissue. They suggest that, like the myeloma protein molecules, the antibody (reagin) molecules similarly bind to the tissue receptors through sites within their Fc regions (Fig. 3). Sites within the Fab region of the cell-bound antibody molecules are thus free to react subsequently with antigen (allergen) in the usual manner. In contrast, the myeloma IgE molecules (like most myeloma forms of the other immunoglobulin classes) cannot combine with antigen (Fig. 3). If, on the other hand, combination with the target cells had been found to occur through the Fab regions of the antibody IgE molecule, it would have been necessary to infer that immediate-type hypersensitivity is an auto-immune phenom-



Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA

Fig. 3 Diagrammatic comparison of the properties of antibody and myeloma γE molecules, showing mode of attachment to target cells (that is by means of sites in the Fc regions). *b*, Mode of interaction of γG type cytolytic antibody molecules with target cells, showing subsequent interaction of complement (c′) with sites located in the Fc regions.

enon involving the production of antibody against self-cell surface antigen (as occurs, for example, in autoimmune haemolytic anaemia.

Thus it is now possible to start explaining how the immunological reactions occurring at tissue mast cell surfaces bring about the pharmacological manifestations characteristic of hypersensitivity reactions of the immediate-type. Whether such reactions are mediated by γE-type antibodies in passively sensitized human tissues (as, for example, in the classical P–K test), or by non-reaginic γG-type antibodies in the heterologous tissues of guinea-pigs passively sensitized with the serum of experimentally immunized animals, the sequence of events is remarkably similar. The antibody plays a crucial role, not only in the initial sensitization process (which, of course, occurs spontaneously in the allergic individual or the actively sensitized guinea-pig), but also in the manner in which it subsequently interacts with specific antigen (allergen) to activate the enzyme system supposedly responsible for effecting the release of histamine, 5-hydroxytryptamine and other pharmacologically active substances. The elucidation of the structural basis of these different, but complementary, functions of tissue-sensitizing antibodies poses a problem of protein characterization. Moreover, it seems likely that studies along these lines on γE globulins will throw light on the mode of action of other classes of immunoglobulin fulfilling other roles on the surfaces of other types of cell (for example, receptor immunoglobulin or lymphocytes).

The emergence of *in vitro* alternatives to the P–K test in humans, and the PCA test in sub-human primates, is facilitating the delineation of the biochemical events set in train by the combination of cell-bound γE antibody and allergen. Such systems are based on passive sensitization by γE antibodies of chopped human lung[33], normal human leucocytes[34], chopped monkey skin[35] or other suitable primate tissue. The histamine released from the washed sensitized cells or tissues as a result of interaction with specific allergen can be measured accurately (down to levels of a few nanograms) by bioassay using isolated guinea-pig ileum, but a spectrofluorometric procedure involving coupling with ophthalaldehyde[36] has been adopted as a satisfactory chemical alternative. These indirect procedures have obvious advantages over the previous pharmacological approach to the study of immediate hypersensitivity reactions, which involved the direct assay *in situ* of the histamine liberated on presentation of the specific antigen to the actively sensitized tissue set up in an organ bath. Their application has provided convincing evidence[37,38] that the allergen-induced release of histamine and other mediations of immediate-type hypersensitivity reactions is accomplished by a multi-step, energy-requiring process to which the glycolytic pathway is essential. There are precise *p*H and temperature requirements, and a dependence on calcium and magnesium ions.

It is important, however, to distinguish this process from cytolytic reactions in which destruction of the cell membrane is effected through the combined agency of anti-cell antibody and complement. In contrast, γE-mediated histamine release has been shown (from K^+ efflux studies[38]) to be an active secretory process which is not intrinsically injurious to the target cell. Thus, there is a fundamental difference in the release mechanism initiated by sensitizing antibodies (for example of the γE type), in the absence of complement, and those more drastic processes induced by cytolytic antibodies (for example of the γG type) with the aid of the complement system. As I have already implied (Fig. 3), the type of process evoked depends on the manner in which the antibody is presented to the cell. Antibodies of the γE type seem particularly suited to binding to histamine-containing cells through sites on their Fc regions, and to possess within their own structures sites capable of direct action on the target cell membrane (or other cell constituents). On the other hand, cytolytic antibodies (whether directed against cell membrane antigen, or coating antigen) react initially through sites within their Fab regions, a subsequent intermolecular interaction between their free Fc regions[39] supposedly leading to the activation of the complement system responsible for the ensuing lysis.

Tertiary and Quaternary Structure

The problem now is to establish how the interaction of the cell-bound γE antibody (reagin) with allergen triggers off the events outlined, which seem (from microscopic studies of viable cell preparations) to be associated with characteristic morphological changes[40] in the target cells, involving the release of their histamine-containing granules. The amino-acid sequencing in progress on the two myeloma IgE preparations should provide important information as might the characterization of the relatively large carbohydrate (11.7%) prosthetic group. Furthermore, studies of the inhibitory capacity of proteolytic cleavage fragments of the myeloma IgE (mentioned earlier) are being extended to smaller and smaller fragments, with the hope of isolating a readily characterizable low molecular weight peptide retaining tissue-binding activity. It is becoming inincreasingly obvious, however, that the peculiar biological properties of γE antibodies (as well as those of the less efficient γG type sensitizing antibodies produced in experimental animals) will be explicable in terms of unusual tertiary and quaternary structural characteristics. It seems likely (as discussed fully elsewhere[41]) that the two structural features which will prove to distinguish sensitizing antibodies will be an ability to bind strongly to complementary tissue sites with no accompanying loss in the conformational integrity of their Fc region (which can soon occur as a result, for example, of relatively mild heat treatment); and the possession of sufficient flexibility within the "hinge regions" to permit the transmission of a critical allosteric change resulting from combination of allergen with sites in the Fab regions of adjacent cell-bound molecules.

There is evidence[42] that antigen-induced conformational changes can occur in free solution, in rabbit antibody directed against commonly used antigens such as bovine serum albumin and horse ferritin, and these lead to the exposure of new antigenic determinants within the Fc regions. It seems reasonable to conclude, therefore, that similar conformational changes could occur in γE-type antibody molecules in combination with specific allergen (as already proposed[43]). Moreover, if the γE antibody is already anchored to the target cell surfaces, through sites within its Fc region, newly exposed side chains could be brought into critical juxtaposition with points on the cell where activation of the appropriate enzyme system would occur (Fig. 4). Indeed there is evidence (from inhibition studies with aggregated IgE) that association of IgE molecules might lead to the formation of "tissue activation sites" which are distinct from the tissue binding sites in monomeric (antibody and myeloma) IgE molecules; and it is possible that activation sites are similarly formed in the Fc regions of adjacent cell-bound sensitizing antibody molecules as a result of the bridging by specific allergen, for which there is increasing evidence[45,46] and which presumably involves the cross-linking of sites within the antibody Fab regions (Fig. 4). The critical question then to be answered is whether groups exposed from the Fc regions of the cross-linked antibody molecules act cooperatively to form the activation site. In this event, the activation site could be linked to the active site of an enzyme, which of course is usually contained within a single polypeptide chain. Alternatively, the side-chains which are supposedly exposed in the Fc regions of the sensitizing antibody molecules might be proved to exert an independent effect on the target cell.

In attempting to decide between these alternatives it is worth considering the results of studies designed to short-circuit the histamine release process initiated by reagin-allergen combination. These involve the use of alternative, artificial, ways of cross-linking the γE molecules, and seem to lead to pharmacological responses similar to those of classical immediate sensitivity reactions. Apart from the pre-aggregation

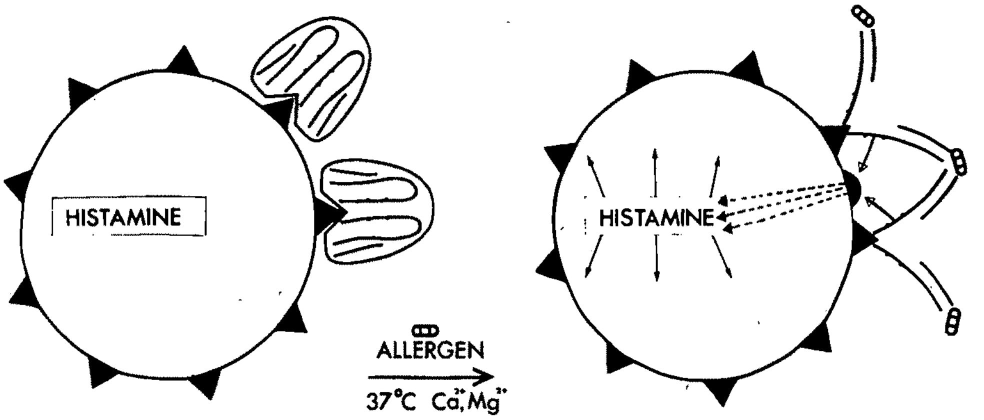


Fig. 4 Postulated mode of interaction between cell-bound γE antibodies and allergen leading to the triggering of release of vasoactive amines (modified from ref. 45).

of the γE molecule before presentation to the target cell (as already mentioned), it is possible to effect histamine release in human or monkey skin by bringing about the cross-linking of the cell-bound γE molecules with antibody directed specifically against determinants within their Fc regions. Thus, it can be demonstrated[46] that the intradermal injection of specific anti-human IgE antiserum induces an immediate oedematous and erythematous reaction similar to that observed in the P–K test.

Tissue Activation Sites

It is suggested that, as in the classical reagin-allergen mediated reaction, the exposure of tissue activation sites within the Fc region of the γE molecule triggers the release of the pharmacologically active mediators. But the cross-linking process which induces the required conformational change is less efficient than that effected by the cross-linking of the Fab regions of the γE antibody molecules by allergen. This might be expected by reference to the model shown in Fig. 5, which suggests that a force applied at position A (at maximum distance from the fulcrum) would be the most effective way of forming the activation site at position S; but application of a force at position B would accomplish the same effect in a less efficient manner. This analogy could offer an explanation of the skin reactions effected by the action of anti-IgE antibody, as well as that elicited by other agents such as protein A (an antigenic component of *Staphylococcus aureus*) which has an unusual capacity for combination with the Fc regions of immunoglobulins[47] and which presumably acts too by cross-linking cell-bound γE molecules[48].

Alternatively, it seems likely that the conformational changes necessary for the formation of tissue activation sites can also occur in non-specifically aggregated IgE (brought about, for example, by lyophilization of monomeric myeloma IgE). As already mentioned, IgE treated in this way is also capable of effecting an immediate release of histamine on injection into human or monkey skin[46]. Thus, perhaps surprisingly, in spite of the non-specific nature of the aggregation process, a conformational change seems to be induced within the Fc regions similar to that resulting from antigen-mediated association of the γE molecules. This is directly analogous, however, to the situation with regard to the association of human (and rabbit) γG molecules where biological activities (for example heterologous skin reactivity and complement fixability), and new antigenic determinants (similar to those formed on specific antigen–antibody combination) are induced by non-specific denaturation treatments. Moreover, recent studies[49] on the nature of the structural changes shown by γG globulins as a result of physical or chemical treatments suggest that here too association is initiated by preliminary (non-covalent) interaction between the Fab regions of the molecule but in this case, of course, without the agency of the antigen. This, it seems, leads to an unfolding of the Fc regions, similar to that occurring in antibody γG molecules on combination with antigen, thereby providing scope for intermolecular Fc interaction with formation of the sites of the various biological activities shown by aggregated γG globulins. Thus what might be expected to be a random aggregation process seems to be a highly ordered polymerization, directed presumably by the initial intermolecular reactions of the Fab regions of the monomer molecules.

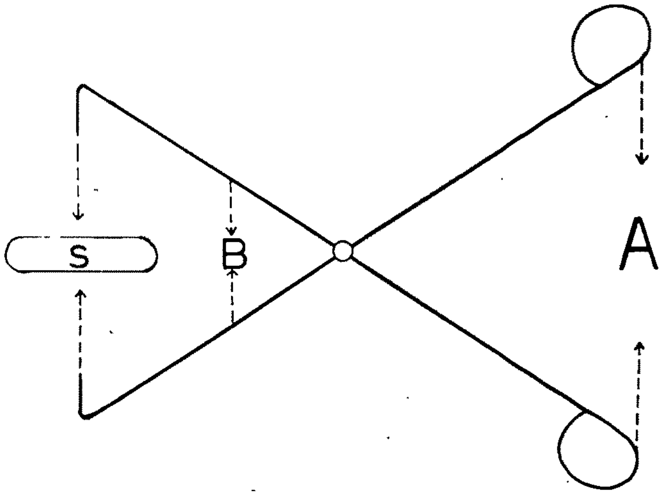


Fig. 5 Model illustrating various methods of inducing an allosteric transformation within cell-bound γE antibody molecules.

The complete characterization of these crucial tertiary and quarternary structural changes could have an important bearing on the explanation of other immunological phenomena. As far as immediate hypersensitivity is concerned, the need now is to characterize the "tissue activation sites" formed in γE globulin molecules on association, and to elucidate the nature of their "substrate" located on the target cells. Various possibilities are being considered. For example, could it be that reagin-allergen combination on the cell surface activates a phospholipase responsible for hydrolysis of membrane phospholipid[50]; or perhaps an esterase is activated, in a similar manner to that implicated in complement mediated cytolytic reactions[51]. Another possibility[52] is the stimulation of the adenyl-cyclase system, which is located on the inner surface of cell membranes (where it catalyses the conversion of ATP to cyclic AMP) and which has been shown to be involved in the action of certain hormones[53]. It could be more than fortuitous that certain polypeptide hormones (such as ACTH) which act in this manner are also potent histamine liberators[54]. It will, however, be important to exclude the simpler possibility that the primary triggering mechanism is a physical shearing of the cell membrane ("rigidification", as one investigator[44] has termed it), brought about by the association of surface-bound γE antibody molecules.

Future Tasks

Although the initiative is now passing to biochemists and biophysicists, many other aspects of the behaviour of γE antibodies require immunological investigation. For example, with the aid of specific antisera prepared against the myeloma proteins, it is possible to begin to identify the sites of binding of γE antibodies on their target cells; as well as their sites of synthesis in human lymphoid tissues. Immunofluorescence studies[55] have provided evidence that γE antibodies are formed locally in the respiratory and gastro-intestinal tract where they probably become involved in allergic states affecting these organs. In any studies of γE antibody synthesis it will be important to answer the fundamental question about the definition of the factors which govern the production of sensitizing rather than immunizing antibodies, Here the use of animal models[56] is particularly revealing, because it is becoming apparent that the reagin-like antibodies produced in species like the rat, the rabbit and the mouse closely resemble the human γE antibodies. The chemical nature of the allergen, and its form and mode of administration are proving important factors in deciding the nature of the resultant antibody response, as is the influence of other classes of antibody produced concomitantly. Through such investigations it is becoming possible to define the immunological basis of "desensitization" (that is hyposensitization), a prophylactic measure which has been practised by clinical allergists for several years and which is thought to encourage the production of γG-type antibodies which bind allergen preferentially (besides producing effects at the cellular level).

Other possible new forms of prophylaxis are suggested by studies[57] on the prevention of the passive sensitization of baboons with allergic human serum by the systemic administration of myeloma IgE, where a refractory state persisting for about a week has been induced by the intravenous injection of about 10 mg protein/kg body weight. The ultimate aim of this approach would be the isolation of a low molecular weight peptide fragment of the myeloma IgE, which retained tissue-binding activity and which could be made available in large amounts by application of modern methods of polypeptide synthesis.

Another advantage to the clinical allergist resulting from the discovery of the myeloma proteins has been the development of methods of measuring IgE concentration which do not rely on tissue or leucocyte sensitization. For example, radioimmunosorbent assay procedures involving the use of anti-IgE[24] or specific allergen[58] coupled chemically to dextran (or some other suitable insoluble carrier such as a cellulose carbonate derivative)[59] have been used to determine the total, or antibody, IgE levels in the sera of patients, and the use of radiolabelled antibody[59] has rendered the single radial diffusion test sufficiently sensitive to detect the relatively low levels of IgE in allergic sera by autoradiography.

It is important to recognize, however, that γE antibodies need not necessarily be deleterious, as suggested, for example, by studies[60] of the immune reactions occurring in the mucous membranes of the rat during the expulsion of an infecting nematode (*Nippostrongylus brasiliensis*). There is evidence of an association between a sharp rise in intestinal mast cells, an alteration of mucosal permeability (effected by vasoactive amines released from the mast cells) and the expulsion of the parasites. It is tempting to conclude, therefore, in this situation that γE antibodies are fulfilling a protective role (the so called "self-cure" mechanism[61]); and it may be significant that one of the few conditions (other than a hypersensitivity of the asthma–hay fever type, and atopic eczema) where very high serum IgE levels have been recorded is in human (as well as animal) parasitic infections.

[1] Frausnitz, C., and Köstner, H., *Zentr. Bakteriol., Parasiteuk.*, Abt. I, **86**, 160 (1921).

[2] Coca, A. F., and Grove, E. F., *J. Immunol.*, **10**, 445 (1925).

[3] Kuhns, W. J., *Proc. Soc. Exp. Biol. Med.*, **108**, 63 (1961).

[4] Augustin, R., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.), 1076 (Blackwell, Oxford, 1967).

[5] Stanworth, D. R., and Kuhns, W. J., *Immunology*, **8**, 323 (1965).

[6] Stanworth, D. R., and Kuhns, W. J., quoted in Stanworth, D. R., *Adv. Immunol.*, **3**, 181 (1963).

[7] Hemmings, W. A., and Brambell, F. W. R., *Brit. Med. Bull.*, **17**, 96 (1961).

[8] Tiselius, A., and Kabat, E. A., *J. Exp. Med.*, **69**, 119 (1939).

[9] Brattsten, I., Colldahl, H., and Laurell, A. H. F., *Acta Allergol.*, **8**, 339 (1955).

[10] Stanworth, D. R., *Immunology*, **2**, 384 (1959).

[11] Franklin, E. C., and Stanworth, D. R., *J. Exp. Med.*, **114**, 521 (1961).

[12] Heremans, J. F., Heremans, M. T., and Schultze, H. E., *Clin. Chim. Acta*, **4**, 96 (1959).

[13] Stanworth, D. R., *Intern. Arch. Allergy*, **28**, 71 (1965).

[14] Vaerman, J. P., Epstein, W., Fudenberg, H., and Ishizaka, K., *Nature*, **203**, 1046 (1964).

[15] Fireman, P., Vannier, W. E., and Goodman, H. C., *J. Exp. Med.*, **117**, 203 (1963).

[16] Ishizaka, K., Ishizaka, T., and Hornbrook, M., *J. Allergy*, **34**, 395 (1963).

[17] Ishizaka, K., and Ishizaka, T., *J. Allergy*, **37**, 169 (1966).

[18] Stanworth, D. R., *Adv. Immunol.*, **3**, 181 (1963).

[19] Rowe, D. S., and Fahey, J. L., *J. Exp. Med.*, **121**, 171 (1965).

[20] Terr, A. I., and Bentz, J. D., *J. Allergy*, **35**, 206 (1964).

[21] Andersen, B. R., and Vannier, W. E., *J. Exp. Med.*, **203**, 117 (1963).

[22] Ishizaka, K., and Ishizaka, T., *J. Allergy*, **42**, 330 (1968).

[23] Ishizaka, K., Ishizaka, T., and Hornbrook, M. M., *J. Immunol.*, **97**, 35 (1966).

[24] Johansson, S. G. O., Bennich, H., and Wide, L., *Immunology*, **14**, 265 (1968).

[25] Johansson, S. G. O., and Bennich, H., *Immunology*, **13**, 381 (1967).

[26] Bennich, H., Ishizaka, K., Ishizaka, T., and Johansson, S. G. O., *J. Immunol.*, **102**, 826 (1969).

[27] Stanworth, D. R., Humphrey, J. H., Bennich, H., and Johansson, S. G. O., *Lancet*, ii, 330 (1967).

[28] Bennich, H., Ishizaka, K., Johansson, S. G. O., Rowe, D. S., Stanworth, D. R., and Terry, W. D., *Bull. World Health Org.*, **38**, 151 (1968).

[29] Ogawa, M., Kochwa, S., Smith, C., Ishizaka, K., and McIntyre, O. R., *New Engl. J. Med.*, **281**, 1217 (1969).

[30] Stanworth, D. R., Humphrey, J. H., Bennich, H., and Johansson, S. G. O., *Lancet*, ii, 17 (1968).

[31] Stanworth, D. R., Housley, J., Bennich, H., and Johansson, S. G. O. (in preparation).

[32] Stanworth, D. R., Housley, J., Bennich, H., and Johansson, S. G. O., *Immunochemistry*, **7**, 321 (1970).

[33] Sheard, P., Killingback, P. G., and Blair, A. M. J. N., *Nature*, **216**, 283 (1967).

[34] Van Arsdel, P. P., and Sells, C. J., *Science*, **141**, 1190 (1963).

[35] Goodfriend, L., and Luhovy, J. I., *Intern. Arch. Allergy*, **33**, 171 (1968).

[36] Shore, P. A., Burkhalter, A., and Cohn, U. H., *J. Pharmacol. Exp. Ther.*, **127**, 182 (1959).

[37] Schild, H. O., in *Biochemistry of the Acute Allergic Reactions* (edit. by Austen, K. F., and Becker, E. L.), 99 (CIOMS Symp., 1968).

[38] Lichenstein, L. M., in *Biochemistry of the Acute Allergic Reactions* (edit. by Austen, K. F., and Becker, E. L.), 153 (CIOMS Symp., 1968).

[39] Stanworth, D. R., and Henney, C. S., *Immunology*, **12**, 1267 (1967).

[40] Hastie, R., *Clin. Exp. Immunol.* (in the press).

[41] Stanworth, D. E., *Immediate Hypersensitivity* (monograph, in preparation).

[42] Henney, C. S., and Stanworth, D. R., *Nature*, **210**, 1071 (1966).

[43] Stanworth, D. R., *Clin. Exp. Immunol.*, **6**, 1 (1970).

[44] Levine, B. B., *J. Immunol.*, **94**, 111, 121 (1965).

[45] De Weck, A. L., and Schneider, C. H., in *Bayer Symp. on Problems in Allergy*, 1 (1969).

[46] Ishizaka, K., and Ishizaka, T., *J. Immunol.*, **100**, 554 (1968).

[47] Forsgren, A., and Sjöquist, J., *J. Immunol.*, **97**, 822 (1966).

[48] Stanworth, D. R., Matthews, N., and Sjöquist, J. (in preparation).

[49] Matthews, N., and Stanworth, D. R., *Proc. Eighteenth Bruges Symp. Protides of Biological Fluids* (in the press).

[50] Fernö, O., Högberg, B., and Uvnäs, B., *Acta Pharmacol.*, **17**, 18 (1960).

[51] Becker, E. L., and Austen, K. F., *J. Exp. Med.*, **124**, 379 (1966).

[52] Lichenstein, L. M., *Proc. Eighth Symp. Collegium Intern. Allergologicum*, Switzerland (1970).

[53] Catt, K. J., *Lancet*, i, 763 (1970).

[54] Jaques, R., *Intern. Arch. Allergy*, **28**, 221 (1965).

[55] Ishizaka, K., and Ishizaka, T., *Clin. Exp. Immunol.*, **6**, 25 (1970).

[56] Patterson, R., *Prog. Allergy*, **13**, 332 (1969).

[57] Stanworth, D. R., *Clin. Allergy*, **1** (in the press).

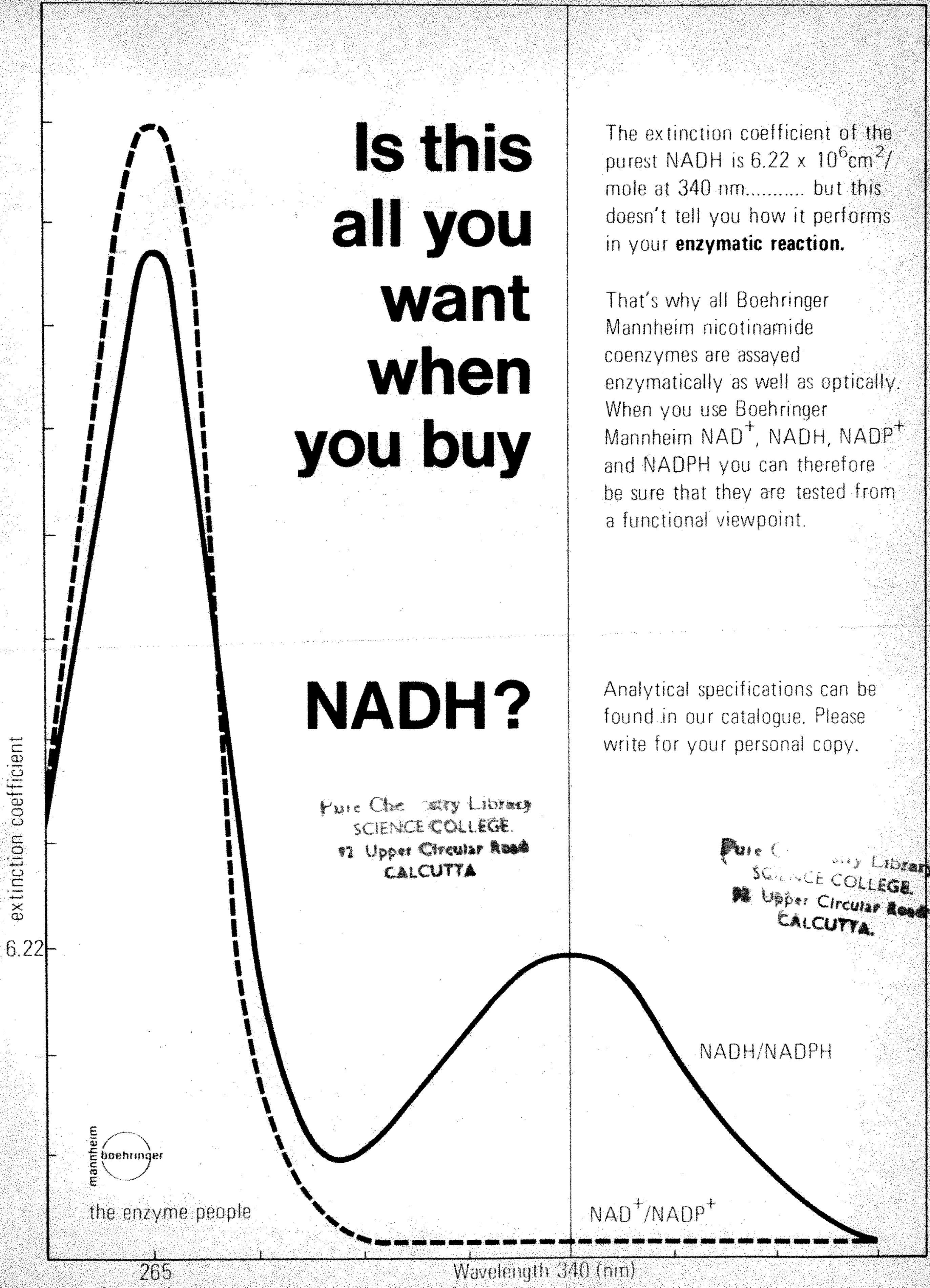
[58] Vide, L., Bennich, H., and Johansson, S. G. O., *Lancet*, ii, 1105 (1967).

[59] Rowe, D. S., *Bull. World. Health Org.*, **40**, 613 (1969).

[60] Miller, H. R. P., and Jarrett, W. F. H. (in the press).

[61] Stewart, D. F., *Austral. J. Agric. Res.*, **4**, 100 (1953).

[62] Bennich, H., and Johansson, S. G. O., *Vox Sang.*, **19**, 1 (1970).



Is this all you want when you buy NADH?

The extinction coefficient of the purest NADH is $6.22 \times 10^6 cm^2$/mole at 340 nm........... but this doesn't tell you how it performs in your **enzymatic reaction.**

That's why all Boehringer Mannheim nicotinamide coenzymes are assayed enzymatically as well as optically. When you use Boehringer Mannheim NAD$^+$, NADH, NADP$^+$ and NADPH you can therefore be sure that they are tested from a functional viewpoint.

Analytical specifications can be found in our catalogue. Please write for your personal copy.

mannheim boehringer
the enzyme people

The Boehringer Corporation (London) Ltd., Bilton House, Uxbridge Road, London W5 2TZ

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA.

Every issue contains:

- authoritative articles and reviews
- assessments of oceanographic instruments
- details of new equipment
- coverage of major conferences and exhibitions
- news of world-wide underwater activities
- keyworded titles of at least 600 recently published articles, papers and reports selected from over 2000 specialist publications
- abstracts of over 70 new patent applications

Published alternate months

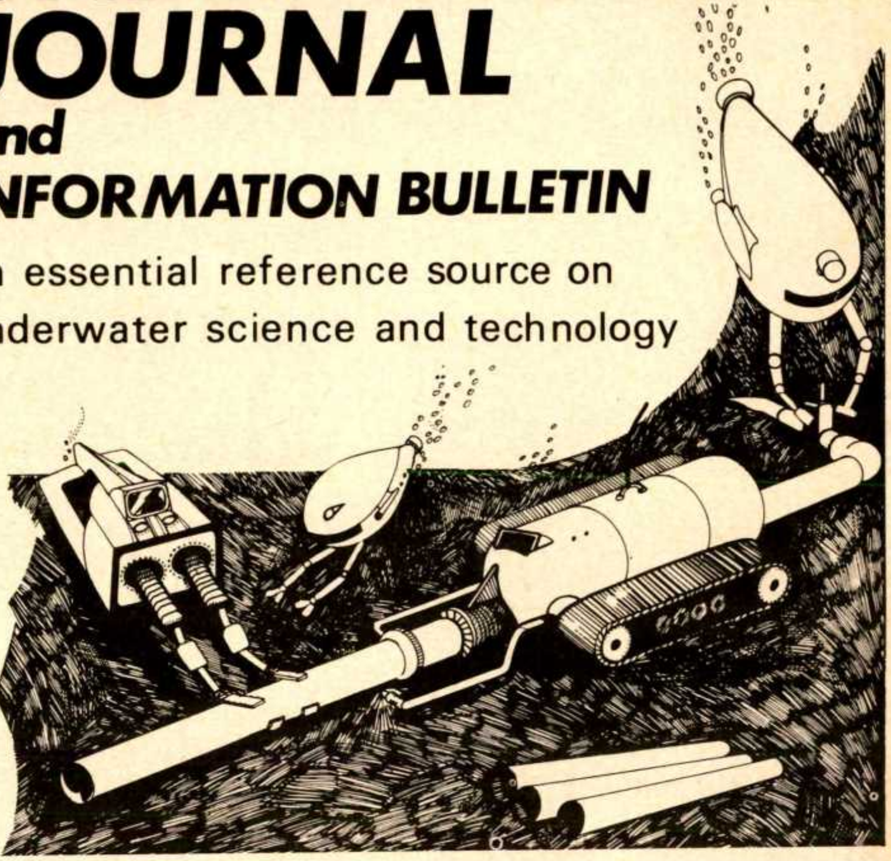
Annual subscription £20 ($50)

Details from:

IPC Science and Technology Press Ltd,
32 High Street, Guildford,
Surrey, England.

UNDERWATER JOURNAL and INFORMATION BULLETIN

an essential reference source on underwater science and technology



ODUM: FUNDAMENTALS OF ECOLOGY

New Third Edition

574 pages. 227 figures with 63 tables. August 1971

£5.00

Here is a thoroughly up-dated review of the principles of ecology, world environments, and ecologic technology. Dr. Odum stresses man's environmental relationships and suggests solutions to his environmental problems.

The book's three parts: *Basic Ecological Principles*, *The Habitat Approach*, and *Applications and Technology* are abundantly cross-referenced so that each can be read, referred to, or taught separately.

This new revision is almost entirely rewritten to reflect today's great advances and increased interest in ecology. Completely new chapters include: Systems Ecology; Pollution and Environmental Health; and Space Travel. Dr. Odum emphasises such topics as crop ecology—world food production—the total energy environment—community development.

By Eugene P. Odum, *Professor of Zoology, University of Georgia.*

LENTZ: CELL FINE STRUCTURE:

An Atlas of Drawings of Whole-Cell Structure

437 pages. 184 illustrations. May 1971. **£4.80**

Information on biological ultrastructure has accumulated at a rapid rate and virtually all cell types have been examined with the electron microscope. Yet, due to the nature of the procedures necessary to view tissues with the electron microscope, it is difficult to obtain a complete picture of an entire cell in a single micrograph. Dr. Lentz has provided a solution to this problem through his new atlas. His collection of hand drawn and finely detailed diagrams permits all the information formerly available only through the study of numerous electron micrographs to be included in a single illustration.

A set of 35 mm black and white slides is available for use with or without the text.

SLIDES:

185 black and White 35 mm slides. August 1971

£31.90

By Thomas L. Lentz, M.D., *Department of Anatomy, Yale University School of Medicine.*

W. B. SAUNDERS COMPANY LTD. **12 Dyott Street London WC1A 1DB**

Cyclic Expression of Blood Group Determinants in Murine Cells and their Relationship to Growth Control

D. B. THOMAS
Department of Immunology, Middlesex Hospital Medical School, London W1

A model for antigenic variation is proposed on the basis of changes in the expression of blood group determinants during the cell cycle.

THERE is a correlation between the growth and surface properties of cells. The division of normal cells in culture is arrested at a critical density[1-3]. This "density-dependent inhibition"[4] of growth is less pronounced for tumours[5] and parallels their degree of malignancy[6,7]. Alteration of the surface membrane of neoplastic cells has been suggested as a mechanism for defective control of growth[1,8]. Consistent with such a proposal is the finding that virally transformed fibroblasts possess surface receptors for plant agglutinins[9,10] which normal fibroblasts express only during mitosis[11].

There are, however, several examples of change in the surface properties of tumours during cell division—an increase in electrophoretic mobility of human osteosarcoma cells[12], a decrease of surface immunoglobulin of human lymphomas[13], a decrease of H-2 alloantigens in the murine cell lines YCAB[14], JLS-V9[15], and the mastocytoma P815Y[16], and an increase of blood group H in HeLa cells during mitosis[17]. Such changes may reflect a common surface property of dividing cells.

The expression of blood group A on primary amnion cells in culture also varies with the growth conditions[17,18]. Franks and Dawson demonstrated that the number of A positive cells in cultures of the rabbit cell line, RK13, was higher during rapid division[19]. It was shown, by clonal selection, that antigen negative cells gave rise to both positive and negative progeny. I describe here the expression of blood group determinants B and H in synchronized cultures of the mouse mastocytoma P815Y and phytohaemagglutinin (PHA)-treated mouse lymphocytes, and propose a model for antigenic variation during cell division.

The mouse mastocytoma P815 strain Y was maintained in suspension culture in Fischer's medium supplemented with 10% horse serum. Cell synchrony was achieved by the double thymidine block procedure[20], as follows: an exponential culture (2×10^5 cells/ml.) was grown for 15 h with 2×10^{-3} M thymidine, 6.5 to 8 h in its absence and a further 8 h with 2×10^{-3} M thymidine. The cells were reincubated in fresh medium at a cell density of $2\text{–}3 \times 10^5$/ml. The degree of synchrony was monitored by serial cell counts and measurement of ^{3}H-thymidine incorporation into DNA. Cells had a mean generation time of 15 h.

Mouse mesenteric node lymphocytes were cultured with PHA (Wellcome Reagents Ltd, reagent grade, reconstituted to 5 ml. with distilled water) in Eagle's minimum essential medium (MEM) supplemented with 10% foetal calf serum. Control cultures were treated with PHA for 3 h before collection.

Cells were stained for blood groups B and H by indirect immunofluorescence. Cell suspensions (2×10^6; 200 µl.) were incubated with an equal volume of antiserum at the appropriate dilution (1/100 to 1/200) for 30 min at 3° C, washed three times with cold MEM, and incubated for 30 min at 3° C with 50 µl. of fluorescein-conjugated rabbit anti-human gamma-globulin (1/20 dilution, previously absorbed with an equal volume of mastocytoma cells). Cells were washed a further four times with cold MEM and examined with a Leitz Ortholux microscope under ultraviolet light. Controls for serological specificity were antisera absorbed with an equal volume of A-positive or B-positive human erythrocytes, or anti-A antiserum. Anti-B antiserum (Lot No. 70–52103) was a gift from Ortho Pharmaceuticals and anti-H antiserum ('Baxter' serum, activity for O and A human erythrocytes) was a gift from Dr K. L. G. Goldsmith, MRC Blood Group Reference Laboratory. For the determination of mitotic index, cells were lysed with 1% sodium citrate and the nuclei washed with cold methanol–acetic acid mixture (3 : 1 v/v). Air-dried smears were stained with May-Grünwald-Giemsa.

In P815Y cells, the expression of blood group determinants B and H was related to growth rate (Fig. 1). Resting cells (late log phase) were negative for B and positive for H. On transfer to fresh medium, an inverse change occurred in antigen expression during the exponential phase of growth. It was decided to study this event during the period of the cell cycle.

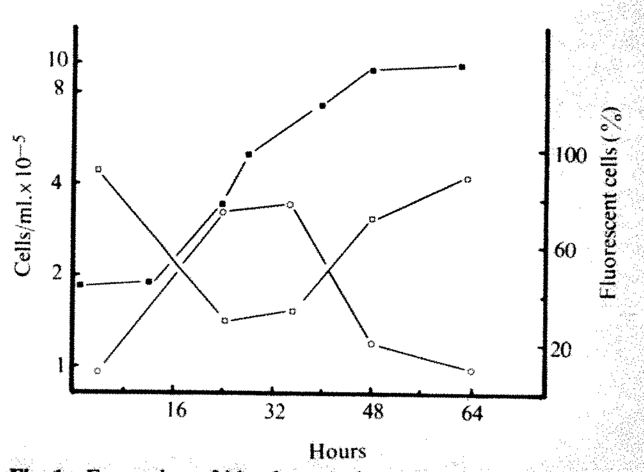


Fig. 1 Expression of blood group determinants B and H in an asynchronous culture of P815Y cells. ■, Cell number; ○, B positive cells; □, H positive cells.

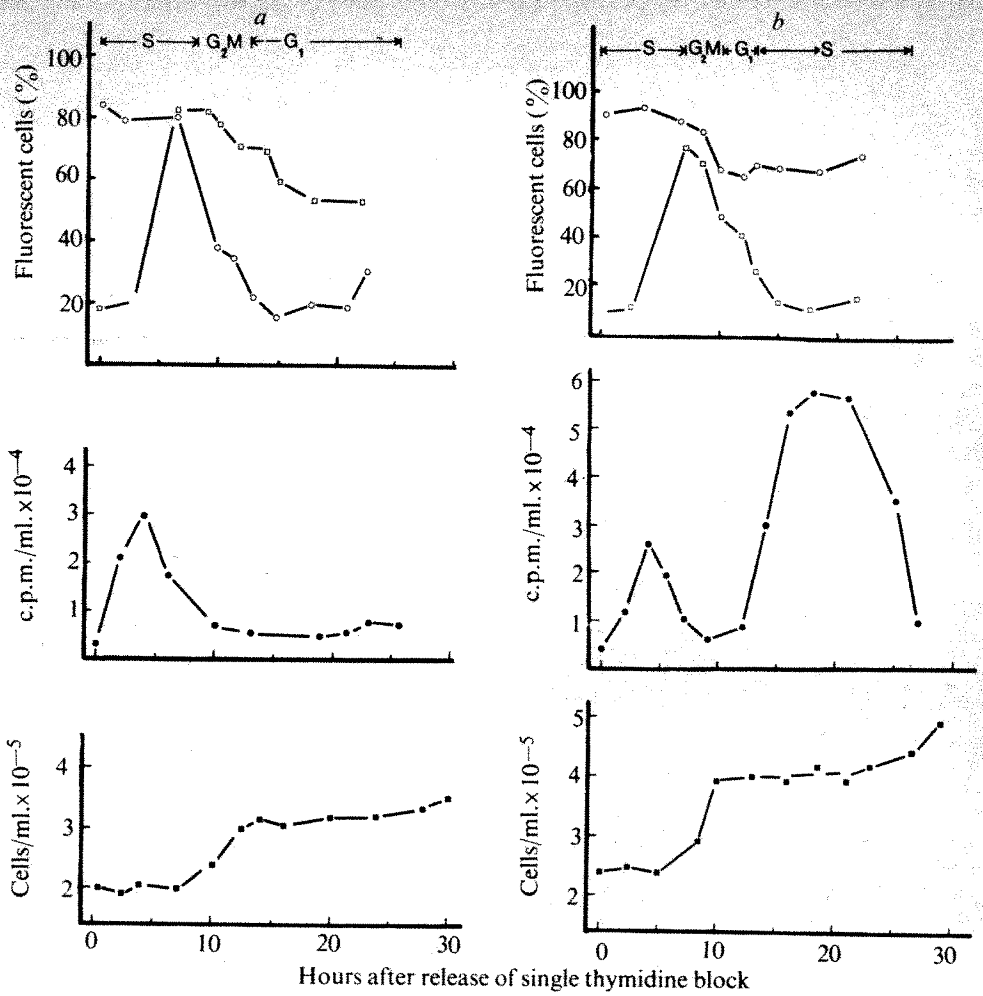


Fig. 2 Expression of blood group determinants B and H in synchronized P815Y cells. Conditions of cell culture were identical apart from the time of the second thymidine addition (7.75 h for *a*; 7 h for *b*). Cell samples (1 ml.) were removed at intervals and incubated with ^{3}H-thymidine (0.4 μCi/ml.) for 30 min. Cells were washed with MEM and the DNA was precipitated with 5% trichloroacetic acid. Precipitates were dispersed in 1% 'Triton X-100' and counted in a liquid scintillation spectrophotometer using a toluene phosphor. Samples of synchronized cells were analysed for cell number (■), ^{3}H-thymidine incorporation (●), blood group B (○), and blood group H (□).

In preliminary experiments, using the double thymidine block procedure, it was possible to obtain synchrony for two cell divisions occasionally (2 in 5). Failure to obtain more than one synchronous division is a common feature of different cell lines and synchrony procedures; the reasons remain obscure[21]. Appearance of a second peak of DNA synthesis was critically dependent on the release period between the first and second thymidine blocks. Also, the degree of synchrony was influenced by cell contact, spinner cultures being more successful than stationary cultures. This is illustrated in Fig. 2 where change in B and H expression was examined in cultures giving one (Fig. 2*a*) or two (Fig. 2*b*) synchronous cell divisions. Conditions of cell culture were identical apart from the time of the second thymidine addition; the sample in Fig. 2*a* was maintained as a stationary culture for the duration of the second thymidine block. In the absence of a second peak of DNA synthesis (Fig. 2*a*), G_1 cells were B^- and H^+. However, this is not always a feature of G_1 and the majority of cells in the second culture (Fig. 2*b*) were B^+ and H^- in this phase. Decay of synchrony and inability to express B was not a consequence of thymidine cytotoxicity. G_1 (B^- and H^+) cells were viable (>98% by dye exclusion tests) and exhibited asynchronous growth after a lag period of several hours. To decide whether the above changes occurred at mitosis or an early part of G_1, cells were released from a single thymidine block and cultured in the presence of the mitotic inhibitor, colcemid (Fig. 3). It is seen that H is maximal at mitosis. No quantitative difference was found for B expression in the interval between thymidine removal and complete mitotic arrest; different samples were equally positive for B at all dilutions of antiserum. Therefore expression of B and H at G_1 is related to the ability of cells to undergo further synchronous division. The difference in staining pattern for blood groups B and H in P815Y cells is illustrated in Fig. 4.

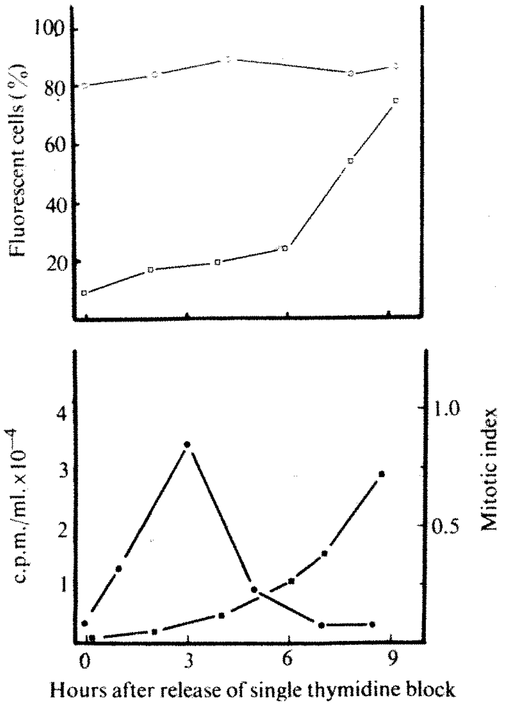


Fig. 3 Expression of blood groups B and H in P815Y cells after release of single thymidine block. An exponentially growing culture (2×10^5 cells/ml.) was exposed to 2×10^{-3} M thymidine for 15 h. Cells were washed and resuspended in fresh medium containing colcemid (0.04 μg/ml.). Samples were removed at intervals and examined for ^{3}H-thymidine incorporation (●), mitotic index (■), blood group B (○), and blood group H (□).

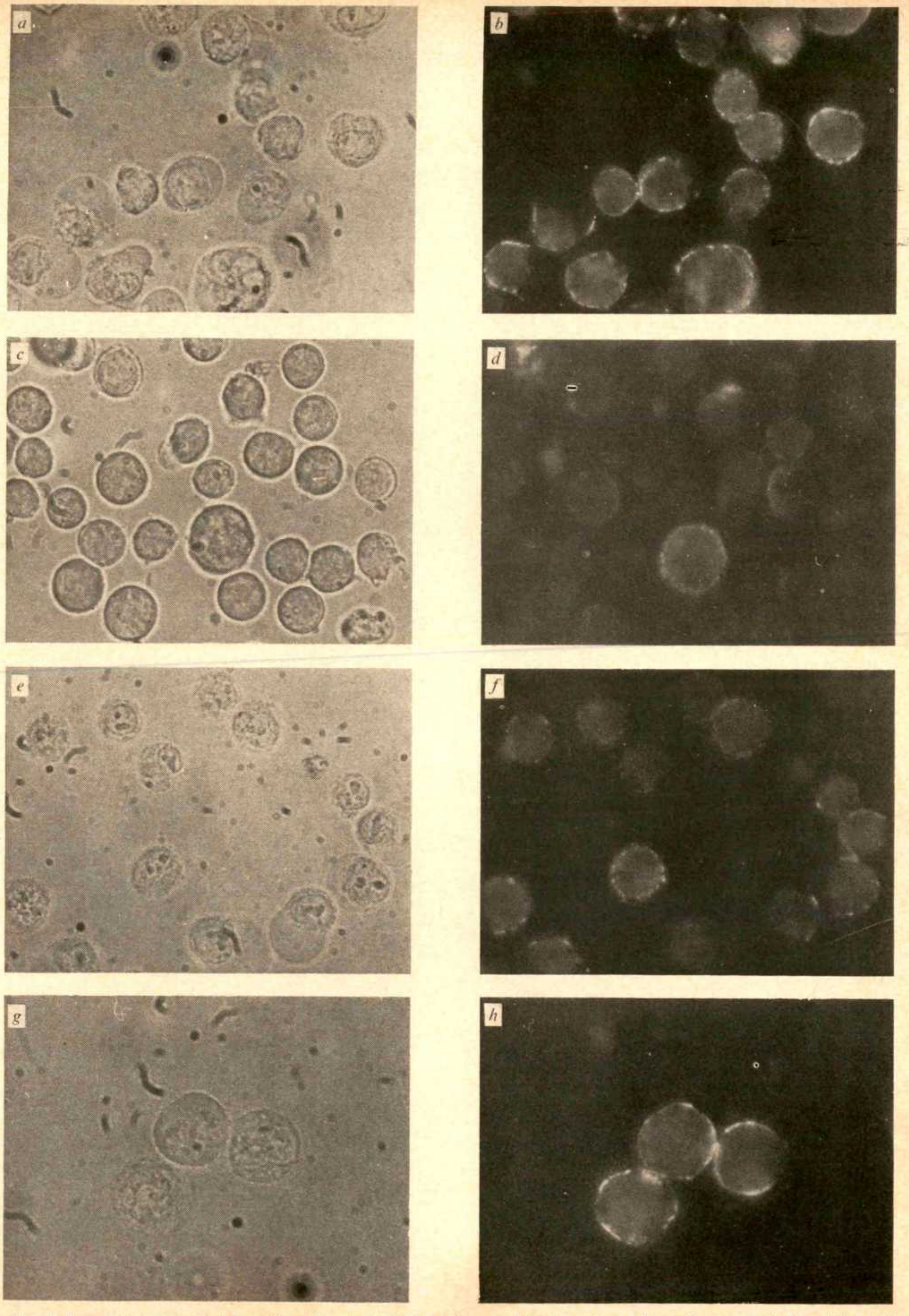


Fig. 4 Immunofluorescent staining of synchronized P815Y cells: *a* and *b*, H positive cells at G_1; *c* and *d*, B positive and negative cells at G_1; *e* and *f*, H positive and negative cells at S; *g* and *h*, B positive cells at S.

Preliminary experiments suggest that a similar change in B expression occurs in synchronized cultures of the murine leukaemia, L1210. Resting cells are B^-, thymidine blocked cells are B^+, and G_1 cells are B^- after one synchronous division. I have excluded the possibility that this restricted expression is only a feature of tumours by showing a similar occurrence for normal mouse by lymphocytes.

Lymphocytes represent a homogeneous population of resting, G_0, cells which divide only on contact with specific antigen or mitogenic agents such as the plant agglutinin, PHA. Also mouse lymphocytes are largely B^- (7–10% B^+ cells in mesenteric lymph nodes). Table 1 shows that B^+ cells were present in lymphocyte cultures after incubation with PHA. The number of positive cells was related to the PHA concentration, being maximal at or near the optimum mitogenic dose (Table 2). At earlier times (48 h, Table 1) the number of B^+ cells exceeded that of ^{3}H-thymidine-labelled cells. This finding suggests that expression of B is an early event of blast cell transformation. Appreciable agglutination of lymphocytes occurred after incubation with PHA and estimation of cell numbers was approximate. However, each cell with a typical lymphoblastoid appearance under phase contrast microscopy was also fluorescent. Appearance of B on PHA-treated lymphocytes is not strain specific; similar results were obtained with the strains Balb/C, CBA, C3H and DBA/2 all of which showed >20% B^+ cells at 48 h of culture. The occurrence of this surface change may prove useful as an additional index of lymphocyte transformation.

It was not possible to examine the spatial relationship of B to H-2 alloantigens on the cell surface by blocking procedures with specific antisera. The pretreatment of B^-, P815Y cells with anti-H-2^d (C3H anti-DBA/2) antiserum gave a positive result on subsequent immunofluorescent staining for blood group B. This may be due to cross-reaction of mouse immunoglobulin with the B determinant.

The chemical basis of serological specificity is established for the ABO system[22]; thus blood group antisera provide useful reagents for detecting the presence of specific sugar residues at the cell surface. The fortuitous cross-reaction of murine antigens with B and H determinants does not imply identity with the parent blood groups. It can be concluded only that a glycolipid or glycoprotein moiety with terminal α-galactosyl 1→3 galactose (B specificity) or α-fucosyl 1→2 galactose (H specificity) residues is present on the cell surface. It is a matter of conjecture whether appearance of B requires *de novo* synthesis or exposure of a pre-existing, cryptic antigen[10]. The restricted expression of B may not be a general feature of murine cells. Thus the thymus has a high mitotic rate, yet only 8–10% of thymocytes are B^+ by indirect immunofluorescence.

Table 1 Appearance of Blood Group B Positive Cells in Mouse Lymphocyte (Balb/c Strain) Cultures after Treatment with PHA (1/100 Dilution)

Time of culture (h)	Control cultures % B^+ cells	Control cultures % ^{3}H-thymidine-labelled cells*	PHA-treated cultures % B^+ cells	PHA-treated cultures % ^{3}H-thymidine-labelled cells*
0	7–10	0	7–10	0
24	3	<1	8–10	<1
48	<2	<1	20–25	7–10
72	<2	<1	20–25	24–28

* Determined by autoradiography after exposure of cells to ^{3}H-thymidine (1 μCi/ml.) for 3 h.

Table 2 Effect of PHA Concentration on the Mitogenic Response and Expression of Blood Group B in Lymphocyte (Balb/c Strain) Cultures at 72 h

PHA concentration	% B^+ cells	^{3}H-thymidine incorporation* (c.p.m./ml. × 10^{-3})
0	<1	0.65
1/500	5–10	6.0
1/200	20–25	19.7
1/100	20–25	35
1/50	<5	3

* Cells (2 × 10^6/ml.) were incubated with ^{3}H-thymidine (1 μCi/ml.) for 3 h and the trichloroacetic acid-insoluble radioactivity determined by scintillation counting.

In P815Y cells, there is an inverse relationship between expression of H-2 alloantigens and blood group H on the one hand, and blood group B on the other. H-2 has been shown to be minimal during the intermitotic period[16], in agreement with a reported variation of H-2 and viral antigens in other murine cell lines[14,15]. Variation in surface antigens during cell division has been accounted for by a limited period of gene transcription and/or decrease in rate of synthesis[14–16]. However, the appearance of a new determinant, B, suggests that decrease of H-2 and H might be due to a conformational change at the cell surface. A similar proposal has been made to account for change in surface charge and electrophoretic mobility during cell division[23].

The expression of H is maximal in synchronized HeLa cells at mitosis, but for surface antigens in other cell lines the peak occurs in G_1[14,15]. Cikes and Friberg have tried to reconcile this difference by suggesting that the appearance of H was an early G_1 event[15]. Yet the present studies show that, according to degree of synchrony (Fig. 2), cells can proceed directly at mitosis from an H^+ state to an H^-G_1 state. Thus the antigenic

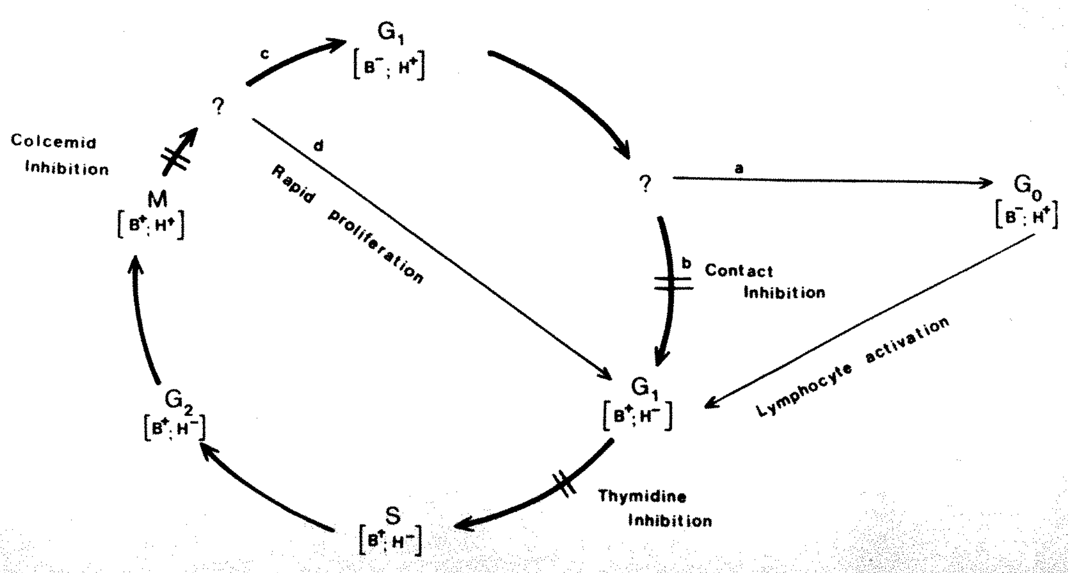


Fig. 5 Proposed model for antigenic variation during the cell cycle. B^+ is α-galactosyl 1→3 galactose; H^+ is α-fucosyl 1→2 galactose.

properties of G_1 cells may reflect a potential for further division.

Model for Antigenic Variation

I wish to propose a model for antigenic variation during the division of P815Y cells and the PHA-induced transformation of mouse lymphocytes (Fig. 5). The evidence in support of the scheme is as follows. (1) Resting cells (G_1 or G_0) are B^- and H^+ and give rise to B^+ and H^- cells during exponential growth (Fig. 1). (2) At mitosis, cells can proceed to a G_1 state which is B^- and H^+ (Fig. 2*a*) or B^+ and H^- (Fig. 2*b*). (3) G_1 cells (B^+ and H^-, Fig. 2*b*) undergo synchronous division while G_1 cells (B^- and H^+, Fig. 2*a*) are restricted to asynchronous growth. (4) Blood group B determinant is expressed on lymphocytes after treatment with PHA, and its appearance precedes DNA synthesis (Table 1).

Accordingly, the ability to express the α-galactosyl 1→3 galactose moiety (B determinant) is a prerequisite for cell division. It is not claimed that changes in B and H expression initiate cell division, but rather that they provide an index of a cell with commitment to mitosis. Two branch points exist in the cycle (a+b; c+d) which may serve as sites of growth control. Escape from growth inhibition is associated with the permanent expression of B at the cell surface (Fig. 2*b*). In limiting conditions of growth, there is an increase in the number of B^- cells (Fig. 1). It is suggested that cells, at mitosis, can be diverted from a path of rapid proliferation (d) to one of growth inhibition (c) with arrest in a G_1 (or G_0)B^- state.

It is known that variation in division time of cells, *in vivo* and *in vitro*, can be accounted for by differences in the G_1 period, the sum of $S+G_2+M$ being constant[24,25]. Also, G_1 is absent for some cells during rapid proliferation[26]. The possibility of two pathways, c and d, for cell division would provide a basis for this variation in the G_1 interval.

Furthermore, it is reasonable to expect that differences in surface properties of rapidly dividing (tumour, embryonic ?) cells and other cells would be most apparent in G_1 and would be minimal in limiting conditions of growth.

I thank Professor I. M. Roitt for advice and encouragement, A. V. T. for helpful discussion, and Mrs G. Stead for preparation of the manuscript. This work was supported by the Medical Research Council.

Received July 5, 1971.

[1] Abercrombie, M., and Ambrose, E. J., *Cancer Res.*, **22**, 525 (1962).
[2] Eagle, H., *Science*, **148**, 42 (1965).
[3] Levine, L., Becker, Y., Boone, C., and Eagle, H., *Proc. US Nat. Acad. Sci.*, **53**, 350 (1965).
[4] Stoker, M. G. P., and Rubin, H., *Nature*, **215**, 171 (1967).
[5] Todaro, G. J., and Green, H. J., *J. Cell Biol.*, **17**, 299 (1963).
[6] Pollack, R. E., Green, H., and Todaro, G. J., *Proc. US Nat. Acad. Sci.*, **60**, 126 (1968).
[7] Aaronson, S. A., and Todaro, G. J., *Science*, **162**, 1024 (1968).
[8] Wallach, D. F. H., *Proc. US Nat. Acad. Sci.*, **61**, 868 (1968).
[9] Burger, M. M., and Goldberg, A. R., *Proc. US Nat. Acad. Sci.*, **57**, 359 (1967).
[10] Burger, M. M., *Proc. US Nat. Acad. Sci.*, **62**, 994 (1969).
[11] Fox, T. O., Sheppard, J. R., and Burger, M. M., *Proc. US Nat. Acad. Sci.*, **68**, 244 (1971).
[12] Mayhew, E., *J. Gen. Physiol.*, **49**, 717 (1966).
[13] Buell, D. N., and Fahey, J. L., *Science*, **164**, 1524 (1969).
[14] Cikes, M., *J. Nat. Cancer Inst.*, **45**, 979 (1970).
[15] Cikes, M., and Friberg, S., *Proc. US Nat. Acad. Sci.*, **68**, 566 (1971).
[16] Pasternak, C. A., Warmsley, A. M. H., and Thomas, D. B., *J. Cell Biol.* (in the press).
[17] Kuhns, W. J., and Bramson, S., *Nature*, **219**, 938 (1968).
[18] Dawson, A., and Franks, D., *Exp. Cell Res.*, **47**, 377 (1967).
[19] Franks, D., and Dawson, A., *Exp. Cell Res.*, **42**, 543 (1966).
[20] Galvazi, G., and Bootsma, D., *Exp. Cell Res.*, **41**, 438 (1966).
[21] Peterson, D. F., and Anderson, E. C., *Nature*, **203**, 642 (1964).
[22] Watkins, W. M., in *Glycoproteins*, BBA Library (edit. by Gottschalk, A.), **5**, 462 (Elsevier, Amsterdam, 1966).
[23] Kraemer, P. M., *J. Cell Biol.*, **33**, 197 (1967).
[24] Cameron, I. L., and Greulich, R. C., *J. Cell Biol.*, **18**, 31 (1963).
[25] Prescott, D. M., *Cancer Res.*, **28**, 1815 (1968).
[26] Robbins, E., and Schraff, M. D., *J. Cell Biol.*, **34**, 684 (1967).

Pure Chemistry L
SCIENCE COLLE
92 Upper Circular
CALCUTTA

Cytostatic Antibody and SV40 Tumour Immunity in Hamsters

KATHLEEN R. AMBROSE, N. G. ANDERSON & J. H. COGGIN, jun.

Molecular Anatomy (MAN) Program, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, and Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37916

Ambrose *et al.* propose a likely scheme for the course of tumour development, as a failure of the host immune system.

THERE is growing support for the view that cancer involves a failure of the immune system of the host[1], and we have set out to investigate the mechanism of this failure in newborn hamsters injected with SV40 virus. For our purposes, this system has the advantage that autochthonous tumours occur reproducibly, that tumourigenesis can be prevented by immunization (which shows that the immune system of the host is functional before the occurrence of tumours) and that all tumours have the same transplantation antigens (TATAS).

Tumours occur reproducibly after a latent period of 90 to 300 days, during which the appearance of tumours can be prevented either by a second SV40 injection or immunization with irradiated SV40 tumour cells[2]. In previous studies, we attempted to delineate the roles of cell-mediated immunity and humoral immunity in this response. The occurrence of cell-mediated immunity is demonstrated by the way in which lymphocytes from immune animals will protect non-immune animals against challenge by tumour cells[3], but the role of humoral immunity is more obscure. Circulating cytotoxic antibodies have been reported in patients recovering from Burkitt's tumour[5], and there is some evidence that what are called blocking antibodies will enhance the growth of tumours, either by reacting with target cells[6] or with immune lymphocytes[7].

To distinguish between cell-mediated and humoral immunity *in vivo*, we have measured quantitatively the growth of tumour cells in small plastic chambers which are implanted in the peritoneal cavity and which are permeable to antibodies, not to cells[8,9]. After several days, the chambers are removed and the viable cells are counted. In non-immune animals, the number of cells increases, but in animals which are immune, growth is retarded or even prevented. So long as the humoral factors have not been isolated in pure form, we prefer to describe humoral factors defined by this method as cytostatic and not cytotoxic.

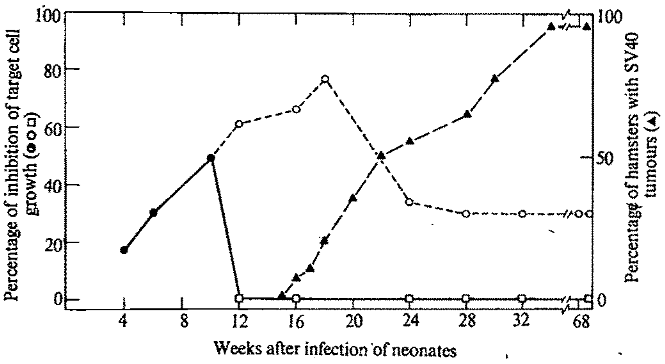


Fig. 1 Detection of cytostasis of SV40 tumour cells in diffusion chambers implanted in hamsters at intervals following neonatal infection with SV40 virus. ●, Cytostatic antibody in all animals; ○ tumour free animals; □, pre-tumour animals; ▲, palpable tumours.

In our experiments, we use animals of the LVG/LAK strain of Syrian golden hamsters. We produce autochthonous tumours by injecting newborn hamsters in the right subscapular space with 0.2 ml. doses of a suspension of SV40 virus (Fig. 1). Palpable tumours begin to appear at the site of injection after about 90 days, and 95% of the hamsters develop tumours after 200–300 days. To immunize adult hamsters so as to interrupt oncogenesis, three 0.2 ml. doses of the same virus suspension are injected at weekly intervals.

Antibody in Latent Period

One series of experiments was designed as a test for circulating antibody in the latent period before the appearance of tumours. After weaning at 21 days, we tested each week a group of ten animals (mixed sex groups) for circulating antibody, using as controls animals injected at birth with green monkey kidney passage control fluid medium containing no SV40 virus. Implanted chambers containing SV40 tumour cells were removed after 5 days and the animals were palpated for tumours at weekly intervals.

In the first 10 weeks of life, there is a surprising growth of circulating antibody in all ten infected animals in each infected group. Thereafter, there is a precipitous decline of circulating antibody in those animals which later develop tumours, with the loss of antibody always preceding the appearance of the tumour. Animals which remain free from tumours after 500 days still have measurable amounts of circulating antibody.

The transient occurrence of circulating antibody implies that the corresponding antigen is functional, which in turn implies that latency cannot be accounted for by dormancy of the virus. Instead, it seems as if transformed cells are produced and that they remain quiescent during latency, and we have been able to demonstrate that such cells do indeed exist.

To this end, we have been able to use the intracellular antigen called the T antigen which is produced when cells are transformed by SV40 virus and which may be detected with fluorescent antibodies[10,11]. The antigen is also produced during lytic infections[12] but is not always associated with oncogenicity, since cells transformed with SV40 virus but lacking T antigen have been found. Even so, the T antigen is a useful marker for cells which have been infected (but possibly not transformed) with SV40.

The inhibition relative to the control target cells is shown in Fig. 1.

Hamsters injected subcutaneously at birth with SV40 were killed in groups of ten, first at 8 h and then at 24 h intervals after injection. The tissue in the rejection area (5–8 mm in diameter) was sectioned and examined for T antigen using fluorescein conjugated antibody (Flow Laboratories). Within 8 h, approximately 10% of the cells examined showed strong diffuse immunofluorescence in the cytoplasm and a bright halo around the nuclear membrane. Cells from control hamsters injected with virus-free culture fluid were uniformly negative. In infected animals, more than 90% of the cells in the connective tissue region showed cytoplasmic fluorescence by 48 h but on the fourth day, few cells showed specific staining in either the nucleus or the cytoplasm, suggesting abortive infection. There was no evidence of cell degeneration. Intra-nuclear fluorescence was seen in a few pockets of six to eight cells after 7 days, but these numbered less than 5% of the cells in the injection area. During the next 17–20 days, cytoplasmic staining disappeared in the T antigen-positive cells and nuclear fluorescence weakened. A new type of large cell never seen in control sections was, however, observed to emerge among the positive cells. These cells, which formed foci and increased in number after 30 days are thought to give rise to tumours.

This work demonstrates that infected and presumably transformed cells persist in fairly large numbers during the latent period and produce the antigen or antigens giving rise to circulating antibody. As competence to produce humoral antibody appears before that for cellular immunity[14], circulating antibody may be produced initially and may inhibit a cellular response afferently or efferently.

To define the role of humoral factors more closely, we ask whether serum containing circulating antibody can inhibit SV40 tumour cell proliferation when administered passively in implantation chambers.

Table 1 Detection of Cytostatic Antibody against SV40 Target Cells following Passive Administration of Serum to Normal Hamsters

Serum donor	Average No. viable cells/chamber after 5 days' incubation *	% cytostasis compared with normal serum control
Normal control	618,000	
Hyperimmunized to SV40 tumour	327,000	47
SV40 tumour bearer	596,333	5

* Chambers seeded with 50,000 viable SV40 tumour cells.

SV40 target cells were incubated for 1 h *in vitro* in sera from normal immunized hamsters and from animals with tumours and then inoculated into diffusion chambers placed in weanling hamsters. Ten experimental and ten control animals were given 0.2 ml. of immune or control serum each day for 4 days. On the fifth day, the chambers were removed and the viable cells counted (Table 1). Normal serum gave a typical cell increase over 5 days, whereas animals receiving serum from SV40-tumour-immune hamsters showed cytostasis comparable with that observed in chambers implanted in immunized donors. In contrast, passive administration of serum from tumour bearers produced only slight inhibition of tumour cell growth. This confirms that a humoral factor plays a part in cytostasis but is absent or markedly decreased in tumour bearers. So far, the correlation between circulating antibody and the absence of a palpable tumour is complete, but the role of the circulating antibody remains obscure.

Course of Antibody Production

Does circulating antibody invariably follow immunization? To test this, the time course of appearance of circulating antibody was followed in adult hamsters after a single intraperitoneal injection of either 10^7 irradiated (5,000 r.) SV40 tumour cells or SV40 virus (Fig. 2). Circulating antibody was detected after five days, increasing to 65% inhibition and falling to a low level, followed by a slow rise in animals immunized with tumour cells. Baboon anti-hamster IgG serum labelled with fluorescein was used to show that cells recovered from chambers at 24 days were coated with IgG while those recovered at 8 days were not. Unfortunately, anti-hamster IgM serum was not available to determine whether the inhibition observed between 4 and 12 days was due to IgM, but it seems reasonable to make this assumption. The SV40 virus response apparently is due only to IgG. Animals immunized to both irradiated tumour cells* and virus were resistant to SV40 tumour cell challenge.

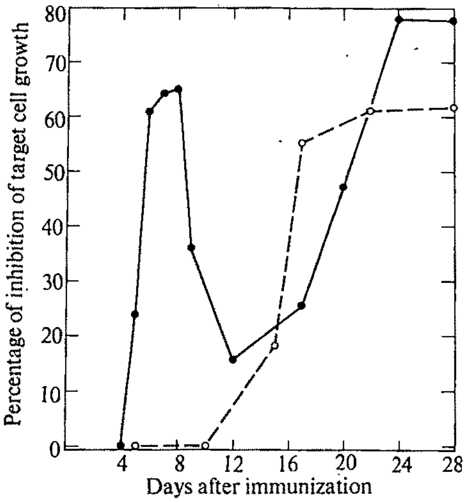


Fig. 2 Time course of appearance of cytostatic antibody against SV40 tumour cells in diffusion chambers in hamsters immunized with either irradiated SV40-transformed tumour cells grown in tissue culture or SV40 virus. ●, SV40 tumour cells (5,000 r.); ○, SV40.

The rapidity of the immune response observed with irradiated cell immunization raises the question of whether a transient circulating antibody response also occurs when nonimmunized animals are challenged with tumour-producing doses of SV40-transformed cells.

SV40 tumour cells (5×10^4 viable cells) were injected subcutaneously in the subscapular region of weanling hamsters. Before this and at 5 day intervals afterwards, diffusion chambers containing SV40 tumour cells were implanted into the peritoneal cavity; and 5 days later the number of tumour target cells was counted (Fig. 3). Circulating antibody was detected by 5 days after injection and persisted for approximately 10 days in all animals. No circulating antibody could be detected in any hamster by 15 days, at which time 25% of the challenged animals had very small palpable masses at the site of tumour cell injection. All animals developed tumours by 20 days.

In this system, therefore, circulating antibody invariably precedes tumour appearance, slows tumour cell growth, and is always present in animals immune to tumour challenge. Does it reappear when tumours are removed ?

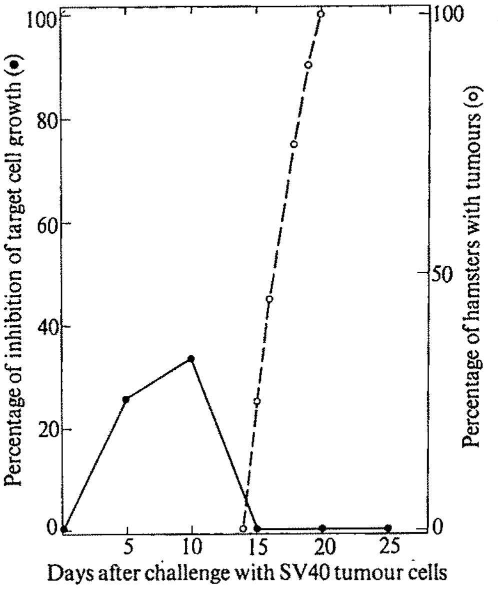


Fig. 3 Transient appearance of cytostatic antibody to SV40 tumour cells in diffusion chambers after implantation of live unirradiated SV40 tumour cells. ●, Cytostatic antibody; ○, palpable tumours.

Postsurgical Immune Response

Four weeks after injection of SV40 tumour cells into adult hamsters, a surgical cure was attempted. All animals had been monitored for circulating antibody, and none was found. Considerable attention to detail is required to achieve complete tumour removal. The cells used to produce tumours were injected subcutaneously in 0.1 ml. (5×10^4 cells) in a shaved area near the dorsal midline. Care was taken to minimize cell leakage or penetration of injected cells into adjacent tissues. When the fibrosarcomas reached 1–2 cm in diameter, the area was again shaved, the skin cleansed, and the tumour with considerable adjacent tissue lifted well away from the trunk and clamped off. The tumour and as much adjacent normal tissue as practical was cut away at the haemostat, and the wound was closed with clips, leaving the skin taut in the area of surgery. With these precautions and with care to avoid tumour cell dispersion *in situ* by vigorous palpation, a 50 to 60% cure rate could be achieved. Animals tumour free for 150 days after surgery were considered cured.

When circulating antibody levels were determined, about half the animals recovered after 10 days, which coincided with high levels of circulating antibody (Fig. 4). Results from all animals were pooled and plotted as a single result for each group, as the variation in cell counts was small for tumour-free animals (±5%).

Basis of Immune System Failure in Cancer

The conclusion that so called tumour-specific transplantation antigens, including those appearing after oncogenic virus infection, may be normal early embryonic antigens re-expressed in the tumour is reviewed in detail elsewhere[15–18]. The idea is based on four lines of evidence: (1) antisera to tumour surface antigens react with a variety of embryonic cells; (2) irradiated early embryonic cells immunize adult animals against a variety of tumours, including transplanted, autochthonous, spontaneous, and viral-induced tumours and elicit humoral and cellular responses against tumour detected *in vitro*; (3) during and after pregnancy circulating antitumour antibodies and lymphocytes are found; and (4) heterologous antisera to a wide variety of chemically induced tumours, after absorption with normal adult donor tissues, react with foetal cells. The para-

* We observed that splenectomy performed before multiple immunization of hamsters with irradiated SV40 tumour cells had no effect on circulating antibody formation, whereas splenectomy following immunization completely eliminated pre-existing levels of antibody. Both splenectomized animal groups resisted subsequent SV40 tumour challenge.

doxical problem inherent in the concept of immunologic surveillance is to reconcile why the foetal allograft, tolerated during normal pregnancy, is not rejected. Does a developing cancer survive by a similar mechanism, albeit outside the placental "barrier"?

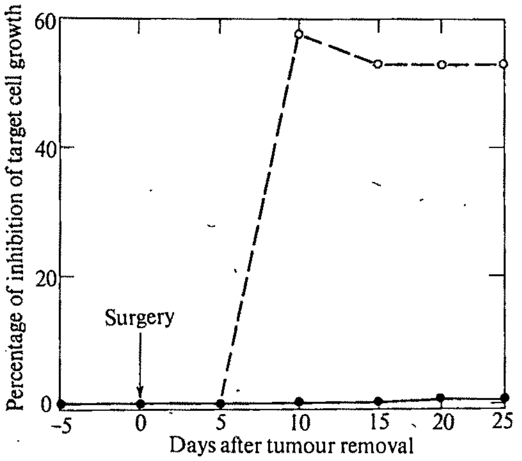


Fig. 4 Appearance of cytostatic antibodies after successful surgical removal of tumours (○) and continued absence of antibodies in surgical failures (●).

A solidly effective immune response to naturally occurring neoplasia may be difficult to achieve in practice, and may actually be effective only for very small groups of cells, as the data presented here suggest. We may divide the time course of tumour induction with SV40 virus into four phases: (1) Initial oncogenicity: a small tumour mass is established following virus infection which exhibits embryonic antigens. The cells may grow unrestricted for a short period (a few hours) before an immune response occurs. (2) Cytostatic immunity: some cells expressing tumour antigens may be destroyed during the initial antibody response; however, tumour cells are present for which the circulating antibodies are cytostatic and not cytotoxic. Numerous studies in our laboratory for many tumour systems in hamsters and mice revealed that *in vivo* humoral responses to tumour cells are cytostatic in nature[8,9,14,15]. It should be noted that the demonstration of cytotoxic antibodies usually requires a specially selected xenotypic complement to be effective[19,20] and that cytotoxicity is demonstrated experimentally *in vitro*. The result is a long latent period of very slow growth. During this period immunization with irradiated cells or virus is effective in preventing tumour formation. (3) Transition to local antigen excess: at some critical tumour mass, antigen production is thought to be sufficient to create a condition of local antigen excess, and rapid tumour growth ensues. As antigen excess becomes widespread, circulating antibody to the antigen becomes undetectable. (In preliminary experiments ^{131}I-labelled, tumour-eluted antibody injected into tumour bearers was rapidly localized in the kidneys as has been shown to occur with soluble antigen-excess complexes.) (4) Uncontrolled growth: once the circulating antibody is outstripped, the tumour mass increases rapidly. After successful surgery, however, the antibody rapidly reappears. Functional lymphocytes are present in tumour-bearing animals capable of destroying tumour cells *in vitro*[21,22], but these fail to eliminate the tumour. This failure most probably results from immunological "confusion" perpetuated by circulating antigen or antigen-antibody complexes at the lymphocyte surface. Circulating antibody may thus play a role as a masking factor preventing activation of both cell-mediated immunity and lymphocyte or macrophage function.

This is the simplest description consistent with the observations. Whether other tumours follow a similar time course remains to be determined. This description does not emphasize cell-mediated immunity, largely because it was not measured. However, both adoptive transfer of immunity with immune lymphocytes and colony inhibition studies have demonstrated a cellular defence mechanism against SV40 virus tumours[3,20,21].

Implications for Human Cancer

Many human cancers, including carcinoma of the cervix and prostate, have long latent periods during which cytostatic antibodies may appear. When rapid growth occurs, the antibody level should drop, to reappear again after successful treatment[22].

If the SV40 model studied here is a valid model for immunologic reactivity to autochthonous tumours in man, then the possibility of detecting cancer in the period of cytostatic antibody control exists.

We have not invoked here a multiplicity of different antibodies including enhancing or special blocking antibodies as distinct from cytotoxic ones. Rather, circulating antibody is thought to both depress tumour growth and prevent immune lymphocytes from being effective. In the latter role, these antibodies may produce enhancement. To evaluate the validity of this view, cytostatic antibodies and tumour surface antigens must be available in a high state of purity.

This work was supported by grants from the US Atomic Energy Commission, the National Institutes of Cancer, General Medical Sciences and Allergy and Infectious Diseases.

Received July 19; revised August 23, 1971

[1] Hellström, K. E., and Hellström, I., *Adv. Cancer Res.*, **12**, 167 (1969).

[2] Coggin, jun., J. H., Larson, V. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. Med.*, **124**, 774 (1967).

[3] Coggin, jun., J. H., Larson, V. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. Med.*, **124**, 1295 (1967).

[4] Hellström, I., Hellström, K. E., and Pierce, G. E., *Intern. J. Cancer*, **3**, 467 (1968).

[5] Herberman, R. R., and Fahey, J. L., *Proc. Soc. Exp. Biol. Med.*, **127**, 938 (1968).

[6] Hellström, I., and Hellström, K. E., *Intern. J. Cancer.*, **4**, 587 (1969).

[7] Hellström, K. E., and Hellström, I., *Ann. Rev. Microbiol.*, **24**, 373 (1970).

[8] Coggin, jun., J. H., and Ambrose, K. R., *Proc. Soc. Exp. Biol. Med.*, **130**, 246 (1969).

[9] Ambrose, K. R., Candler, E. L., and Coggin, jun., J. H., *Proc. Soc. Exp. Biol. Med.*, **132**, 1013 (1969).

[10] Pope, J. H., and Rowe, W. P., *J. Exp. Med.*, **120**, 121 (1964).

[11] Rapp, F., Kitahara, T., Butel, J. S., and Melnick, J. L., *Proc. US Nat. Acad. Sci.*, **52**, 1138 (1969).

[12] Levin, M. J., Oxman, M. N., Diamandopoulos, G. T., Levine, A. S., Henry, P. H., and Enders, J. F., *Proc. US Nat. Acad. Sci.*, **62**, 589 (1969).

[13] Diamandopoulos, G. T., Tevethia, S. S., Rapp, F., and Enders, J. F., *Virology*, **34**, 331 (1968).

[14] Hutchin, P., Amos, D. B., and Prioleau, W. H., *Transplantation*, **5**, 68 (1967).

[16] Coggin, jun., J. H., Ambrose, K. R., Bellomy, B. B., and Anderson, N. G., *J. Immunol.* (in the press).

[17] Coggin, jun., J. H., Ambrose, K. R., and Anderson, N. G., *Proc. First Conf. and Workshop on Embryonic and Fetal Antigens in Cancer* (DTIE, AEC, Oak Ridge, Tenn., to be published).

[18] Ambrose, K. R., Anderson, N. G., and Coggin, jun., J. H., *Nature* (in the press).

[19] Hellström, I., Hellström, K. E., Pierce, G. E., and Yang, J. P. S., *Nature*, **220**, 1352 (1968).

[20] Tevethia, S. S., Crouch, N. A., Melnick, J. L., and Rapp, F., *Intern. J. Cancer*, **5**, 176 (1970).

[21] Dierlam, P. J., Anderson, N. G., and Coggin, jun., J. H., *Proc. First Conf. and Workshop on Embryonic and Fetal Antigens in Cancer* (DTIE, AEC, Oak Ridge, Tenn., to be published).

[22] Morton, D. L., Eilber, F. R., Joseph, W. L., Wood, W. C., Trahan, E., and Ketcham, A. S., *Ann Surg.*, **172**, 740 (1970).

LETTERS TO NATURE

PHYSICAL SCIENCES

Decrease in Upper-atmosphere Rotation Rate at Heights above 350 km

THE average rate of rotation of the upper atmosphere can be evaluated from the small changes in inclinations of satellite orbits to the equator[1], and results obtained by analysing about thirty orbits have indicated[2] that the rotation rate increases from about 1.1 revolutions day^{-1} at a height of 200 km to about 1.4 revolutions day^{-1} at a height of 350 km. Various explanations of this "super-rotation" have been proposed[3–9], in terms of aerodynamic or electrodynamic theory, or both.

Recently, several values of upper-atmosphere rotation rate at heights above 370 km have been obtained[10,11], and these show that the increase with height does not continue beyond about 350 km. Instead the rotation rate seems to decrease to about 1.0 revolutions day^{-1} at 420 km and 0.7 revolution day^{-1} at 500 km. Fig. 1 shows results from thirty-two satellite orbits. The new values are those from the satellites Ariel 3 (1967–42A) at 390 and 500 km, from 1963–27A at 390 km and from 1968–86A at 410 km. Other previously unpublished results in Fig. 1 are from Cosmos 307 rocket[12] (1969–94B) at 240 km, and a preliminary value from Cosmos 316 (1969–108A) at 155 km.

If the upper atmosphere rotated at a constant angular rate, it would exert its greatest effect on a non-circular satellite orbit when the perigee was near the equator, and would have scarcely any effect when the perigee was at apex, that is, at maximum latitude. Consequently, as a first approximation, rotation rates obtained from orbital analysis can be regarded as averages over latitudes up to about half the satellite's orbital inclination. The four points on Fig. 1 at heights above 370 km are all obtained from near-polar orbits, whereas the two high values at heights near 360 km are from orbits with inclinations of 33° and 51°, which can be regarded as sampling the rotation up to latitudes of about 20° to 25° only. Thus if the equatorial rotation is particularly rapid, it would be appropriate for the line on the right in Fig. 1 to be faired in to the line on the left at a height of about 300 km, leaving the high values near 360 km out on a limb. If the rotation rate is independent of latitude, the join should be made at a greater height, near 350 km. Either method of joining gives a maximum rotation rate, that is maximum west-to-east winds, at a height between 300 and 350 km, which is near the height of maximum electron concentration in the F-layer of the ionosphere. A maximum rotation rate near this height might be expected if the rate is governed by electrodynamic forces, as suggested by Allan[3], Rishbeth[6] and Volland[9].

The decrease in the mean atmospheric rotation rate at heights between 400 and 500 km is not surprising. Pressure gradients and electrodynamic forces should be less important in the exosphere at heights of 500 to 1,000 km, where the neutral molecules, which are still numerically dominant, tend to pursue independent ballistic paths. Rapid rotation would imply that molecules in direct orbits travel considerably faster, on average, than those in retrograde orbits; but the faster molecules are more likely to escape, and this escape of the "high-velocity tail" should reduce the rotation rate in the exosphere, perhaps to much less than 1 revolution day^{-1}.

The wind pattern in the upper atmosphere at heights up to 500 km has an important influence on upper atmosphere heating, and analyses of orbits now being determined with

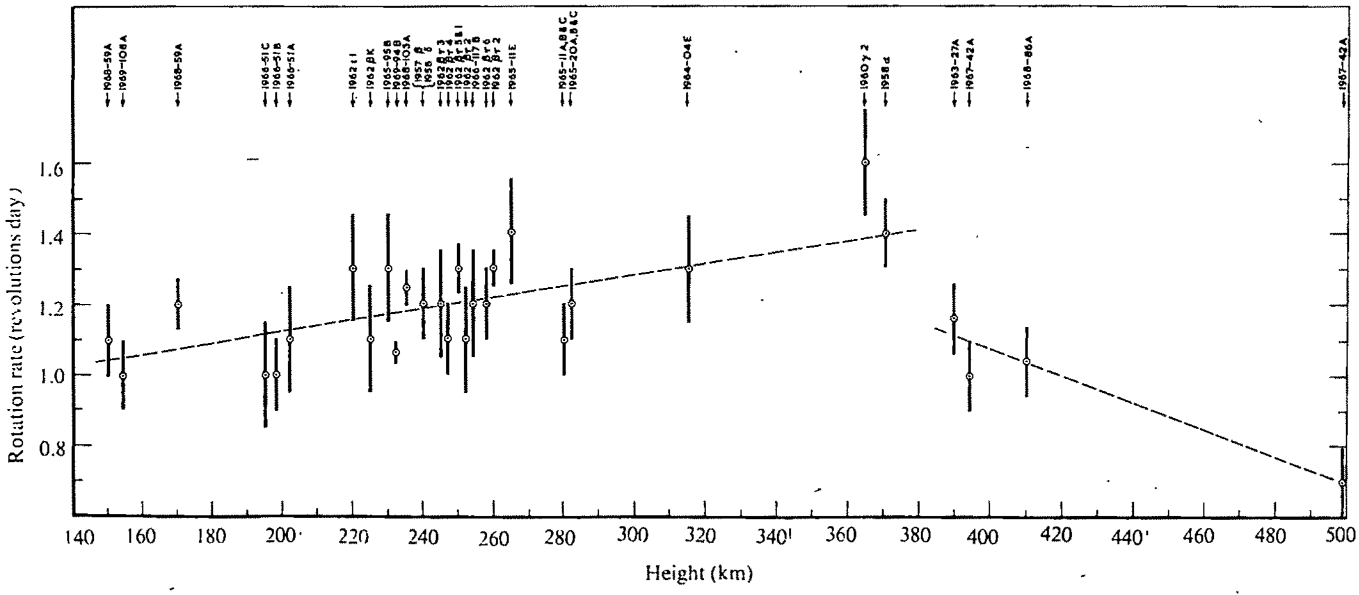


Fig. 1 Mean upper-atmosphere rotation rate derived from analyses of thirty-two satellite orbits.

the aid of the very accurate observations from the Hewitt cameras at Malvern and Edinburgh is yielding information on the variations of the zonal wind speed, which is likely to depend on local time as well as latitude. Results from Cosmos 307 rocket[12] and Cosmos 316 (unpublished data) suggest that the rotation rate sometimes departs greatly from its average value. This conclusion is in accord with recent measurements of very strong neutral winds, especially in auroral regions at times of geomagnetic disturbance when winds of more than 500 m s^{-1} have been recorded[13,14].

D. G. KING-HELE

Royal Aircraft Establishment,
Farnborough,
Hampshire

Received August 23, 1971.

[1] King-Hele, D. G., *Theory of Satellite Orbits in an Atmosphere* (Butterworths, London, 1964).
[2] King-Hele, D. G., Scott, D. W., and Walker, D. M. C., *Planet. Space Sci.*, **18**, 1433 (1970).
[3] King-Hele, D. G., and Allan, R. R., *Space Sci. Rev.*, **6**, 248 (1966).
[4] Challinor, R. A., *Planet. Space Sci.*, **17**, 1097 (1969); **18**, 1485 (1970).
[5] Cole, K. D., *Planet. Space Sci.*, **19**, 59 (1971).
[6] Rishbeth, H., *Planet. Space Sci.*, **19**, 357 (1971).
[7] Weinstein, D. H., and Keeney, J., *Nature*, **231**, 109 (1971).
[8] Isakov, M. N., Morosov, S. K., and Schnoll, E. E., COSPAR Meeting, Seattle, June 1971.
[9] Volland, H., *Nature*, **232**, 248 (1971).
[10] Gooding, R. H., *Nature Phys. Sci.*, **231**, 168 (1971).
[11] King-Hele, D. G., *Royal Aircraft Establishment Technical Report*, 71171 (1971).
[12] Hiller, H., *Royal Aircraft Establishment Technical Report*, 71151 (1971).
[13] Rees, D., *J. Brit. Interplanet. Soc.*, **24**, 233 (1971).
[14] Feess, W. A., COSPAR meeting, Seattle, June 1971.

Use of Astronomical Telescopes to measure Aerosol Pollution

ASTRONOMICAL observatories are particularly suited for studies of the background aerosol content of the atmosphere for four reasons. (1) The observatories are usually in remote locations away from the lights and smoke of cities. (2) Most observatories have standard equipment designed to correct the measured light fluxes of stars for the loss of brightness in the passage through the atmosphere (in other words, to correct for atmospheric extinction). (3) There is good global coverage of observatories. (4) Observations of the extinction of starlight by the atmosphere have been made for nearly forty years and are available for re-analysis. The last point is particularly important because there are no other reliable records of the condition of the atmosphere in remote locations.

In this article we compare data from three astronomical observatories[1] with other measurements of the scattering of light by aerosols at clean air locations. The scattering coefficients obtained by the different methods support other evidence for the existence of a background level of aerosols in the atmosphere, and also establish the astronomical telescope as a potentially valuable tool for monitoring aerosol pollution.

Extinction measurements are made photoelectrically by observing the apparent brightness of standard stars at different zenith angles[2]. Charlson *et al.*[1] have examined data from seven observatories for atmospheric extinction, but data that is statistically meaningful has so far only been obtained for three observatories.

The three observatories are Rattlesnake Ridge (Washington), Kitt Peak National Observatory (Arizona), and Cerro Tololo Interamerican Observatory (Chile). Rattlesnake Ridge Observatory is about 30 km west of the nearest centre of population, Richland, which has almost no polluting industry, and is at an altitude of 1,080 m in a clean, nearly desert, climate. Kitt Peak is at 2,064 m in a desert climate about 75 km west of the nearest centre of population (Tucson), and Cerro Tololo, by far the least polluted site, is at an altitude of 2,195 m about 450 km north of the nearest centre of population, Santiago.

The third column of Table 1 shows the geometric mean of the ratio of the extinction coefficient due to scattering from the total column of air between the observatory and the star $(\bar{b}_{\text{scat}})$ to the Rayleigh scattering by air (b_{Rayleigh}). These values have been interpolated to a wavelength of 500 nm to standard temperature and pressure. Absorption was neglected because it is expected to be very small compared with scattering. Charlson *et al.*[1] reported extinction k in magnitudes at the zenith so the following conversion was used to obtain the scattering coefficient $\bar{b}_{\text{scat}}$

$$\bar{b}_{\text{scat}} = \frac{0.92\,k}{X}$$

where X is the optical depth for scattering above the observatory ($\simeq 8$ km for a sea level observatory). The extinction due to ozone absorption in the Choppuis band was removed from this data assuming an optical depth for ozone of 0.25 cm at Cerro Tololo and 0.35 cm at the other sites. The super and subscripts in Table 1 are the 90% confidence limits. Rattlesnake Ridge showed much more variation than Kitt Peak or Cerro Tololo. These ratios are consistent with nephelometer and sunphotometer measurements at similar locations giving ratios of 1.5 on Mt Olympus, Washington, and 1.95 at Point Barrow, Alaska[3].

Table 1 Light Extinction Properties of the Atmosphere over Three Observatories

Location (altitude)	Effective wavelengths	$\bar{b}_{\text{scat}}/b_{\text{Rayleigh}}$ at 500 nm (No. of nights measured)		α total			Aerosol only α (aerosol)	
			$\bar{\alpha}_{RV}$	$\bar{\alpha}_{VB}$	$\bar{\alpha}_{BU}$	$\bar{\alpha}_{RV}$	$\bar{\alpha}_{VB}$	$\bar{\alpha}_{BU}$
Rattlesnake Ridge, Washington (1,080 m)	$R\sim 693$ nm $V\sim 537$ nm $B\sim 440$ nm $U\sim 365$ nm	$2.0^{+0.1}_{-0.9}$ (20 nights)	$3.8^{+1.4}_{-1.8}$	$2.1^{+1.6}_{-0.8}$	$3.3^{+0.9}_{-0.7}$	$3.6^{+1.4}_{-3.0}$	$0.6^{+3.2}_{-1.9}$	$1.4^{+3.1}_{-1.6}$
Kitt Peak, Arizona (2,064 m)	$R\sim 693$ nm $V\sim 537$ nm $B\sim 440$ nm $U\sim 365$ nm	$1.7^{+0.5}_{-0.3}$ (24 nights)	$3.4^{+1.7}_{-1.5}$	$2.2^{+0.7}_{-0.5}$	$3.8^{+0.2}_{-1.4}$	$2.9^{+2.6}_{-1.9}$	$-0.4^{+0.8}_{-3.0}$	—
Cerro Tololo, Chile (2,195 m)	$V\sim 553$ nm $B\sim 440$ nm $U\sim 366$ nm	$1.3^{+0.4}_{-0.3}$ (17 nights)	—	$3.8^{+1.2}_{-1.9}$	$4.1^{+0.8}_{-0.7}$	—	$1.5^{+3.5}_{-3.0}$	$3.2^{+3.3}_{-1.3}$

The astronomical measurements were made at several different wavelengths (see Table 1) that were defined using broad band (100 nm) glass filters. The wavelength dependence of the atmospheric scattering coefficient due to both aerosol and Rayleigh scatter can be described by

$$\overline{b}_{\text{scat}} \propto \lambda^{-\alpha\text{total}}$$

where αtotal is an empirically determined exponent having a value 4 in pure air. Similarly, the contribution to extinction from aerosol scatter alone can be described by

$$\overline{b}_{\text{scat}}\,(\text{aerosol}) \propto \lambda^{-\alpha(\text{aerosol})}$$

where α(aerosol) has a typical value of 1.3 for urban air. Volz has related α(aerosol) to size distributions of the form

$$\frac{\mathrm{d}N}{\mathrm{d}\log r} = Cr^{-\beta}$$

where dN is the number of particles in the logarithmic radius increment d log r, C is a function of the total amount of aerosol and β is an exponent of about 3 for urban air.

Within a range of $2 < \beta < 6$ the following relationship applies

$$\beta = a(\text{aerosol}) + 2$$

Table 1 lists the geometric mean values of αtotal and α(aerosol) obtained from the total extinction coefficients at the indicated wavelengths. For example, α_{RV} is measured between the red (693 nm) and the visible (537 nm for Cerro Tololo), and similarly for the other αs with subscript R implying "red", B implying "blue", and U implying "ultraviolet". The effective wavelengths for each site are shown in Table 1.

These wavelengths, incidentally, correspond to those used in the multiwavelength nephelometer results of ref. 4, although of course the telescope determinations refer to the entire line of sight whereas the nephelometer gives a measurement at a point.

In the rest of Table 1 the aerosol contribution has been isolated using the aerosol scattering coefficient $\overline{b}_{\text{scat}}\,(\text{aerosol}) = \overline{b}_{\text{scat}} - b_{\text{Rayleigh}}$. In some cases negative values of α(aerosol) are obtained, when the extinction due to the aerosol component increases rather than decreases with increasing wavelength, and indeed more than half the nights at all three locations showed anomalous, that is, negative, values. This type of scattering behaviour has also been observed by Quenzel *et al.*[5] in clean oceanic air during the Meteor Expedition. This type of scattering must be due to an aerosol size distribution different from the simple power law with β between 2 and 6. On very rare occasions anomalous aerosol extinction may even dominate the strong Rayleigh scattering wavelength dependence (once in a blue Moon). Because of the wide variation in the α(aerosol) at all three locations, much more data will be required to characterize the wavelength dependence of background aerosol.

All the data reported[1] were reduced completely by computer from original raw intensity measurements. The probable error of a single reduced intensity measurement is typically less than 2% at 500 nm. This uncertainty increases slightly toward shorter wavelength. Because the extinction typical at an observatory of altitude 4,000 foot is only about 20% at this wavelength, it is possible for the extinction measurements to be uncertain by ±10%. As the actual measurement error is usually less than 1%, we can credit most of the uncertainty to the fact that the standard stars are measured at random times and random zenith angles throughout the night, and are reduced by assuming no time or spatial variation in extinction during the night. Both time and spatial variation often do occur, however, due to unobserved clouds. As a ±10% error in extinction is related to only a 2% error in the intensity of the star at that wavelength, this is sufficient for most routine astronomical photometry. Better extinction measurements (a 1% error in extinction) could be obtained in the future by using a local intensity standard (such as a radioactive light source) so that one measurement in space and time could be used to calculate the extinction of the light from a standard star. In calculating the wavelength dependence, the uncertainty in extinction exerts a strong influence on α, especially when $\Delta\log b_{\text{scat}}$ is very small. In the same way a slightly incorrect choice of effective wavelengths of the filters also exerts an influence on α. The determination of the effective wavelengh for the broad band filters is at present complicated by the fact that the standard stars observed usually have different spectral distributions, usually with significant slopes in the wavelength region being measured, and include many different patterns of absorption lines. Using the mean values of $\overline{b}_{\text{scat}}$ shown in Table 1 the error in $\overline{b}_{\text{scat}}$ and wavelength can combine to make as much as a 60% error in the measured αs.

We conclude that astronomically determined measurements of atmospheric extinction are consistent with other measurements in clean air locations. These data provide information that can be used to characterize the background aerosol in the atmosphere and changes due to world-wide air pollution. So far, not enough old data has been reduced to establish possible secular changes in global atmospheric extinction, although Hodge[6] has found a significant increase in atmospheric extinction over the Mt Wilson observatory in California in the past fifty years.

This work was done as part of the ASTRA project funded by the National Science Foundation.

William M. Porch
Edward J. Mannery
Robert J. Charlson
Paul W. Hodge

University of Washington,
Seattle,
Washington 98105

Received February 1; revised June 24, 1971.

[1] Charlson, R. J., Hodge, P. W., Lucke, P. B., Mannery, E. J., and Snow, T. P., *Project ASTRA Publication No. 2* (Univ. Washington, Seattle, 1970).
[2] Hardie, R. H., in *Astronomical Techniques* (edit. by Hiltner, W. A., (Univ. Chicago Press, Chicago, 1962).
[3] Porch, W. M., Charlson, R. J., and Radke, L. F., *Science*, **170**, 315 (1970).
[4] Ahlquist, N. C., and Charlson, R. J., *Atmospheric Environment*, **3**, 551 (1969).
[5] Quenzel, H., *J. Geophys. Res.*, **75**, 2915 (1970).
[6] Hodge, P. W., *Project ASTRA Publication No. 4* (Univ. Washington, Seattle, 1970); *Nature*, **229**, 549 (1971).

Exchange of SO_2 between the Atmosphere and Natural Waters

Little is known about the rate of exchange of gas between the atmosphere and natural waters (such as oceans, rivers, lakes and airborne water droplets), and most work so far has concentrated on the more common atmospheric constituents, N_2, O_2, CO_2 and water vapour. The transfer of trace components, such as oxides of sulphur and nitrogen, has received almost no attention. This article is an attempt to predict the principal factors controlling the exchange of SO_2 across air–water interfaces in the natural environment.

Gas exchange rates are often given either as an exchange constant (k = flux of gas/concentration difference), or its reciprocal, which measures the "resistance" of the interface to gas transfer. Danckwerts[1] has shown that the overall resistance of a gas–liquid interface may be interpreted as the sum of the resistances of the individual phases:

$$\frac{1}{K_1} = \frac{1}{k_1} + \frac{1}{H\,k_2} \qquad (1)$$

$1/K_l$ is the total resistance on a liquid phase basis, $1/k_l$ and $1/k_g$ are the individual resistances of the liquid and gas phases and H is the Henry's law constant, where

$$H = \frac{\text{equilibrium concentration in gas phase}}{\text{equilibrium concentration of unionized dissolved gas in liquid phase}}$$

For a sparingly soluble gas, the liquid phase resistance term in equation (1) is much larger[2,3] than that for the gas phase. This is partly a reflexion of the large value of H for such a gas, and partly due to the fact that the molecular diffusivity (and hence diffusive transport) of most gases is about two orders of magnitude less in water than it is in air. By contrast the exchange of water vapour, in common with evaporation–condensation of any pure liquid, is controlled by resistance in the gas phase. The behaviour of SO_2 is likely to fall between these two extremes. One reason for this is the moderate solubility of the gas in water, which makes the gas phase resistance term larger than for a less soluble gas. Another factor is that the value of k_l may be increased because of the chemical reactivity of the gas in solution. Provided that the liquid is not too acidic, SO_2 molecules entering the water will be rapidly hydrated and then ionized. Transport of SO_2 near the liquid side of the interface may then be enhanced because the normal dissolved gas concentration gradient will be supplemented by the gradient of ionic species. Such enhancement of the value of k_l for a chemically reactive gas has been observed by Hoover and Berkshire[4] in laboratory studies using CO_2. These authors have derived the following expressions for calculating the degree of enhancement to be expected.

$$\alpha\, k_{l\,(\text{inert})} = k_{l(\text{reactive})} \qquad (2)$$

where

$$\alpha = \frac{\tau}{(\tau-1) + \left\{\tanh\left[\left(\frac{k^*\tau}{D}\right)^{\frac{1}{2}} \frac{D}{k_{l(\text{inert})}}\right] \Big/ \left(\frac{k^*\tau}{D}\right)^{\frac{1}{2}} \frac{D}{k_{l(\text{inert})}}\right\}} \qquad (3)$$

α is the ratio of the k_l values for a reactive and an inert gas exchanging under identical conditions, τ is the ratio of total to ionic forms of the gas in solution, k^* is the hydration rate constant for the gas, and D is the molecular diffusivity of the dissolved gas molecules.

Although derived for CO_2 exchange, equations (2) and (3) should also be applicable to SO_2 transfer. As the hydration rate constant for SO_2 ($\sim 10^6$ s^{-1}) is much higher than that for CO_2 ($\sim 10^{-2}$ s^{-1}) the exchange enhancement for SO_2 should be considerably more pronounced than the enhancement found for CO_2.

If equations (1) to (3) are to be used to estimate exchange rates for SO_2 under environmental conditions, typical values must be found for $k_{g(SO_2)}$ and $k_{l(\text{inert})}$ The value for $k_{l(\text{inert})}$ must then be multiplied by the appropriate value of α to obtain $k_{l(SO_2)}$. The value of α depends critically on τ, which in turn is a function of the pH of the water. To cover most environmental situations, I shall calculate α for pH values 2 to 9.

Using the data given by Schooley[5], a mean value for $k_{g(H_2O)}$ over the oceans of about 3,000 cm h^{-1} may be derived. Values over smaller bodies of water (lakes, rivers and so on) will almost certainly be less than this, but a value of 3,000 cm h^{-1} will be assumed for the present. It will further be assumed that k_g for SO_2 and H_2O are approximately equal. A typical value for $k_{l(\text{inert})}$ seems to be more difficult to obtain. Downing[6] gives values between 0.4 and 7.5 cm h^{-1} for O_2 in various freshwater environments although, exceptionally, values as high as 300 cm h^{-1} have been recorded. Measurements at sea lie in the region 4 to 36 cm h^{-1} (my work to be published). A reasonable guess would seem to be about 10 cm h^{-1} for $k_{l(\text{inert})}$. It will be shown that the values chosen for $k_{g(SO_2)}$ and $k_{l(\text{inert})}$ are not very critical to the conclusions to be drawn from the calculations.

Table 1 shows the results of the calculations in which it is assumed that $H = 3.8 \times 10^{-2}$ (ref. 7), $D = 2 \times 10^{-5}$ cm^2 s^{-1}, $k^* = 3.4 \times 10^6$ s^{-1} (ref. 8).

Table 1 Calculation of the Ratio of the Gas to the Liquid Phase Resistance

pH	α	$k_{l(SO_2)}$ cm h^{-1}	$\frac{1}{k_{l(SO_2)}} = r_l$ h cm^{-1} × 10^5	$\frac{1}{H\,k_{g(SO_2)}} = r_g$ h cm^{-1} × 10^3	r_g/r_l
2	2.7	27	3,700	8.8	0.24
2.8	11.7	117	855	8.8	1.03
3	18.0	180	560	8.8	1.57
4	169	1,690	59	8.8	14.92
5	1,376	13,760	7.3	8.8	120.6
6	2,884	28,840	3.5	8.8	251.4
7	2,966	29,660	3.4	8.8	258.8
8	2,967	29,670	3.4	8.8	258.8
9	2,967	29,670	3.4	8.8	258.8

The final column in Table 1 gives the ratio of the gas to the liquid phase resistance. At a pH of 2.8 both resistances are approximately equal but when the pH is below this value the liquid phase resistance is more important than the resistance of the gas phase. Above a pH of 2.8 the gas phase resistance becomes increasingly dominant. The exchange of SO_2 is interesting because it changes from liquid to gas phase control as the pH is raised. By contrast, although the r_l value for CO_2 decreases with increasing pH (due to the ionic gradient effect) it is always greater than the value of r_g. Thus exchange of CO_2 is principally controlled by the resistance of the liquid phase.

For a number of years chemical engineers have been making measurements of SO_2 transfer rates in packed columns[9,10]. Their experimental results seem to support the ideas outlined above, in that both the gas and liquid phase resistances appear to exert an appreciable effect on the overall resistance.

The pH of most natural waters in surface environments is between 4 and 9. It can therefore be concluded from the calculations set out above that, for almost all environmental air–water interfaces, gas phase resistance should control the SO_2 exchange rate. This conclusion would not have been substantially affected by using rather different values for $k_{g(SO_2)}$ and $k_{l(\text{inert})}$. Under calm conditions in both air and water, $k_{g(SO_2)}$ and $k_{l(\text{inert})}$ might be 1,000 cm h^{-1} and 2 cm h^{-1} respectively; in this case r_g and r_l would have been equal at a pH of about 3.0. By contrast, under turbulent conditions in both phases ($k_{g(SO_2)} = 5{,}000$ cm h^{-1}, $k_{l(\text{inert})} = 30$ cm h^{-1}), r_g and r_l become equal when the pH is about 2.5. Thus for a wide range of mixing conditions, pH values greater than about 4 imply that gas rather than liquid phase resistance will control the exchange of SO_2. The behaviour of SO_2 in crossing a natural air–water interface will therefore tend to resemble that of water vapour rather than O_2 or CO_2.

Two tentative conclusions may be drawn from this result. First, the vertical gradients of SO_2 and water vapour (humidity deficit) close to the sea surface should be similar. Second, the mean residence time of an SO_2 molecule in the atmosphere should be of the same order as that for a water molecule (~ 10 days, ref. 11). This contrasts with the much larger residence time for a CO_2 molecule (~ 7 yr, ref. 12); in this case, exchange is controlled by resistance in the liquid phase. A residence time of about 10 days for SO_2 is in reasonable agreement with the mean value of 5 days given by Junge[11].

I thank Mr A. C. Chamberlain of AERE, Harwell, for helpful discussions and comments on the manuscript.

P. S. Liss

School of Environmental Sciences,
University of East Anglia,
Norwich, NOR 88C

Received June 17; revised August 23, 1971.

[1] Danckwerts, P. V., *Gas–Liquid Reactions*, 145 (McGraw-Hill, London, 1970).

[2] Bolin, B., *Tellus*, **12**, 274 (1960).

[3] Skirrow, G., in *Chemical Oceanography* (edit. by Riley, J. P., and Skirrow, G.), **1**, 227 (Academic Press, London, 1965).

[4] Hoover, T. E., and Berkshire, D. C., *J. Geophys. Res.*, **74**, 456 (1969).

[5] Schooley, A. H., *J. Mar. Res.*, **27**, 335 (1969).

[6] Downing, A. L., in *River Pollution* (edit. by Klein, L.), **2**, 224 (Butterworths, London, 1962).

[7] Terraglio, F. P., and Manganelli, R. M., *J. Air Pollut. Control Assoc.*, **17**, 403 (1967).

[8] Eigen, M., Kustin, K., and Maass, G., *Z. Phys. Chem.*, **30**, 130 (1961).

[9] Adams, F. W., *Ind. Eng. Chem.*, **25**, 424 (1933).

[10] Whitney, R. P., and Vivian, J. E., *Chem. Eng. Prog.*, **45**, 323 (1949).

[11] Junge, C. E., *Air Chemistry and Radioactivity*, 3 (Academic Press, London, 1963).

[12] Craig, H., *Tellus*, **9**, 1 (1957).

Determination of the Density of Seawater

KREMLING has recently described[1] a promising new method for the determination of the density of seawater. The agreement he demonstrates between his results and those based on the equation of state, determined by Knudsen as a function of salinity and temperature, is even better than he suggests. This arises from an error in Kremling's interpretation of the new definition of salinity.

Salinity, S, is now defined by the International Tables[2] in terms of conductivity. The best estimate of chlorinity[3] is obtained by using the relation $\mathrm{Cl} = S/1.80655$ and not the Knudsen equation. One thus arrives at a lower value for Cl than estimated by Kremling which is most marked for the lower values of Cl. When the densities are recalculated one finds that Kremling's estimates are reduced by 0.20 at $S = 5\%$ and by 0.009 at $S = 20\%$ with linear interpolation between. Application of this correction suggests complete agreement in the mean with Knudsen and exemplifies once again the great quality of standards measurements in the early years of the century.

J. CREASE

National Institute of Oceanography,
Wormley,
Godalming,
Surrey

Received August 9, 1971.

[1] Kremling, K., *Nature*, **229**, 109 (1971).

[2] *International Oceanographic Tables* (National Institute of Oceanography of Great Britain and UNESCO, 1966).

[3] Cox, R. A., Culkin, F., and Riley, J. P., *Deep-Sea Res.*, **14**, 203 (1967).

Structure of Fibrous Carbon

THE existence of fibrous or whisker carbon has been reported in both synthetic studies of graphite[1,2] and in naturally occurring graphite[3]. The synthetic studies involved decomposition of hydrocarbons over metals[1] and decomposition of carbon monoxide over iron[2] and, particularly in the second of these studies, the occurrence of metal catalyst within the fibre was observed. Furthermore, it has been established[1] that the average orientation of the carbon crystallites is with their (0002) orientation parallel to the axis of the fibre. In this communication we describe the use of high resolution electron microscopy to establish the precise relationship between the orientation of the graphite planes and the catalyst particle within the fibre.

A nickel foil was heated to 700° C in 600 mm Hg of methane or propane for several hours and the ensuing film of graphite was stripped from the foil surface by immersion in concentrated HCl. The film of graphite was sufficiently thin to be studied by transmission electron microscopy and it contained outcrops of fibrous carbon as well as polycrystalline and high quality graphitic carbon. Fig. 1*A* shows the appearance of a typical carbon fibre formed in an atmosphere of methane, the very high width to length ratio of the fibres formed in methane being characteristic of this hydrocarbon. Fig. 1*B* is an enlargement of part of the previous figure so that the (0002) lattice planes can be seen. There are three principal regions of the fibre: in the centre is an irregular particle of what is believed to be metal (in this case it would be nickel) and surrounding this is a highly crystalline carbon with the (0002) lattice planes parallel to the surface of the metal particle; on the periphery of the fibre there is a narrow region of amorphous carbon. The first point of interest is the close parallelism of the graphite layer planes with the surface of the metal particle, because it has been reported

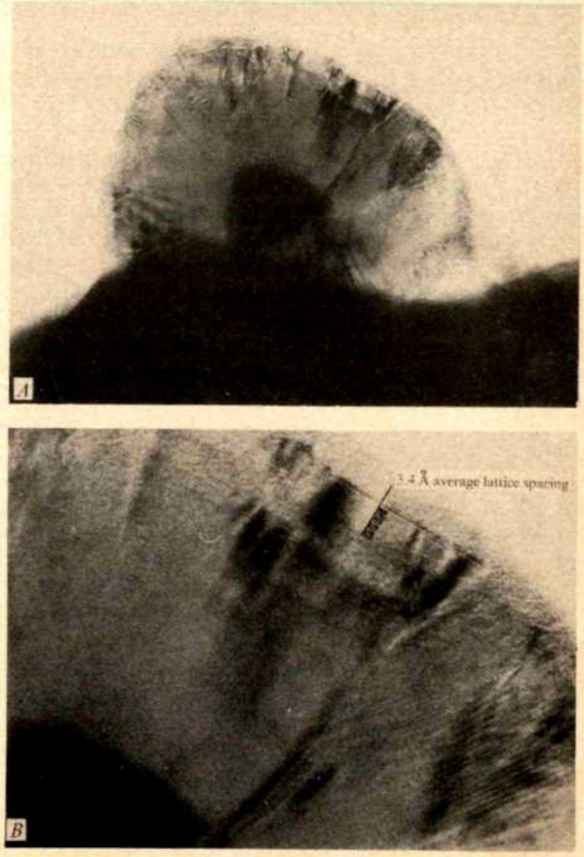


Fig. 1 *A*, Fibre produced by methane pyrolysis over nickel (× 312,000); *B*, Enlargement of a region of Fig. 1*A*, with (0002) lattice planes delineated for identification (× 1,000,000).

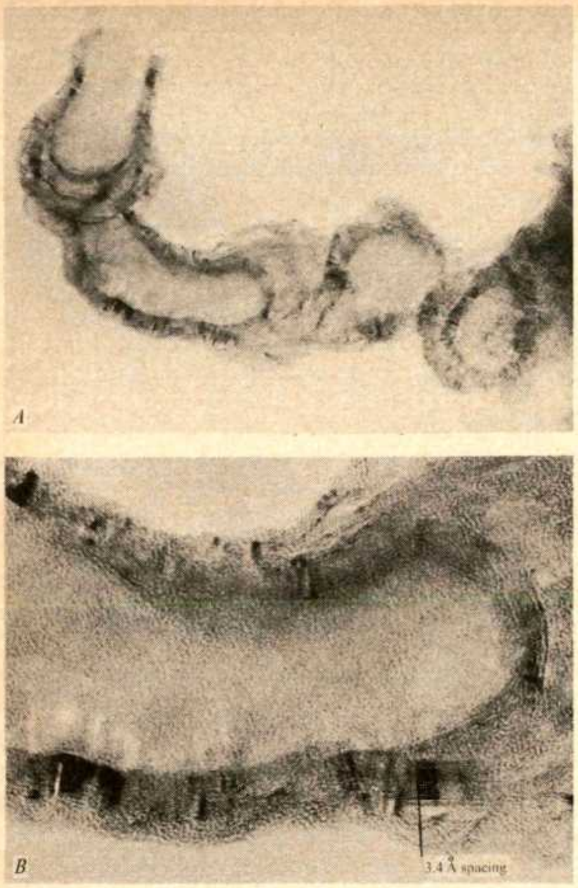


Fig. 2 *A*, Fibre produced by propane pyrolysis over nickel (× 312,000); *B*, Enlargement of a region of Fig. 2*A* with (0002) lattice planes delineated for identification (× 1,000,000).

that there is a preference for graphite growth on the (110) faces of nickel[4]. The irregular shape of the metal particle precludes the exposure of any single lattice plane all around its surface and yet there are no marked irregularities in the carbon fibre that would suggest a preferred direction of growth. From the intensity variations in the original micrograph, we also conclude that this fibre is approximately hemispherical in shape.

Fig. 2*A* is a carbon fibre formed by propane pyrolysis. No metal particle was observed in this fibre but the open nature of the end of the fibre and possibly along the centre indicates that the metal was removed by the preparative treatment in concentrated HCl. Fig. 2*B* is an enlargement of part of this fibre. Areas of resolved (0002) graphite lattice planes are again distinguishable throughout the fibre even at its root where it has grown from the main carbon deposit. The overall shape of this fibre, however, is markedly different from fibres produced by methane and the arcing that occurs periodically along the central region of the fibre suggests that the direction of growth bends in and out of the plane of the micrograph. The average direction of the (0002) lattice planes is, however, parallel to the axis of the fibre or, to be more precise, the direction is parallel to the areas where metal particles were believed to have been situated. The difference between these fibres and those produced from methane could be explained by the mobility of the metal particle in the case of the propane fibres; a much longer and more distorted fibre would then be produced as the metal moved away from the main carbon deposit.

The structure of these fibres—in particular, that produced by methane pyrolysis—raises several questions about the mechanism of their formation. It is difficult to imagine that the metal could exert catalytic influence up to 300 Å away from the metal surface and yet it is equally difficult to explain a mechanism that requires diffusion of active species perpendicular to the layer planes of graphite. Furthermore the micrograph in Fig. 1 shows that where there are faults in the layer plane structure which might provide an easy channel for diffusion, there is no marked production of carbon at the end of these channels as would be expected if a diffusion mechanism predominated. This argument is equally valid whether active species are considered to diffuse out or hydrocarbon molecules are considered to diffuse in, thus depositing the carbon layers adjacent to the metal surface and building up the fibre from the inside. We suggest that, as each carbon layer is laid down, atomically dispersed nickel is incorporated within this layer. In this form, catalytic deposition could take place on the outer layers of the growing fibre and would continue for some distance away from the metal although there would be a dilution of the metal atoms by the subsequent layers laid down. If this mechanism is applicable to general graphite formation, a dilution of nickel would be reached at some distance away from the metal surface where it is no longer possible for laminar graphite to be laid down; this has been observed by ourselves and by other workers[5]. At this stage the crystallite size of the carbon that is deposited is very small, and the crystallites are randomly oriented with respect to each other. This mechanism is further supported by oxidation studies on pyrolytic graphites that have been laid down by this technique. After removal of the graphite by oxidation there remain agglomerates of nickel that have been dispersed within the graphite[1], the agglomeration obviously being caused by the heating necessary for the oxidation reaction because agglomerates were not observed in the unreacted material.

We thank Dr J. L. Hutchison for assistance with the electron microscopy and the United Kingdom Atomic Energy Authority for financial assistance to B. G.

T. BAIRD
J. R. FRYER
B. GRANT

Department of Chemistry,
University of Glasgow,
Glasgow W2

Received August 18, 1971.

[1] Robertson, S. D., *Carbon*, **8**, 365 (1970).
[2] Ruston, W. R., Warzee, M., Hennaut, J., and Waty, J., *Carbon*, **7**, 47 (1969).
[3] Patel, A. R., and Deshapande, S. B., *Carbon*, **8**, 242 (1970).
[4] Presland, A. E. B., Roscoe, C., and Walker, P. L., jun., *Third Conference on Industrial Carbons and Graphite*, London, 1970.
[5] Blau, G., and Presland, A. E. B., *Third Conference on Industrial Carbons and Graphite*, London, 1970.

BIOLOGICAL SCIENCES

Requirement of Thymus-dependent Lymphocytes for Potentiation by Adjuvants of Antibody Formation

THE mode of action of immunological adjuvants is of theoretical as well as practical importance. Adjuvants can not only increase immune responses; they can also bring about change from one type of immune response to another. For example, doses of bovine serum albumin (BSA) that in the absence of adjuvant induce tolerance, in the presence of adjuvants induce antibody formation[1,2]. To understand how adjuvants switch on antibody formation it may be necessary to determine which of the cells involved in the immune response are affected by adjuvants. Earlier experiments[3-5] led to the conclusion that the cells initially involved in the action of adjuvants are macrophages. This interpretation was based on observations that particulate adjuvants (such as bacteria) are ingested by macrophages but not by lymphocytes. Non-particulate or particulate adjuvants taken up by macrophages in culture and injected into syngeneic mice increased the antibody response of the recipients to two proteins (*Maia squinada* haemocyanin or bovine serum albumin (BSA)); in contrast, adjuvants taken up by lymphoid cells used to reconstitute immune responses in irradiated recipients had no demonstrable effect on antibody formation.

It is now clear that immune responses to many antigens require co-operation of thymus-dependent (T) and bone marrow derived (B) lymphocytes[6], the former possibly functioning as an antigen-concentrating system while the latter give rise to cells which can synthesize and release antibody[7,8]. If it is accepted that adjuvants act initially on macrophages, which are not themselves immunocompetent, two hypotheses can be considered. According to the first, adjuvants increase the probability of direct interaction of macrophages containing antigen and B lymphocytes capable of producing antibody, by-passing the T lymphocytes. According to the second, adjuvants increase the effectiveness of the interaction of macrophages and T lymphocytes, and this results in greater stimulation of the appropriate B lymphocytes to produce specific antibody. In an attempt to decide between these hypotheses we have compared the effects of adjuvants on antibody production against BSA in normal young adult CBA male mice and CBA mice deprived of T lymphocytes by neonatal thymectomy followed by two injections at 5 and 6 weeks of 0.2 ml. of rabbit anti-mouse lymphocyte serum (ALS) in some experiments, or thymectomy at 8 weeks followed by 850 r. whole-body irradiation and reconstitution with syngeneic bone marrow[9]. As an additional control, thymectomized (TX), irradiated and reconstituted mice receiving also a syngeneic thymus graft under the renal capsule[10] have been used.

The primary immunization was by intraperitoneal inoculation with 1 mg centrifuged BSA in 0.5 ml. saline; 20 days later the mice were bled for determination of primary antibody responses. The same day they were injected with 100 μg of BSA intraperitoneally, and blood samples were taken 10 days later for determination of secondary antibody responses. As adjuvants 2 μg of purified *E. coli* lipopolysaccharide (LPS) or 0.4 μg of *Bordetella pertussis* vaccine (Wellcome) were administered together with the antigen in saline. Freund's incomplete adjuvant (ICFA—mineral oil, Bayo F, containing mannoside—mono-oleate, Difco), or Freund's complete adjuvant (FCA–ICFA with 100 μg of *Myobacterium butyricum*/ml.) or 5 mg of 'Retinol' in ICFA were injected intraperitoneally 1 h after administration of antigen in saline. Antibody was assayed by the Farr test[1,4]. The antigen-binding capacity for each group of six mice (except for those marked with an asterisk, which contained 5) is presented as the geometric mean of the antigen-binding capacity (ABC) expressed as $\log_{10}$, together with the standard error (s.e.) calculated from the variance.

Table 1 Serum Antibody against Bovine Serum Albumin in Normal and T Cell-Deprived Mice with and without Adjuvants

Treatment	Restoration	Adjuvant	Primary ABC	s.e.	Secondary ABC	s.e.
None	—	—	−0.42	0.14	0.57	0.15
None	—	LPS	0.34	0.08	1.61	0.17
None	—	Pertussis	0.24	0.06	1.73	0.26
None	—	ICFA	0.38	0.14	1.32	0.19
None	—	CFA	0.83	0.18	1.62	0.23
None*	—	'Retinol'	1.23	0.22	2.17	0.26
TX, ALS	—	—	−1.04	0.12	−0.96	0.12
TX, ALS*	—	LPS	−1.15	0.23	−1.12	0.18
TX, ALS	—	Pertussis	−1.32	0.14	−0.83	0.25
TX, ALS	—	ICFA	−1.37	0.11	−0.37	0.12
TX, ALS	—	CFA	−1.22	0.06	−0.93	0.15
TX, ALS	—	'Retinol'	−1.49	0.13	−0.82	0.21
TX, X-ray	Marrow	—	−1.36	0.19	−1.06	0.09
TX, X-ray	Marrow	LPS	−1.19	0.17	−1.27	0.13
TX, X-ray	Marrow	Pertussis	−1.08	0.08	−1.56	0.24
TX, X-ray	Marrow, thymus	—	−0.22	0.15	0.21	0.10
TX, X-ray	Marrow, thymus	LPS	0.13	0.09	1.26	0.08
TX, X-ray*	Marrow, thymus	Pertussis	0.27	0.06	1.34	0.27

Representative results are shown in Table 1. In mice depleted of T cells by either procedure antibody responses to BSA are decreased, as previously described by Taylor[11], and the disparity between normal and T cell-deprived mice is greater when the primary immunization is made with adjuvant. In mice reconstituted with a thymus graft marked stimulation of antibody formation by adjuvants is again seen.

It therefore seems that T lymphocytes are required for potentiation of antibody formation against BSA by adjuvants. It has been suggested[3–5] that one of the effects of adjuvants may be to bring about the release from macrophages of a factor which stimulates proliferation of lymphocytes, and that when antigen is presented at this time there is an increased probability of antibody formation rather than tolerance induction. In keeping with this, it has been found[12] that vitamin A, which is an efficient adjuvant but is not in itself immunogenic, produces marked blast transformation and proliferation of cells in the thymus-dependent areas of draining lymph nodes, whereas substances, such as paraffin oil, without adjuvant activity have at most slight effects on the thymus-dependent areas of lymph nodes. These findings are consistent with the possibility that one of the effects of adjuvants is to increase the effectiveness of the interaction of macrophages and T lymphocytes. The macrophages concentrate antigen, which is presented to T lymphocytes in highly immunogenic form along with a stimulus for the latter to proliferate. This greatly increases the probability that T lymphocytes will mount an immune response, which in turn allows them to co-operate in antibody production by B lymphocytes. But whether or not the interaction of macrophages with T cells is augmented by adjuvants it seems that stimulation of T cells is an important aspect of the mechanism of action of at least some adjuvants. It is of interest to note that injections of allogeneic cells may also provide a general stimulus to T cell proliferation and overcome the requirement for co-operation by populations of T cells specifically stimulated by antigen in the potentiation of an immune response[13].

Part of this work was supported by grants to the Chester

Beatty Research Institute from the Medical Research Council and the Cancer Research Campaign.

A. C. ALLISON

Clinical Research Centre,
Harrow,
Middlesex

A. J. S. DAVIES

Chester Beatty Research Institute,
London SW3 6JB

Received June 9, 1971.

[1] Mitchison, N. A., *Proc. Roy. Soc.*, B, **161**, 275 (1964).
[2] Dresser, D. W., and Mitchison, N. A., *Adv. Immunol.*, **8**, 129 (1968).
[3] Unanue, E. R., Askonas, B. A., and Allison, A. C., *J. Immunol.*, **103**, 71 (1969).
[4] Spitznagel, J. K., and Allison, A. C., *J. Immunol.*, **104**, 119 (1970).
[5] Spitznagel, J. K., and Allison, A. C., *J. Immunol.*, **104**, 128 (1970).
[6] Roitt, I. M., Greaves, M. F., Rorrigiani, G., Brostoff, J., and Playfair, J. H. L., *Lancet*, ii, 367 (1969).
[7] Mitchell, G. F., and Miller, J. F. A. P., *J. Exp. Med.*, **128**, 821 (1968).
[8] Mitchison, N. A., in *Sixth Intern. Immunopathol. Symp.* (edit. by Miescher, P.) (Schwabe, Basel, 1970).
[9] Davies, A. J. S., Leuchars, E., Wallis, V., and Koller, P. C., *Transplantation*, **4**, 438 (1966).
[10] Leuchars, E., Cross, A. M., and Dukor, P., *Transplantation*, **3**, 28 (1965).
[11] Taylor, R. B., *Transplant. Rev.*, **1**, 114 (1969).
[12] Taub, R. N., Krantz, A. R., and Dresser, D. W., *Immunology*, **18**, 171 (1970).
[13] Benacerraf, B., in *Sixth Intern. Immunopathol. Symp.* (edit. by Miescher, P.) (Schwabe, Basel, 1970).

Variations of the Expression of HL-A Antigens on Human Diploid Fibroblasts *in vitro*

ALL kinds of human nucleated cells so far tested express on their surface the HL-A antigens which form the major histocompatibility system in man[1]. These antigens, which are easily detected on lymphocytes and on platelets of peripheral blood, have also been found on other human cells[2–9]. In long term human cell cultures, it has been possible to demonstrate the remarkable stability of the HL-A antigens. Analogous observations have been reported in mouse regarding the antigens controlled by H-2 histocompatibility system[10]. We report here results obtained from the study of ten varieties of human fibroblasts, which enabled us to analyse the behaviour of the antigens of the HL-A system in cell cultures of limited duration[11].

Taking advantage of skin graft experiments performed in our laboratory[12], we obtained fibroblasts through standard procedures, from skin biopsies of healthy volunteers belonging to families whose tissues had been extensively and repeatedly typed in lymphocytotoxicity and complement fixation on platelets (Table 1).

Fibroblasts were typed using Terasaki's lymphocytotoxicity technique, slightly modified[13]. The dose of immune sera was increased, the fibroblast suspension was adjusted to 1,000 cells per test, and the reading was made by dye exclusion.

The antisera used (Table 2) came from either multiparous women, immunized or multiple transfused individuals. Each specificity was defined by one to five reagents. All the results obtained with the reagents defining a same specificity were concordant.

Each specificity detected was tested directly against either the corresponding antiserum, or a cross-reacting antiserum[14]: for example, anti-HL-A1, which recognized HL-A3, and HL-A11 (refs. 15, 16), anti-HL-A5 which recognizes Da6, Da20, Da24, Te18 (ref. 17), anti-HL-A7 which recognizes BB (15). Except with fibroblast 109′ (Table 1), the fibroblast specificities detected were always the same as those determined in corresponding lymphocytes[8,9]. The HL-A antigens progressively disappeared (Table 3). The loss of expression of these antigens did not seem to be random, and occurred by locus, and not by haplotype. In nine cases out of ten, the antigens controlled at the second locus were the first to disappear.

Table 1 Comparison of the HL-A Antigens detected on the Lymphocytes and the Fibroblasts from a Same Donor

Donors	HL-A genotypes of donors of lymphocytes and fibroblasts
32	*HL-A2*, BB[a]/*HL-A9*, *HL-A5*
35	*HL-A9*, *HL-A5*/*HL-A3*[d], Da20[b]
46	*HL-A10*, *HL-A7*/*HL-A3*[d], —
212	*HL-A3*[a], *HL-A7*/*HL-A10*, BB[a]
GUI . . .	*HL-A2*, *HL-A7*/*HL-A3*, Da6[b]
33	*HL-A9*, *HL-A5*/HL-A11[c], Da18[e]
115	*HL-A2*, Te18[b]/*HL-A3*, Da24[b]
154	*HL-A2*, —/*HL-A2*, AA[e]
44[f]	*HL-A2*, HL-A12[e]
109[g]	HL-A2,—/HL-A1, HL-A8
109′[g]	(HL-A7) (HL-A1) (HL-A5)

In each case, the formulae show the genes at the two haplotypes, which determine the corresponding antigens on the lymphocytes.

—, Genes, the products of which are not serologically detected. Italic types show the products of the genes which have been serologically found on fibroblasts. a, BB antigens, not determined directly, positive reaction with anti-HL-A7 due to the cross-reaction BB, HL-A7. b, Antigens Da20, Da6, Te18, Da24, not determined directly for shortage of available antisera, positive reaction with anti-HL-A5 due to the cross-reaction between the former specificities and HL-A5 antigen. c, Antigen HL-A11, not tested directly, positive with anti-HL-A1, due to cross-reaction HL-A11, HL-A1. d, Antigen HL-A3, tested directly by anti-HL-A3, but positive reaction with anti-HL-A1, due to cross-reaction between the specificities HL-A3, HL-A1. e, Antigens Da18, AA, HL-A12, specificities not tested for shortage of available antisera. f, Genotype not determined. g, Complete disparity between the groups determined on lymphocytes 109 and fibroblasts 109′.

Table 2 Immune Sera used for the Determination of HL-A Antigens on Fibroblasts and Lymphocytes

Specificities	Antisera		Doses used (µl.) On fibroblasts			On lymphocytes	
First locus							
HL-A2	Hisl.	Multiple-transfused	5	3		1	
	Mari.	Multiparous	3	1		0.06	
	2.12.260	Multiparous	5	3		0.5	
	27.11.317	Multiparous	5	3		0.5	
HL-A9	Eluate		0.06	0.03	0.015	0.015	
	Dup.	Multiple-transfused	0.25			0.03	0.015
HL-A1	Gius.	Multiple-transfused	0.06	0.03		0.03	0.015
	110	Multiparous	0.12	0.06		0.12	0.06
	Hayer	Multiple transfused	0.25	0.12		0.25	0.12
	Eluate		0.12	0.06		0.06	0.03
HL-A3	Eluate		0.12	0.06		0.06	0.03
HL-A10	Loui.	Immunized	5	3		0.25	
	Gran.	Multiple-transfused	0.25			0.06	
Second locus							
HL-A5	Plati.	Multiple-transfused	0.5	0.25		0.06	0.03
	Goud.	Multiple transfused	0.5	0.25		0.06	0.03
	4.12.401	Multiparous	5	3		0.5	
	9.12.017	Multiparous	5	3		0.5	
	Eluate		0.10	0.06		0.015	
HL-A7	Eluate		0.2	0.1		0.06	0.03
	25.11.262	Multiparous	5	3		0.5	
	Barr.	Multiple-transfused	0.5	0.25		0.12	0.6
	Fitou		1	0.3		0.16	0.1

Table 3 Time of Disappearance of HL-A Antigens on Fibroblasts in Culture

	Strains of fibroblasts	32$_h$				33$_h$				44^F_h		GUI . . . $_h$			
Approxi- time scale	Initially detected antigens	First locus		Second locus		First locus		Second locus		First locus	Second locus	First locus		Second locus	
	Number of passages	HL-A2	HL-A9	BB^a	HL-A5	HL-A9	HL-A11^c	HL-A5	Da18^e	HL-A2	HL-A12	HL-A2	HL-A3	HL-A7	Da6^b
	Passage														
Two months	20	+	+	+	+	+	+	+		+		+	+	+	+
	21														
	22	+	+	+	+										
	23														
	24	+	+	+	−										
	25														
	26					+	+	+				+	+	+	+
	27														
	28														
	29											+	+	+	+
	30	+	+	+	−	+	+	+		+					
Two months	31														
	32	+	+	+	−										
	33									+					
	34	+	+	+	−										
	35														
	36														
	37	+	+	+	−					+					
	38														
	39	+	+	+	−							+	+	+	+
	40	+	+	+	−	+	+	−		+					
Six months	41	+	+	−	−										
	42	+	−	−	−	+	+	−		+		+	+	+	+
	43														
	44														
	45	+	−	−	−	+	+	−		+					
	46	+	−	−	−							+	+	+	+
	47														
	48	−	−	−	−					+					
	49														
	50	−	−	−	−					−					
	51											+	+	+	+
	52					+	−	−							
	53														
	54														
	55					+	−	−							
	56										●				
	57														
	58			●											
	59											+	+	−	−
	60														
	61					+	−	−				+	+	−	−
	62					−	−	−							
	63					−	−	−				+	+	−	−
	64														
	65														
	66											+	+	−	−
	67							●							
	68													○	

	Strains of fibroblasts	46$_h$				212				35$_h$				109'^{g}				154				115			
Approxi- time scale	Initially detected antigens	First locus		Second locus		First locus		Second locus		First locus		Second locus		First locus		Second locus		First locus		Second locus		First locus		Second locus	
	Number of passages	HL-A10	HL-A3^d	HL-A7	—	HL-A3^d	HL-A10	HL-A7	BB^a	HL-A9	HL-A3^d	HL-A5	Da20^b	HL-A1	—	HL-A7	HL-A5	HL-A2	HL-A2	—	AA^e	HL-A2	HL-A3	Te18^b	Da24^b
	Passage																								
Two months	20					+^{i}	+	+	+	+	+^{i}	+	+	+		+	+	+	+			+	+	+	+
	21	+	+^{i}	+																					
	22																					+	+	+	+
	23																								
	24					+^{i}	+	+	+					+		+	+	+	+						
	25																								
	26									+	+^{i}	+	+					+	+			+	+	+	+
	27																								
	28	+	+^{i}	+										+		+	−					+	+	+	+
	29					+^{i}	+	+	+																
	30													+		+	−	+	+						
Two months	31																								
	32					+^{i}	+	+	+	+	+^{i}	+	+					+	+						
	33																					+	−	+	+
	34	+	+^{i}	+		+^{i}	+	+	+	+	+^{i}	−	−												
	35									+	+	−	−									+	−	+	+
	36	+	+^{i}	+		+	+	+	+	+	+	−	−												
	37																								
	38	+	+^{i}	+		+	+	−	−	+	+	−	−					+	+			+	−	+	+
	39	+	+	+		+	+	−	−																
	40					+	+	−	−					+		+	−	+	+						
Six months	41																								
	42					+	+	−	−															○	
	43									−	+	−	−												
	44	+	+	+										+		+	−								
	45							○		−	+	−	−												
	46	+	+											+		−	−								
	47									−	+	−	−												
	48																	+	+						
	49																								
	50	+	+	+						−	+	−	−	+		−	−								
	51																								
	52									−	+	−	−					+	+						
	53													+		−	−	+	+						
	54	+	+	−						−	−	−	−					+	+						
	55									−	−	−	−												
	56																								
	57	+	+	−												○									
	58																			○					
	59																								
	60	+	+	−																					
	61																								
	62											●													
	63			○																					
	64																								
	65																								
	66																								
	67																								
	68																								

+, Passages when antigens were found. −, Disappearance either of specificity or of a cross-reaction demonstrating this specificity. a, b, c, d, e, f, g, see Table 1. h, Six fibroblasts were studied for the first time at a passage number not exactly determined (between fifteen and twenty-five—estimated at twenty in the table). i, Antigen HL-A3 recognized by an anti-HL-A1 anti-serum, because of the cross-reaction HL-A3, HL-A1 ;+ without i indicates the persistency of a positive reaction with anti-HL-A3 and the negativation of the reaction with anti-HL-A1.

In three cases—fibroblasts 46, 212, 35 (Table 3)—the HL-A3 antigen was recognized both by anti-HL-A3 immune serum, and by the anti-HL-A1 reagent, and we could observe that the serological negativation of the cross-reaction preceded that of the direct reaction.

The disappearance of histocompatibility antigens occurred together with the senescence of the cultures and preceded cell degeneration and death. The presence of all the HL-A antigens on the fibroblasts coincided with a phase of normal cellular multiplication with a generation time of about 24 h. In six cases out of ten, the partial disappearance of the antigens controlled by the second locus occurred after a number of passages which varied with the strains of the fibroblasts studied, and corresponded to a phase of an apparently normal cellular multiplication. There was, however, an increasing generation time from 24 h to 96 h and sometimes even more. On the contrary, in four cases out of ten, though the cells survived after the disappearance of all the HL-A antigens, the multiplication stopped rapidly, the morphological aspect of the culture was that of a complete degeneration of the strain, rapidly followed by death. Degeneration could be diagnosed by a decrease in the cell number and morphologically by the presence on the cells of aggregates of cytoplasmic origin, and by the loss of their initial spindle-shaped oblong form.

Concordance has already been observed on lymphocytes, and on fibroblasts regarding certain given groups. Our study confirms the presence of HL-A antigens on the surface of fibroblasts, in spite of their lesser sensitivity to immunological lysis, probably in relation with a lesser concentration of antigenic sites. It implies the concept of a large distribution of antigens on human tissues, and underlines the importance of their role in transplantation. Our results differ from those of other authors[8] on the stability of the HL-A antigens on human fibroblasts in culture: more specificities of the second locus have been observed and for a longer time.

As we have explained, the serological behaviour of the products, which are controlled by the first and the second loci, is different when these products are studied in the course of time on cultured human diploid fibroblasts. By loss of specificity we mean the disappearance at a given moment of a specificity which could previously be detected in a reproducible manner. Our experimental conditions do not allow us to determine whether it is a question of qualitative or quantitative loss of antigens, but absorption experiments are in progress. It is possible that antigens are still present, but can no longer be detected by immune sera. In nine out of ten fibroblast strains, however, the products controlled by the second locus were the first to become serologically undetectable. It is unlikely that the antisera used to determine the antigens at the first locus are more sensitive than those applied to the antigens of the second locus, for comparison of the titres of the immune sera shows that the mean activity is the same in the two cases. It thus seems that the stability of the antigens controlled by the first and second HL-A loci is different, and that the products of the former are more stable in our experimental conditions. It is still possible that at a certain stage of cell degeneration following multiple passages, some of these antigens may become more susceptible to trypsin action, because of a general change in the cell membranes. The asymmetry observed between the products controlled by the first and second HL-A loci can be compared with that observed between the D and K regions of mouse H-2 locus, shown *in vitro* by the mixed lymphocyte test[18], and *in vivo* by graft versus host reaction[19]. Furthermore, cross-reactions are a general phenomenon observed in the serology of the HL-A system[14]; this experiment, which shows that the loss of cross-reactivity is independent of that of direct reactivity, suggests that the antigenic determinants responsible for the direct specificity and those responsible for cross-reactions are distinct.

Finally, our results lead us to discuss the possible biological role of the HL-A system: in four cases out of ten, either degeneration or cell death (fibroblasts 32, 33, 44, 35) rapidly followed once the point was reached at which specificity in the HL-A system was no longer detectable. On the other hand, in the other six cases (fibroblasts GUI, 46, 212, 154, 109′, and 115), where at least one of the specificities controlled by the two loci of the HL-A system was still detected, no sign of cellular senescence was observed except an increased generation time. Indeed, from the results obtained, it is not possible to confirm that there is a relationship between loss of HL-A antigens and cell degeneration. But if these results are compared with those obtained for the mouse H-2 locus in which all the strains have at least one set of antigens of the H-2 locus[20-23], it becomes tempting to speculate that HL-A antigens behave in the same fashion. The practical interest of the detection of HL-A antigens is first their role as genetic markers, yet with a certain limitation regarding their stability of expression during the cell senescence period; and second, the fact that it makes it possible to define the identity of strains and to determine their age.

We thank Dr J. Colombani for helpful criticism, and Mrs Roche for her help with the English manuscript.

M. SASPORTES
C. DEHAY
M. FELLOUS

Institut de Recherches sur les Maladies du Sang,
Hôpital Saint Louis,
75 Paris 10

Received January 15; revised April 30, 1971.

[1] Dausset, J., Colombani, J., Legrand, L., and Fellous, M., *Histocompatibility Testing*, 53 (Munksgaard, Copenhagen, 1970).
[2] Yunis, E. J., Yunis, J. J., and Brand, R. G., *Blood*, **25**, 1 (1965).
[3] Metzgar, R. S., Flanagan, J. F., and Zmijewski, C. A., *J. Immunol.*, **95**, 494 (1965).
[4] Milgrom, F., and Kano, F., *Histocompatibility Testing*, 179 (Munksgaard, Copenhagen, 1965).
[5] Papermaster, V. M., Papermaster, W. B., and Moore, G. E., *Fed. Proc.*, **28**, 379 (1969).
[6] Engelfriet, C. P., Heersche, J. N. M., Eijsvoogel, V. R., and Van Loghem, J. J., *Vox Sang.*, **11**, 625 (1966).
[7] Melief, C. J. M., van der Hart, M. A., Engelfriet, C. D., Eijsvoogel, V. P., and van Loghem, J. J., *Vox Sang.*, **15**, 187 (1968).
[8] Miggiano, V. C., Nabholtz, M., and Bodmer, W. F., *Histocompatibility Testing*, 623 (Munksgaard, Copenhagen, 1970).
[9] Thorsby, E., and Lie, S., *Vox Sang.*, **15**, 44 (1968).
[10] Klein, D., Merchant, D. J., Klein, J., and Shreffer, D. C., *J. Nat. Cancer Inst.*, **44**, 11 (1970).
[11] Hayflick, L., *Exp. Cell Res.*, **37**, 611 (1965).
[12] Dausset, J., Rapaport, F. T., Legrand, L., Colombani, J., and Marcelli-Barge, A., *Histocompatibility Testing*, 381 (Munksgaard, Copenhagen, 1970).
[13] Terasaki, P. I., and McClelland, J. D., *Nature*, **204**, 998 (1964).
[14] Colombani, J., Colombani, M., and Dausset, J., *Histocompatibility Testing*, 79 (Munksgaard, Copenhaben, 1970).
[15] Svejgaard, A., *Ser. Haematol.*, **2**, 3 (1969).
[16] Thorsby, E., and Kissmeyer-Nielsen, F., *Vox Sang.*, **17**, 102 (1969).
[17] Colombani, M., Colombani, J., Dastot, H., Mayer, S., Tongio, M., and Dausset, J., *Rev. Franç. Etud. Clin. Biol.*, **14**, 995 (1969).
[18] Rychlikova, M., Demant, P., and Ivanyi, P., *Folia Biol.*, **16**, **3**, 218 (1970).
[19] Demant, P., *Folio Biol.*, **16**, **4**, 273 (1970).
[20] Klein, E., *Nobel Symp.* (1965).
[21] Klein, E., and Moller, E., *J. Nat. Cancer Inst.*, **31**, 347 (1963).
[22] Moller, E., *J. Nat. Cancer Inst.*, **33**, 979 (1964).
[23] Cann, H. M., and Herzenberg, L. A., *J. Exp. Med.*, **117**, 267 (1963).

Fluorescent *Y* Screening of Hospitalized Newborns

QUINACRINE dihydrochloride and quinacrine mustard have been used to identify chromosomes at metaphase[1,2] and the *Y* chromosome in interphase[3-6]. We now report a preliminary survey of blood smears for *Y* chromosome fluorescence from all

Table 1 Characteristics of the Infants Studied

Case	Gestation (weeks)	Birth weight (g)	Admission diagnosis	*Y* chromosome	Congenital defects	Final outcome
1	35	1,680	Umbilical omphalocoele Respiratory distress	*XYY*	Mongoloid facies Umbilical omphalocoele Cong. bilat. hydronephrosis VSD PDA	Died age 2 days
2	40	3,630	Suborbital dermoid	Large	Frontal encephalocoele Bilat. choanal atresia	Alive Surgical correction
3	31	1,340	Prematurity (twin of case 4)	Small	Nil	Alive
4	31	1,845	Prematurity (twin of case 3)	Small	Nil	Alive
5	40	2,400	Cleft lip and palate	Small	Bilat. cleft lip and palate Undescended testis	Alive
6	41	2,780	Ambiguous genitalia	Normal	Hypospadias Imperforate anus	Alive Colostomy
7	36	1,770	Prematurity Ambiguous genitalia	Normal	Marked hypospadias	Alive

newborn males transferred to this hospital from February to April 1971.

Blood smears were air-dried, fixed for 5 min in 100% methanol, dried, stained in 0.5% quinacrine dihydrochloride ('Atebrine', Gurr) for 5 min, rinsed in running tap water, dried, mounted in phosphate buffer (*p*H 4.5) and examined under a Zeiss photomicroscope with an HBO 200 lamp, excitor filter BG 12 and barrier filter 53/44.

Blood smears were taken from 140 male and five female infants. Ten lymphocytes and usually ten neutrophils were examined from each smear without prior knowledge of the sex or clinical status of the infant. *Y* chromosome abnormalities or normal variants, confirmed by karyotypes, were detected in five infants by this fluorescence study (Table 1 and Fig. 1). A further two infants, admitted specifically for definitive sex determination because of ambiguous genitalia, had normal male fluorescence patterns and karyotypes (Table 1 and Fig. 1).

In the *XYY* smear (case 1), three cells with two fluorescent spots (F-spots) were seen in the initial twenty leucocytes. On further screening, approximately 30% of 100 cells examined had two F-spots, and none with a single large F-spot. F-spots were noticed to be very large in almost all leucocytes from case 2 (large *Y* chromosomes).

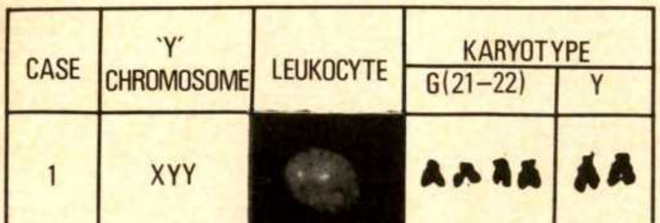


Fig. 1 Comparison of leucocyte *Y* fluorescence pattern with karyotype. The fluorescent spot in leucocytes from cases 3–5 was present but difficult to illustrate because of its small size.

Smears from cases 3, 4 and 5 had definite F-spots but these were so small and difficult to see that they might have been classified as negative (female). Smears from the five females had no F-spots and were easily identified.

Two cells in each of two other smears contained two F-spots and were shown to be false positives by karyotypes. In the remaining smears, 83% of lymphocytes (and 65% of neutrophils) contained an F-spot of normal size(Fig. 1, cases 6, 7). Karyotypes were not made on any of these males.

This study shows that *Y* chromosome abnormalities and variants can be rapidly detected by this fluorescence technique and then confirmed by karyotype. Parallel chromosome studies were not done and the number of false negatives is unknown. This study might suggest that the number of false negatives is small because a large proportion of cases with interesting *Y* chromosomes were found. This was, however, a selected population, hospitalized because of neonatal problems. Whether the large proportion of interesting *Y* chromosome cases was related to selection of this type of patient or was a chance finding will have to be determined by further studies.

Identification of male nuclear sex by the fluorescent technique is a valuable addition to the sex chromatin test in cases of ambiguous genitalia.

We thank Dr Paul Swyer for his cooperation. This work was supported by the Ontario Cancer Treatment and Research Foundation.

PETER K. LEWIN
PATRICK E. CONEN

Departments of Pediatrics and Pathology,
The Hospital for Sick Children,
555 *University Avenue,*
Toronto 101

Received June 2, 1971.

[1] Caspersson, T., Farber, S., Foley, G. E., Kudynowski, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., *Exp. Cell Res.*, **49**, 219 (1968).
[2] Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell Res.*, **58**, 141 (1969).
[3] Zech, L., *Exp. Cell Res.*, **58**, 463 (1969).
[4] Pearson, P. K., Bobrow, M., and Vosa, C. G., *Nature*, **226**, 78 (1970).
[5] Polani, P. E., and Mutton, D. E., *Brit. Med. J.*, **1**, 138 (1971).
[6] Conen, P. E., Lewin, P. K., and Vakil, D., *Canad. Med. Assoc. J.*, **104**, 925 (1971).

Scrapie in Immunologically Deficient Mice

ALTHOUGH the agent responsible for scrapie is classified as a slow virus, many features distinguish it from a typical virus[1-3], one of which is its apparent failure to elicit an immune response. Attempts to demonstrate antibody by neutralization[4,5], complement fixation[4], precipitation[4] and immunofluorescence[4,6] have been unsuccessful, but the role of cell-mediated immunity in scrapie has been less well studied.

Neonatal thymectomy did not alter the incubation period or pathology in one small series of mice inoculated intracerebrally with scrapie[2]. We have studied the course of scrapie produced by intraperitoneal inoculation in thymectomized mice, lethally irradiated and reconstituted with foetal liver cells. Although they were markedly depleted of thymus-derived (T) lymphocytes and were incapable of rejecting allogeneic skin grafts, their response to the scrapie agent was not different from that of normal mice.

CBA/He mice of both sexes were used: group I was thymectomized at 4 weeks old, irradiated with 900 r. and reconstituted with syngeneic foetal liver cells; group II received 900 r. and was reconstituted with foetal liver cells; group III was untreated. Four weeks later they all received skin grafts from A strain donors and after 9 days half the animals in each group were inoculated intraperitoneally with 0.25 ml. of 10^{-1} dilution of normal BSVS mouse brain; the rest received 0.25 ml. of 10^{-1} dilution of brain from BSVS mice with scrapie; these materials were supplied by Dr R. L. Chandler.

Cell-mediated immune function was followed by assessment of skin allograft survival. In addition the percentage of cells bearing the θ allo-antigen (a marker for T lymphocytes[7]) and the microscopic appearance of the lymphoid organs were studied in two animals from each group, 7 months after inoculation (Table 1). Animals in group I retained skin allografts throughout the experiment, had very few θ-bearing cells in their blood and were markedly depleted of lymphocytes in the thymus-dependent areas of spleen and lymph nodes[8]. In contrast, mice in groups II and III rejected their skin grafts after 13.6 and 10.3 days respectively, had normal numbers of θ-bearing blood lymphocytes and normal lymphoid organs.

Mice were observed five or six times a week for the appearance of scrapie following the criteria of Dickenson *et al.*[9]. No mice inoculated with normal brain developed signs of scrapie, but those injected with scrapie-affected brain developed scrapie and either were killed in extremis or died within 230 days of inoculation. Many of the immunologically deficient mice in group I died before developing signs of scrapie. None of these mice had neuropathological lesions consistent with scrapie during the clinical phase of the disease.

Table 2 shows that scrapie began between 163 and 170 days in all three groups. There was no significant difference between mean times of death of 190 and 205 days seen in groups I and III respectively; they were even closer than appears because four of the mice in group I were killed when judged to be preterminal in order to obtain satisfactorily histology and peripheral blood for determination of θ-bearing lymphocytes. Although the extent and magnitude of the nervous system lesions were not compared in detail, the neuropathological changes revealed by haematoxin and eosin or Cajal were consistent with scrapie in all three groups. Thus we could demonstrate no appreciable difference in the clinical and pathological aspects of scrapie in immunologically deficient mice depleted of T lymphocytes when compared with normal mice. This suggests that cell-mediated immunity neither contributes to the pathogenesis of scrapie nor plays a significant role in the host's defence against the agent.

Table 1 Cellular Immune Status

Group	Treatment	Skin allograft survival in days (mean)	% θ-bearing cells in blood*	Lymphoid* organs
I	Thymectomy 900 r. Reconstituted with foetal liver	Greater than 200	4, 17	Lymphocyte depletion in in thymus-dependent areas
II	900 r. Reconstituted with foetal liver	9–19 (13.6)	77, 68	Normal
III	None	9–14 (10.3)	74, 73	Normal

* Evaluated after 7 months.

Table 2 Time Course of Scrapie

Group	No. of mice	Onset of disease (days)	Time from inoculation to death (days) Range	Mean
I	7	163–170	174–200	190
II	16	163–170	200–223	215
III	24	163–170	173–228	205

This work was supported in part by the National Multiple Sclerosis Society of the United States.

D. E. McFarlin
M. C. Raff
E. Simpson
S. H. Nehlsen

National Institute for Medical Research,
Mill Hill, London NW7

Received June 9, 1971.

1 Latarjet, R., Muel, B., Haig, D. A., Clarke, M. D., and Alper, T., *Nature*, **227**, 1341 (1970).
2 Gibbons, R. A., and Hunter, G. D., *Nature*, **215**, 1041 (1967).
3 Field, E. J., *Intern. Rev. Exp. Pathol.*, **8**, 129 (1969).
4 Gibbs, J. G., Gajdusek, C. C., and Morris, J. A., *Public Health Service Publication* No. 1378, 195 (1965).
5 Haig, D. A., and Clarke, M. C., *Public Health Service Publication* No. 1378, 215 (1965).
6 Chandler, R. L., *Vet. Rec.*, **71**, 38 (1959).
7 Raff, M. D., *Nature*, **224**, 378 (1969).
8 Parrott, D. M. V., De Sousa, M. A. B., and East, J., *J. Exp. Med.*, **123**, 191 (1966).
9 Dickinson, A. G., Meikle, V. M. H., and Fraser, H., *J. Comp. Pathol.*, **78**, 293 (1968).

Extraction of Prostaglandins from Human Blood

EXISTING methods for extracting prostaglandins from tissues or seminal plasma with 70–90% ethanol[1] or acetone[2] give poor and inconsistent recoveries from blood. Equilibrium dialysis and recovery experiments with labelled and unlabelled prostaglandins show that this is due to binding with precipitated serum albumen (unpublished results of W. G. U.). Plasma globulins and intact blood cells do not seem to interact with the prostaglandins. When precipitation of protein is avoided by using 40–50% ethanol acidified with formic acid, the prostaglandins can be extracted quantitatively into chloroform. The method is as follows: (1) centrifuge heparinized or citrated blood; (2) mix 1 volume each of plasma and saline with 2 volumes of ethanol (analytical grade); (3) extract twice with petrol (boiling point 40°–60° C, 2 × 2 volumes) to remove neutral fats including carotene; (4) adjust to *p*H 3–3.5 with formic acid (1–3% v/v); (5) extract twice with amounts of chloroform each equal to the total volume in stage 4; (6) evaporate chloroform in a thin film evaporator at 30° C; (7) redissolve residue in 10 ml. chloroform and evaporate to aid the removal of formic acid; (8) blow oxygen-free nitrogen through vessel until odour of formic acid is gone; (9) dissolve in Krebs solution for bioassay or in appropriate solvent for chromatography.

The plasma should be either fresh or frozen (−10° C in the present experiments). Plasma stored in glass and freeze-dried whole plasma were found to contain acidic biologically active material which was not a prostaglandin. Formic acid is used in stage (4) to achieve the desired *p*H because its *pKa* is near *p*H 3 and because its volatility allows easy removal; the amount of acid needed varies with different samples of plasma. Chloroform is used to extract the alcoholic solution (stage 5) since only a small amount of alcohol or water enters the chloroform phase; diethyl ether and ethyl acetate used in other procedures are miscible with 50% ethanol.

The resulting extract in stage (9) is still crude, and contains bilirubin, phospholipids and traces of neutral fats in addition to the prostaglandins. Substantial amounts of haematin will also be present if haemolysis has occurred. These impurities do not affect the biological assays but they can affect the rate at which prostaglandins run on thin-layer chromatography. Phospholipid material can be removed by precipitation in cold acetone[3,4], but this is undesirable because a biologically active unidentified material is formed probably from the phospholipid fraction. This might account for the high blood levels of prostaglandin reported by Hickler[4]. The extracts can, however, be purified suitably for thin-layer chromatography by evaporating the chloroform, dissolving the residue in 10 ml. of 70% ethanol and shaking briefly with 250–500 mg Fuller's earth. After diluting the supernatant to 50% with water and acidifying with formic acid, the prostaglandins re-extract quantitatively into chloroform. This procedure removes haematin and phospholipids, but not bilirubin.

Assays of PGE$_1$, E$_2$, F$_{1\alpha}$ and F$_{2\alpha}$ on the rat fundus[5] and of PGA$_1$ and A$_2$ in anaesthetized cats and rats[6,7] gave recoveries of 102% (range 72–140%, 52 experiments) from human blood containing 0.01–1 μg/ml. added prostaglandin. The levels of prostaglandin activity in peripheral venous blood from fifteen normal males was found to be 1.6 ± 0.9 ng/ml. (assayed as prostaglandin E$_2$ ± s.d.).

We thank Dr J. E. Pike, Upjohn Ltd, Kalamazoo, for prostaglandins, Professor D. A. van Dorp, Unilever, Vlaardingen, for ^{3}H-PGE$_1$, and the Wellcome Trust and King's College Hospital Research Trust for support (W. G. U. and I. F. S.).

WILLIAM G. UNGER
IAN F. STAMFORD
ALAN BENNETT

Department of Surgery,
King's College Hospital Medical School,
London SE5

Received May 13; revised July 1, 1971.

[1] Samuelsson, B., *Biochim. Biophys. Acta.*, **84**, 218 (1964).
[2] Ambache, N., *Biochem. Pharm.*, **12**, 421 (1963).
[3] Hanahan, D. J., Dittner, J. C., and Warashina, E., *J. Biol. Chem.*, **228**, 685 (1957).
[4] Hickler, R. B., *Prostaglandin Symp. of the Worcester Foundation for Experimental Biology* (edit. by Ramwell, P. W., and Shaw, J. E.), 279 (Interscience, New York, 1968).
[5] Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344 (1957).
[6] Horton, E. W., and Jones, R. L., *J. Physiol.*, **200**, 56P (1969).
[7] Crowshaw, K., McGiff, J. C., Strand, J. C., Lonigro, A. J., and Terragno, N. A., *Fed. Proc.*, **29**, 645 Abs. (1970).

New Porous Biomaterials by Replication of Echinoderm Skeletal Microstructures

AN important aspect of materials science is the development of new porous solids for prosthetic applications, especially for synthetic teeth and bone replacement. To be acceptable, such biomaterials must meet some rather demanding specifications, among which are high strength and compatibility with body fluids or tissues. The structure of the porous medium is important as the pores must be both interconnected and have the proper size, shape and uniformity to permit ingrowth and attachment of body tissue. The problem lies in attaining the optimum combination of these properties to yield a high strength to weight ratio material, with a microstructure which best facilitates firm attachment of tissue. Ideally these materials should be easily machined or fashioned into particular shapes for different individual implants, and should be relatively inexpensive.

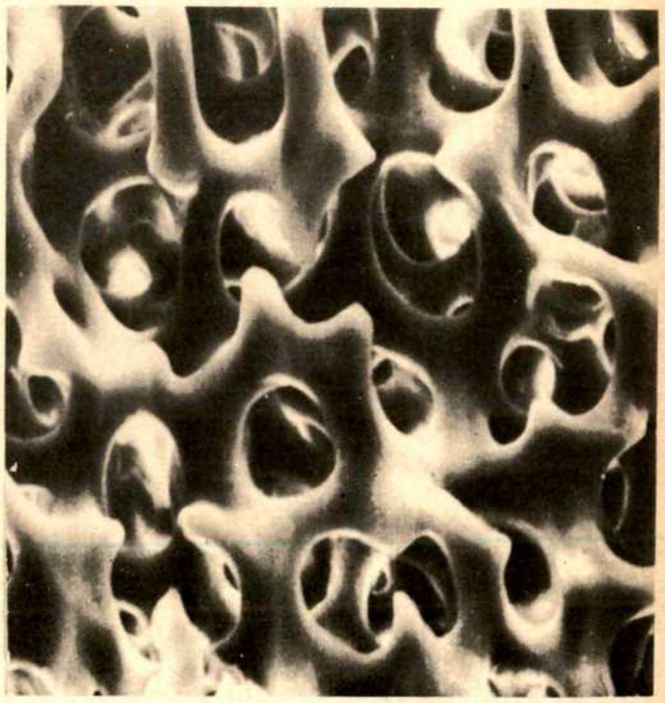


Fig. 1 Three dimensional fenestrate structure of skeletal high-magnesium calcite secreted by echinoderms. Photomicrograph by scanning electron microscopy. The diameter of the pores is approximately 10–15 μm

Ceramics show considerable promise in that strength and inertness requirements are likely to be met. Such materials have high compressive strength, do not corrode and have a specific gravity similar to that of bone[1,2]. But because of the difficulties of creating the ideal pore structure artificially, we propose a new technique of replicating the microstructure found in the CaCO$_3$ skeletal elements of echinoderms. Using these methods it seems possible to select a natural microstructure with the most desirable geometry or morphology for a particular application, and then reproduce this structure by replacing the original skeletal material with one possessing adequate physical, chemical and mechanical properties.

The mineral phase of echinoderm ossicles is high-magnesium calcite[3] which is secreted in optical continuity. Each spine of a sea urchin or echinoid, for example, is indistinguishable from a single crystal by X-ray diffraction or optical methods. The skeletal calcite, however, is permeated by interconnecting channels forming a fenestrate microstructure in spite of the single crystal nature of the inorganic phase (Fig. 1). In life, these passageways are filled with various organic cells and body fluids[4]. Donnay and Pawson[5] have described this structure as a "periodic minimal surface" which divides space into two interpenetrating regions, each of which is a single yet multiple connected domain. The two regions, however, have no connexion with each other and the surface is the interface

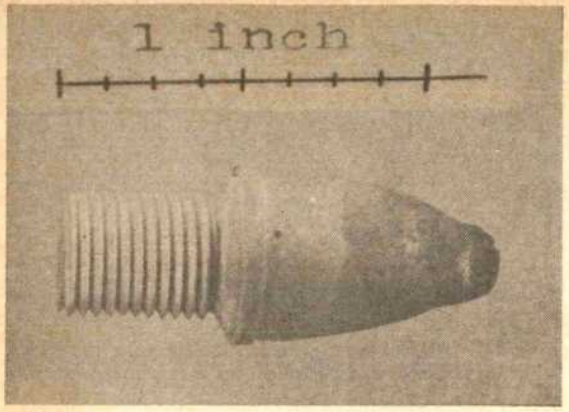


Fig. 2 Screw thread machined from a spine of the echinoid *Heterocentrotus trigonarius*.

between the skeletal calcite phase and the soft-tissue organic phase. Maximum contact for crystal growth is provided by this structure, which according to Donnay and Pawson[5] has not been found in nature other than in echinoderm skeletons.

Although the fenestrate microstructure is found in almost all echinoderm skeletal ossicles[5-7], few of these are large enough for practical applications. Spines of the echinoid *Heterocentrotus* are of sufficient size and ready availability to constitute a suitable source of material for replication. The genus *Heterocentrotus*, commonly referred to as the "slate-pencil urchin", is widely distributed and common in the Indo-Pacific area. The external morphology of the spines ranges from rather slender, pencil-like bodies with a tricarinate tip, to very thick, cylindrical or club-like forms. The length and width of the larger primary spines may exceed 160 and 13 mm respectively.

Although echinoderm skeletons are characterized by a unique and potentially useful microstructure, the calcium carbonate of which they consist is highly unsuitable for prosthetic applications. Calcite is considerably softer (H=3, Mohs scale) than the hydroxyapatite (H=5) found in bones and teeth. Other undesirable properties of calcite include high solubility, perfect rhombohedral cleavage, and decomposition in the presence of acids. In spite of its high degree of cleavage, however, the high-magnesium calcite of echinoderm ossicles is easily machined without damage, as illustrated by the threaded echinoid spine in Fig. 2. The very properties that preclude the use of this mineral in human implants, however, facilitate reproduction of the microstructure in other materials.

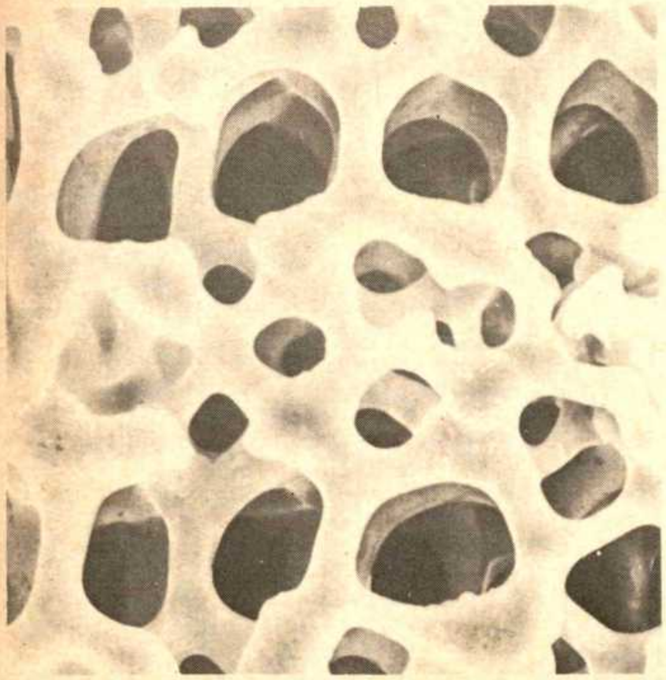


Fig. 3 Scanning electron photomicrograph of an epoxy resin negative replica of the microstructure from a spine of *Heterocentrotus trigonarius*.

Three dimensional negative replicas, made successfully from both epoxy resin and sodium silicate, reproduce the exact morphology of the void phase. Before vacuum impregnation, spines of *Heterocentrotus* are immersed in 5% sodium hypochlorite to remove organic matter, rinsed with distilled water and dried at 100° C. General Electric No. 3255 'Permafil', catalysed with 0.8% tertiary butyl perbenzoate, is used to prepare the epoxy resin replica. Air is removed from the pore volume in vacuum and replaced by the resin which effectively fills all pores. After curing the impregnated samples for 24 h at room temperature, the original calcite of the skeletal structure is removed by dilute (1 : 10) HCl. The same procedure is used to prepare the sodium silicate negative replicas except that the curing time is extended to 4 days. Epoxy and sodium silicate replicas are illustrated in Figs. 3 and 4.

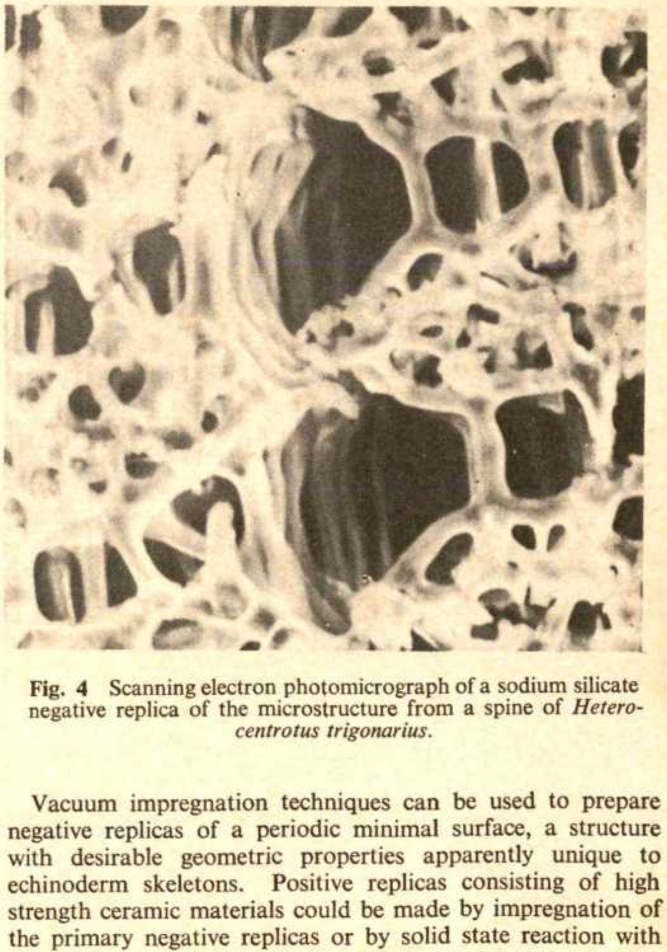


Fig. 4 Scanning electron photomicrograph of a sodium silicate negative replica of the microstructure from a spine of *Heterocentrotus trigonarius*.

Vacuum impregnation techniques can be used to prepare negative replicas of a periodic minimal surface, a structure with desirable geometric properties apparently unique to echinoderm skeletons. Positive replicas consisting of high strength ceramic materials could be made by impregnation of the primary negative replicas or by solid state reaction with the high-magnesium calcite. Phosphate-bonded alumina ceramic, for example, is particularly suitable in that it is well tolerated by tissues, does not elicit an immunologic response, and allows the attachment of fibrous connective tissue[8]. The concept of replicating natural microstructures for special applications offers considerable potential for the development of new porous biomaterials with an optimum combination of strength, density and infiltrability.

We thank Mr Michael Zeigler for assistance. This research was supported by the Advanced Research Projects Agency (E. W. W.) and the National Science Foundation (J. N. W.).

JON N. WEBER
EUGENE W. WHITE
JANA LEBIEDZIK

Materials Research Laboratory,
Pennsylvania State University,
University Park,
Pennsylvania 16802

Received June 11, 1971.

[1] Smith, L., *Arch. Surg.*, **87**, 653 (1963).
[2] Klawitter, J. J., Hulbert, S. F., Talbert, C. D., and Fitts, C. T., in *Use of Ceramics in Surgical Implants* (edit. by Hulbert, S. F., and Young, F. A.) (Gordon and Breach, 1969).
[3] Weber, J. N., *Amer. J. Sci.*, **267**, 537 (1969).
[4] Pilkington, J. B., *J. Mar. Biol. Assoc. UK.*, **49**, 857 (1969).
[5] Donnay, G., and Pawson, D. L., *Science*, **166**, 1147 (1969).
[6] Nichols, D., and Currey, J. D., in *Cell Structure and its Interpretation* (edit. by McGee-Russell, S. M., and Ross, K. F. A.) (Arnold, London, 1968).
[7] Weber, J. N., Greer, R. T., Voight, B., White, E. W., and Roy, R., *J. Ultrastructure Res.*, **26**, 355 (1969).
[8] Bhaskar, S. N., Cutright, D. E., Knapp, M. J., Beasley, J. D., Perez, B., and Driskell, T. D., *Oral. Surg.*, **31**, 282 (1971).

Spontaneous Rhythms in Physiological Control Systems

MEAN arterial blood pressure in man undergoes spontaneous and broadly repetitive fluctuations with a typical period of about 10 s. We present an explanation for these fluctuations which we believe may also be applicable to the vasomotor activity associated with thermoregulation and therefore widely relevant.

Fig. 1*a* illustrates the two broadly periodic fluctuations in the arterial blood pressure of a seated adult breathing voluntarily. One of these is related to respiration; the other occurs spontaneously, probably originates in vasomotor action, and is loosely described as a Traube wave which can also occur during periods of apnoea[1]. We believe that this spontaneous oscillation is intrinsic in the sino-aortic reflexes for regulating blood pressure, as a consequence of system nonlinearity, and that it is subject to entrainment[2] by the respiratory rhythm. The two oscillations can exist simultaneously provided that the respiratory period exceeds that of the spontaneous oscillation. But if the subject breathes at a higher frequency than that of the spontaneous oscillation, only the respiratory component remains, unless the respiration is too shallow to produce the effect (Fig. 1*b*). Detailed spectral analysis of the blood pressure confirms this observation.

The magnitude of the external signal, here respiration, required to entrain the spontaneous oscillation depends on the relative frequency of external signal and spontaneous oscillation and is much greater for signal frequencies appreciably below that of the spontaneous component than for frequencies in the region of, or even appreciably greater than, that of the spontaneous component. It has been possible to accommodate the physiological facts and observations in a model[3] (Fig. 2*a*). Disturbances such as postural changes, or respiratory movements, can affect the system; respiratory disturbances are thought to contribute additively because the arteries in which the baroreceptors are located pass through the intrathoracic cavity. Because the arterial walls are elastic, variations in intrathoracic pressure produce redistributions in arterial blood volume, hence a change in distension of the blood vessels which in turn determine baroreceptor activity. Even in the absence of any disturbance, however, the model will continue to oscillate spontaneously.

With systems structured in this way, entrainment is known to be possible with adequate magnitudes of disturbance (Fig. 2*a* and *b*). The gain K_{NL} of the non-linear element to a sinusoidal signal input is inversely proportional to the signal amplitude, as far as the output component at the same frequency is concerned, because the output magnitude is fixed by the limits between which it switches. The overall gain round the feedback loop is $K_{NL}\,KG(j\omega)$; to meet the Nyquist criterion for stability the Nyquist plot of this loop gain must pass within the point $(-1,0)$ at ω_0, the frequency of spontaneous oscillation. Fig. 2*b* shows the frequency response of the linear part $KG(j\omega)$ of this loop gain at various frequencies. Thus the

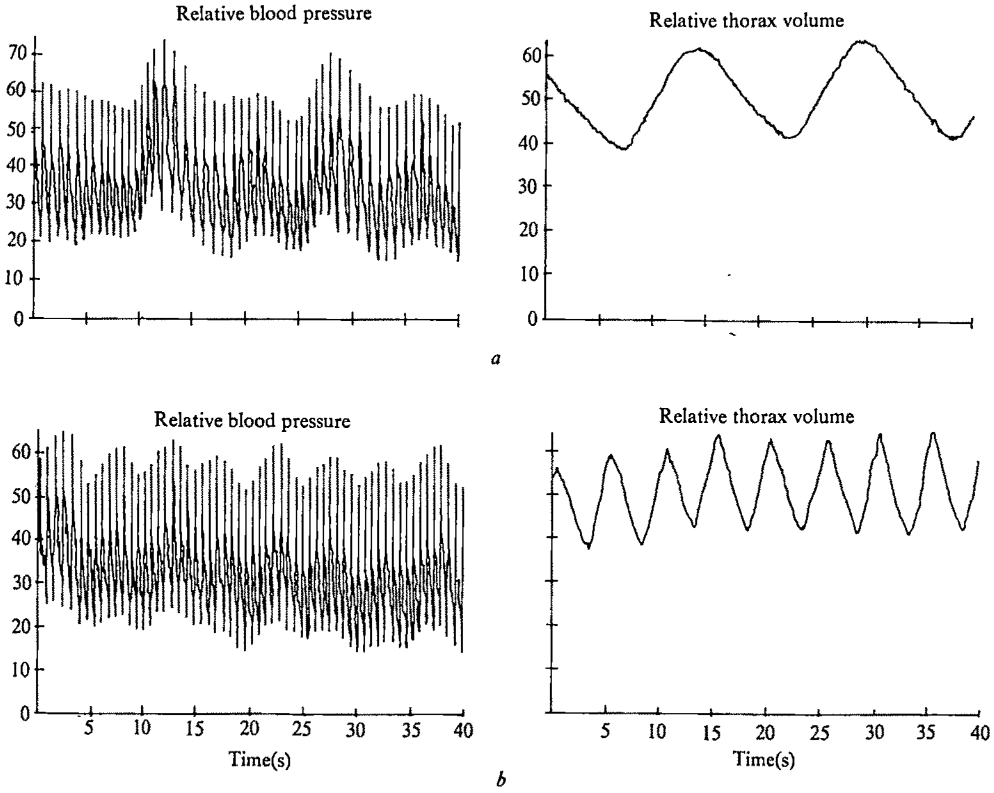


Fig. 1 *a*, A typical record of arterial blood pressure of a seated resting adult. The respiratory period is 15 s and there are two dominant low frequency components in the arterial pressure waveform, one of which has the same period as the respiration. *b*, The respiratory period is 5 s and a component of the same period only is evident in the arterial pressure waveform.

critical point of instability is reached when the value of $KG(j\omega_0)$, shown by X_0, is equal to $-1/K_{NL}$; but the latter is under the control of an externally applied signal. Consequently if the magnitude $-1/K_{NL}$ is made greater than $KG(j\omega_0)$ by applying a sufficiently large external periodic disturbance (with frequency, ω_1 say) (Fig. 2*a*) spontaneous oscillation will cease and the system responds only to the disturbance. The magnitude of disturbance needed to produce this condition, entrainment, is proportional to the magnitude A_1 determined according to the graphical method of Johnson[5], which evaluates the vector difference $[X_1-X_0]$ or $K[G(j\omega_1)-G(j\omega_0)]$. If the frequency response KG of the linear elements circles in towards the origin at higher frequencies as shown (this is broadly true for vascular smooth muscle and other biological effectors), the magnitude of the disturbance required to produce entrainment is much larger for disturbance frequencies (ω_1, say) appreciably below the frequency of spontaneous oscillation than for frequencies (ω_2, say) near, or even appreciably above, that of the spontaneous oscillation (compare A_1 with A_2).

We consider that this property of "selective entrainment" will account for our experimental observations. As another consequence of its nonlinear nature, the model can simulate the high degree of control accuracy of the blood pressure control system. For example, in the normal adult, a positive Valsalva manoeuvre leading to a 60% reduction in cardiac output (which in the absence of nervous regulation would produce a corresponding decrease in mean arterial blood pressure) may often produce only about 5% decrease in mean blood pressure[6]. Similarly, the output of the model will deviate by only 3% from the reference when the corresponding effect is simulated (this is done by decreasing the parameter K by 60%). It would not be possible to achieve this degree of accuracy if the nonlinear element were replaced by a linear block because of the stability restrictions defined by the Nyquist criterion[7].

This raises the possibility that a similar mechanism applies in other biological control systems—especially those with a high degree of control accuracy and periodic oscillations in the controlled variable. This can be tested by looking for "selective entrainment" of the spontaneous component by a periodic disturbance introduced as close as possible to the sensors of the system. We have applied this technique in an investigation of the human thermoregulatory system. Burton[8] has ascribed the slow fluctuations (period of 15 s or greater and above) seen in human digital plethysmograms to the action of the thermoregulatory system. We have studied such a digital plethysmogram signal obtained from the small finger of a normal resting adult seated in a room maintained at 19° C. Apart from the faster components of period less than 15 s, which are probably due to the action of the blood pressure control system described above, the signal comprises only slow fluctuations thought to represent activity of the thermoregulatory system.

If the subject places one hand alternately in containers of hot (40° C) and cold (18° C) water for fixed intervals (say, 10 s, giving a 20 s period), the waveform of the digital blood flow signal is found to be quite different from the spontaneous signal. It comprises only a substantial component having the same period as the stimulus; "selective entrainment" seems to occur because in this case entrainment is possible while a similar magnitude stimulus of longer period, say, 2 min, is found to be ineffective.

We suggest that the spontaneous fluctuations in peripheral blood flow are due to adjustments in the cutaneous circulation by the thermoregulatory system. With the body core temperature at 37° C, thermal variations in the extremities are handled

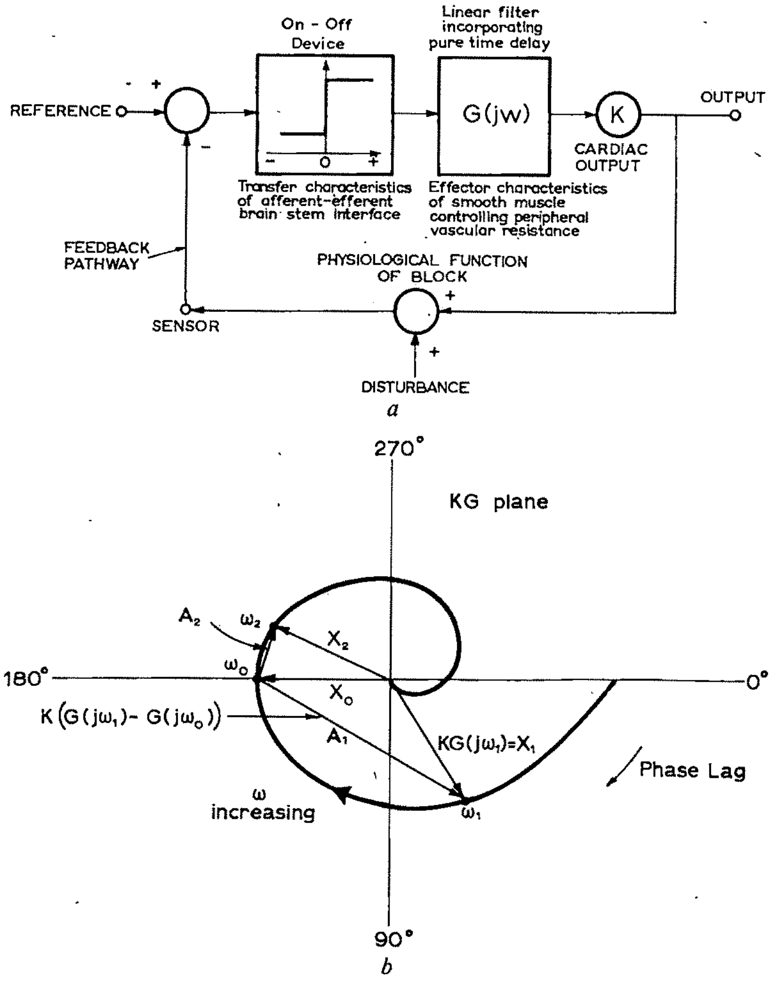

On - Off Device
Linear filter incorporating pure time delay
REFERENCE
- +
-
G(jw)
K
OUTPUT
CARDIAC OUTPUT
– 0 +
Transfer characteristics of afferent-efferent brain stem interface
Effector characteristics of smooth muscle controlling peripheral vascular resistance
FEEDBACK PATHWAY
PHYSIOLOGICAL FUNCTION OF BLOCK
+
SENSOR
+
DISTURBANCE
a
270°
KG plane
A₂
ω₂
X₂
180°
ω₀
X₀
0°
K(G(jω₁)- G(jω₀))
A₁
KG(jω₁)=X₁
Phase Lag
ω increasing
ω₁
90°
b

Fig. 2 *a*, Block diagram of a highly simplified version of the blood pressure control system incorporating only peripheral vascular resistance control. The on–off device represents the transfer characteristics of the afferent–efferent brain stem interface determined by Weidinger *et al.*[4] *b*, Showing the minimum magnitude of the external disturbance at each frequency required in order to entrain the spontaneous oscillation (after Johnson[5]).

by the peripheral system. Selective entrainment occurs when a periodic thermal stimulus is applied, implying that the thermoregulatory system operates on a similar nonlinear basis to the blood pressure control system.

We acknowledge the support of the Medical Research Council and the Leverhulme Trust.

B. W. HYNDMAN
R. I. KITNEY
B. McA. SAYERS

*Engineering in Medicine Laboratory,
Department of Electrical Engineering,
Imperial College of Science and Technology,
London SW7*

Received June 22; revised October 29, 1970.

[1] Dornhorst, A. C., Howard, P., and Leathart, G. L., *Circulation*, **6**, 553 (1952).
[2] Minorsky, N., *Nonlinear Oscillations* (Van Nostrand, London and New York, 1962).
[3] Hyndman, B. W., thesis, Univ. London (1970).
[4] Weidinger, H., Fedina, L., and Kehrel, H., *Pflügers Arch.*, **278**, 229 (1964).
[5] Johnson, C. D., in *Nonlinear Automatic Control* (by Gibson, J. E.), 422 (McGraw-Hill, London and New York, 1963).
[6] Fox, I. J., Crowley, jun., W. P., Grace, J. B., and Wood, E. H., *J. Appl. Physiol.*, **21**, 1553 (1966).
[7] Nyquist, H., *Bell System Tech. J.*, **11**, 126 (1932).
[8] Burton, A. C., *Amer. J. Physiol.*, **129**, 565 (1939).

Decreased Feeding after Injections of Amino-acids into the Hypothalamus

BUT for two exceptions[1,2], attempts to inhibit feeding behaviour by direct intrahypothalamic administration of glucose have been unsuccessful[3-6]. The effects of other nutrients have yet to be assessed. We have found that microinjections of balanced amino-acid solutions into dorsolateral perifornical hypothalamic tissue, where consummatory behaviours are obtained with electrical stimulation, inhibit feeding in rats.

Nine male albino rats with unilateral electrode-cannulae aimed for perifornical hypothalamic tissue were used. Each animal also had a bipolar electrode aimed at the corresponding area in the contralateral side of the hypothalamus for another study. They were caged individually and allowed free access to powdered lab chow and water except during short deprivation periods before feeding tests, and the cages received light for 12 h each day.

Initially the behaviour of each animal was observed during electrical stimulation (50 Hz, sine wave, 8–46 μA) of the brain underlying the tip of the electrode-cannulae and for chemically induced feeding after cannulae-directed injections of 65 mM L-noradrenaline bitartrate dissolved in 90 mM NaCl in volumes of 1 μl. Then the effects of intrahypothalamic injections of amino-acids were determined on three separate occasions. In the first two runs, the effects were compared with those of urea; in the third, they were compared with those of NaCl and D-glucose. Amino-acids and glucose were injected as 10% w/v solutions; urea and NaCl were injected as 4.2% w/v and 2.0% w/v solutions (approximately equiosmotic to the amino-acid solution). Each 10 g of the amino-acid mixture contained 0.33 g histidine HCl, 0.79 g lysine HCl, 0.29 g tryptophan, 0.72 g methionine, 0.72 g phenylalanine, 0.91 g leucine, 1.14 g isoleucine, 0.82 g valine, 0.72 g threonine, 2.60 g sodium glutamate, 0.31 g glutamine and 0.65 g glycine. L-Isomers were used. During all tests intrahypothalamic injections (1 μl. each) were given 1 h 30 min and 5 min before the rats were given access to food. For the first run, food was removed 30 min before the start of the dark period and returned 1 h later; for the second and third runs, food was removed 1 h after the start of the light period and returned 6 h later.

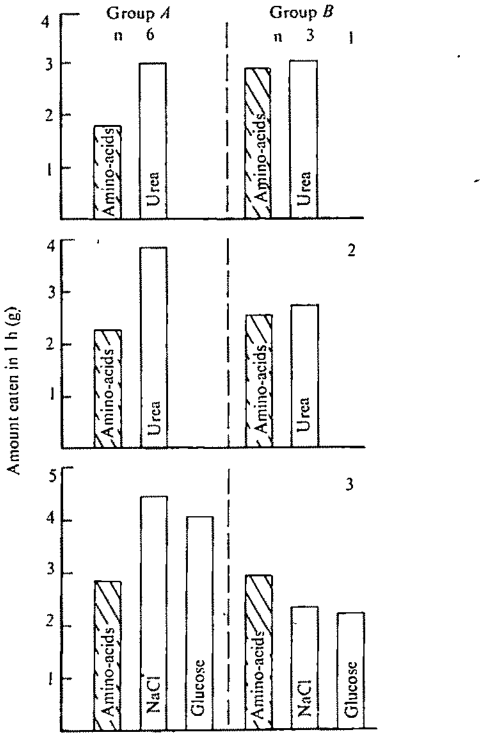


Fig. 1 Mean 1 h food intakes during three runs (see text).

Mean 1 h food intakes for the three runs are presented in Fig. 1. In six subjects, amino-acids depressed feeding more than control injections during every test (group *A*) ($Ns=6$, $Ps<0.2$; randomization test for mixed pairs); in three they did not (group *B*). In no case did the injections produce visually distinguishable abnormalities in behavour. The two groups also exhibited differential responses to injections of noradrenaline bitartrate and to electrical stimulation of tissue surrounding the tips of the cannulae. After noradrenaline injections, rats in group *A* ate 1.1 g (mean value) more than after saline injections; rats in group *B* ate only 0.4 g more ($n_1=6$, $n_2=3$, $V=1$, $P<0.5$; Mann Whitney test). Five rats in group *A* showed consummatory behaviour[7] during electrical stimulation through the electrode-cannulae and also learned to self-stimulate for the current; the remaining subject in group *A* only self-stimulated. Animals in group *B* exhibited flight during electrical stimulation and none exhibited appetitive behaviour or self-stimulation. Histological studies indicated that cannulae tips of rats in group *A* were located in dorsolateral perifornical hypothalamus, where consummatory behaviour has been obtained with electrical stimulation, while those of group *B* were in the far lateral hypothalamus or in mid-lateral hypothalamus near the ventral surface of the brain.

These data complement recent reports that high systemic levels of amino-acids depress feeding more than comparable levels of the other major nutrients[8,9] and suggest that circulating amino-acids depress feeding by a relatively direct action on hypothalamic neurones which are active during feeding behaviour. But the data do not necessarily imply the existence of a discrete sensory system which is responsive to blood amino-acids. Some amino-acids which are absorbed into the brain from the bloodstream[10] slow neural firing rates nonspecifically[11-13]. After the consumption of protein-rich meals, all neural activity, as well as that necessary for the facilitation of feeding, may be so affected. In any case, it is not yet known whether the observed effect reflects the operation of a physiological control mechanism.

This research was supported by a National Science Foundation postdoctoral fellowship, a NIGMS postdoctoral fellowship and by the Medical Research Council.

JAAK PANKSEPP
D. A. BOOTH

Laboratory of Experimental Psychology,
University of Sussex,
Brighton BN1 9QY

Received April 22; revised May 12, 1971.

[1] Herberg, L. J., *Nature*, **187**, 245 (1960).
[2] Booth, D. A., *J. Comp. Physiol. Psychol.*, **65**, 13 (1968).
[3] Epstein, A. N., *Amer. J. Physiol.*, **199**, 969 (1960).
[4] Wagner, J. W., and DeGroot, J., *Amer. J. Physiol.*, **204**, 483 (1963).
[5] Fisher, A. E. *Sci. Amer.* **210**, 60 (1964).
[6] Grossman, S. P., *Fed. Proc.*, **27**, 1349 (1968).
[7] Valenstein, E. S., Cox, V. C., and Kakolewski, J. W., *Psych. Rev.*, **77**, 16 (1970).
[8] Adair, E. R., Miller, N. E., and Booth, D. A., *Commun. Behav. Biol.*, **2**, 25 (1968).
[9] Panksepp, J., *Psychon. Monog. Suppl.* (in the press).
[10] Henschen, A., and Söderberg, U., in *Structure and Function of Inhibitory Neuronal Mechanisms* (edit. by von Euler, C., Skoglund, S., and Söderberg, U.) (Pergamon, Oxford, 1968).
[11] Werman, R., Davidoff, R. A., and Aprison, M. H., *Nature*, **214**, 681 (1967).
[12] Curtis, D. R., Hösli, L., Johnston, J. H., and Johnston, G. A. R., *Exp. Brain Res.*, **5**, 235 (1968).
[13] Curtis, D. R., Hösli, L., and Johnston, G. A. R., *Exp. Brain Res.*, **6**, 1 (1968).

Teratogenicity Studies of Methadone HCl in Rats and Rabbits

METHADONE maintenance was reported as a treatment for heroin addiction in 1965[1], and more than 10,000 patients are now maintained on the drug at more than 100 centres throughout the United States (unpublished Lilly Survey, 1970). Wallach *et al.*[2] observed that although women experienced abnormal menses during heroin addiction, regular menstruation and ovulation resumed with daily methadone treatment. In these studies several pregnancies were followed in which methadone maintenance was initiated before or during pregnancy with no apparent effect.

Guidelines for the clinical investigational use of methadone in maintenance programmes in the United States have been established only recently[3]. Because methadone was introduced as a narcotic analgesic several years before pre-clinical teratological studies were done, data about the effect of methadone in the pregnant animal are limited. In view of the lack of animal reproduction studies involving the drug, and the limited documented clinical experience with methadone in pregnant patients, the recommended treatment for the pregnant addict is hospitalization and narcotic withdrawal.

Foetal anomalies and decreased litter size were observed in hamsters after females had received large subcutaneous doses of methadone on the eighth day of gestation[4]. We now present data for species more commonly used in teratological studies. Rats and rabbits were given doses ten to fifty times the average daily human maintenance dose.

Methadone HCl (*dl*-6-dimethylamino-4,4-diphenyl-3-heptanone hydrochloride) was administered to pregnant Harlan albino rats and Dutch belted rabbits as previously described[5]. The beginning of gestation (day 0) was determined in the rabbits by artificial insemination. The rats were mated, and the time of conception was noted by the presence of a copulatory plug. The rats and rabbits, in groups of twenty-five and fifteen respectively, were given daily oral doses of distilled water, or 20 or 40 mg/kg of methadone by gavage during organogenesis and early foetal development (gestation days 6–15 in the rat, 6–18 in the rabbit). Doses of 60 mg/kg, given in preliminary studies, induced unacceptable levels of toxicity (maternal death and/or foetal resorption). Foetuses were recovered before term by Caesarean section, and examined for external abnormalities and visceral or skeletal defects.

In rats, methadone produced distinct maternal toxicity similar to that of morphine[6,7]. Hypertonia of skeletal muscle, which persisted up to 4 h after dosing, was often accompanied at the larger dose by catalepsy and respiratory depression; eight of the rats given methadone (three at the smaller dose) died of respiratory insufficiency. In rabbits, 40 mg/kg doses of methadone induced mild sedation during the early phases of the study. Two does on this regimen did not complete the test—one died on the twelfth day of gestation with signs of anorexia, weight loss and snuffles; the other aborted on the twenty-fifth day of gestation. The reproductive status of the animals that died was normal (necropsy evaluation); pregnancy was confirmed in seven of the eight rats and both rabbits.

In general, the treated rats ate and gained less than the control rats; in the rabbits, food intake was reduced in both the control and treated animals during the dosing period. These effects were not marked and were considered to be inconsequential in view of the negative teratological findings.

Pregnancy rates (Table 1) were depressed among animals on the 40 mg/kg regimen, but there was no sign of any impairment of reproduction. Normal litter sizes and numbers of corpora lutea indicated that the reduction in pregnancies was an isolated finding, unrelated to methadone treatment. Moreover, the fertility findings were not significantly variant from our cumulative control data (fertility in rats: range 70–100%, mean 90%; rabbits: range 40–100%, mean 80%). In treated rats resorption was slightly more frequent than in the controls; this was attributed to maternal toxicity.

Table 1 Uterine and Foetal Parameters

	Females pregnant	Mean No. foetuses ±s.e.	Resorptions Per total No. implantation sites	Resorptions Females affected*	Mean foetal weight ±s.e. (g)
Rats					
Control	24/25	12.2±0.6	15/308	10/24	3.87±0.08
20 mg/kg methadone	25/25	13.0±0.4	20/306	14/22	3.69±0.05
40 mg/kg methadone	19/25	12.5±0.7	26/214	11/15	3.66±0.07†
Rabbits					
Control	15/15	5.1±0.5	8/84	6/15	27.2±1.2
20 mg/kg methadone	14/15	6.3±0.7	9/97	6/14	25.5±1.0
40 mg/kg methadone	11/15	5.7±0.6	1/52	1/9	29.8±0.9

* Based on pregnant females that survived.
† Not significant at 0.05 level, Dunnett's *t* test, Weil's number of independent sampling units[8,9].

Table 2 Foetal Abnormalities

	No. of foetuses examined	
	Rats	Rabbits
Control	293	76
20 mg/kg methadone	286	88
40 mg/kg methadone	188	51
Cumulative control	9,016	1,620

	Incidence of abnormality			
	External examination	Visceral examination		Skeletal examination
Rats*	Rudimentary tail	Internal hydrocephalus	Hydronephrosis	12 Ribs
Control			24/107	1/186
20 mg/kg methadone	1/286		12/103	
40 mg/kg methadone		1/66	6/66	
Cumulative control	1/9,016	8/3,739	478/3,739	1/5,277

	External examination	Visceral examination		Skeletal examination			
Rabbits	Varus forepaws	Internal hydrocephalus	Hydro-nephrosis	13 Ribs	Sternebrae fused or extra	Sternal bars misaligned	Incomplete development cranial bones
Control		1/76		38/76	3/76	1/76	
20 mg/kg methadone	1/88 †	1/88 † ‡	1/88	50/88	5/88	1/88	1/88 † ‡
40 mg/kg methadone				26/51	6/51		
Cumulative control	2/1,620	2/1,620	1/1,620	444/1,620	17/1,620	11/1,620	22/1,620

* 1/3 of each litter was designated for visceral examination, 2/3 for skeletal examination. † Littermates. ‡ Same foetus.

Defects observed in the foetuses (Table 2) were representative of our control rat and rabbit populations. In the rats rudimentary tail, hydrocephalus and twelve ribs occurred as single, isolated defects; hydronephrosis was seen in foetuses of each treatment group, but most frequently in the control progeny. In the rabbits there was a low incidence of varus forepaws, hydrocephalus and hydronephrosis. Thirteen ribs, a common anatomical variation of our rabbits, and a few minor skeletal defects were observed as frequently in the control foetuses as in those of the methadone regimens.

Doses of 20 or 40 mg/kg of methadone administered orally to rats caused hypertonia of skeletal muscle and respiratory depression; some of the treated rats died of respiratory insufficiency. In rabbits, oral doses of 40 mg/kg induced mild sedation. Reproduction was not impaired in the rats or rabbits regardless of maternal condition. No drug-related defects were observed in the foetuses, and we concluded that methadone HCl in the doses which we used was not teratogenic in the rat or rabbit.

Janet K. Markham
John L. Emmerson
Norris V. Owen

Toxicology Division,
Eli Lilly and Company,
PO Box 708,
Greenfield, Indiana 46140

[1] Dole, V. P., and Nyswander, M., *J. Amer. Med. Assoc.*, **193**, 646 (1965).
[2] Wallach, R. C., Jerez, E., and Blinick, G., *Amer. J. Obstet. Gynec.*, **105**, 1226 (1969).
[3] Edwards, C. C., *Federal Register*, **36**, 6075 (1971).
[4] Geber, W. F., and Schramm, L. C., *Pharmacology*, **11**, 248 (1969).
[5] Emmerson, J. L., Owen, N. V., Koenig, G. R., Markham, J. K., and Anderson, R. C., *Toxicol. Appl. Pharmacol.*, **19**, 471 (1971).
[6] Scott, C. C., and Chen, K. K., *J. Pharmacol. Exp. Therap.*, **87**, 63 (1946).
[7] Chen, K. K., *Ann. NY Acad. Sci.*, **51**, 83 (1948).
[8] Dunnett, C. W., *Biometrics*, **20**, 482 (1964).
[9] Weil, C. S., *Food Cosmet. Toxicol.*, **8**, 177 (1970).

Impairment of Recent Memory by Marihuana and THC in Rhesus Monkeys

Several studies with humans have indicated that marihuana and its active ingredient, delta-9-tetrahydro cannabinol (THC), have disruptive effects on short term memory[1-3]. Of the few animal studies on THC, only that of Scheckel *et al.*[4] addressed the question of memory impairment. The results of that study were attributed to lack of motivation. The study described here was designed to elucidate the effects of marihuana on short term memory in rhesus monkeys using an automated delayed matching-to-sample task. In our laboratory, monkeys have been trained to puff cigarettes for a water reward, and thus we are able to study the effects of marihuana smoke while avoiding the set problems associated with human subjects. Another group of monkeys, trained on a similar memory task, was administered oral THC. Significant memory impairment was established with both modes of administration.

In the first experiment, two adult male rhesus monkeys, trained to smoke cigarettes[5], were placed on a two-task procedure for water reward. A smoking apparatus was attached to a 'Plexiglas' panel covering a 14 inch square opening on one side of each monkey's 36 inch cubic home cage. The smoking procedure has been described[6]; the only modification here was the absence of a dummy air tube, so that the monkeys were forced to puff smoke rather than having a choice of smoke versus air. A matching test was also attached to a 'Plexiglas' panel covering a 14 inch square opening on an adjacent side of each monkey's cage. The matching test has also been described elsewhere[7]. The combined smoking–matching procedure operated as follows: first, the monkey was required to make thirty puffs on the smoking machine (a fixed ratio 30 contingency was used throughout); this behaviour extinguished a light next to the puffing tube and signalled the start of the matching test. The monkey was then presented with a green or red sample light for 3 s on the matching panel. Touching a centre tube under the sample light turned off the light and, after a predetermined delay, two side

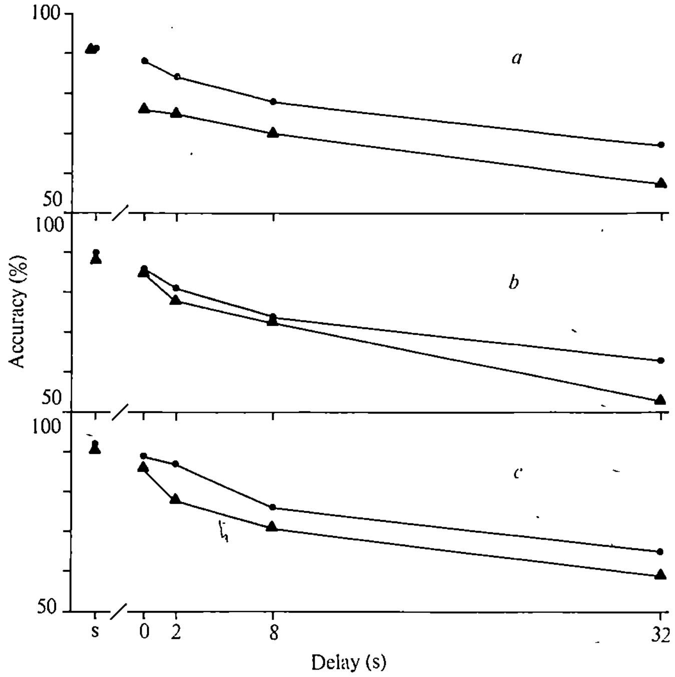


Fig. 1 Effect of THC on delayed oddity performance (s = simultaneous). ●, Control; ▲: *a*, 2.0 mg/kg THC; *b*, 1.0 mg/kg THC; *c*, 0.5 mg/kg THC.

lights appeared, one green and one red. Touching the solenoid of the light which matched in colour the previous sample light resulted in a 2 ml. water reward. If an error was made, or if no response was made after 8 s, the side lights were turned off and the light of the smoking machine appeared, signalling the start of the smoking test again. The monkeys were tested on a total of three delays in the matching test: 0 s, 5 s and 30 s; however, only two delays were used during the same session (either 0 and 30 s, 5 and 30 s, or 0 and 5 s). The delays were programmed randomly and the delay combinations were presented in a balanced order to control for possible sequence effects. Testing was programmed automatically by Digibit logic circuits as well as by punched tape devices, all of which were located in a separate control room.

The monkeys were tested in the two-task procedure 7 days a week and received all their water during a daily 4 h testing session (starting at 1030 h). On all days the monkeys were given access to thirty cigarettes. On control days the subjects received tobacco cigarettes (Raleigh plains). On drug days (Tuesdays and Fridays) thirty marihuana cigarettes, rolled in the laboratory, were used, with tobacco plugs at either end. Each cigarette contained approximately 0.5 g of marihuana, obtained from *Cannabis sativa*, Mexican female, 1969, which was supplied by DHEW, Public Health Service, NIMH (analysis: THC, 1.5%; cannabidiol, 0.12%). The effect of marihuana on each monkey's performance on each delay was tested an average of six times. The total number of smoking puffs per session, the total number of matching trials taken, as well as the accuracy on each delay, were recorded and analysed. Drug days and control days were compared, the day immediately preceding the drug day being considered as the control day. Chi-square tests were used to compare statistically the differences.

The results (data for two monkeys combined) are presented in Table 1. Accuracy was lowered but not significantly impaired by marihuana at 0 s ($P > 0.2$) and 5 s (just missed significance with $P = 0.06$). On the 30 s delay, however, accuracy was significantly impaired ($P < 0.01$) by marihuana. The rate of puffing smoke as well as the rate of responding on the matching test were both significantly decreased ($P < 0.05$ in both cases). (There were no significant differences between the two monkeys on any performance measure in any drug or control condition.)

Table 1 Effect of Marihuana Smoking on Delayed Matching Performance

Accuracy %	Marihuana	Control
0 s	99	100
5 s	89	94
30 s	68	74
Total puffs	4,614	5,795
Total matching trials	150	198

In the second experiment, four adult rhesus monkeys (different monkeys from those used in the first experiment) were used, all with extensive previous experience on the memory task. The task was similar to the matching test used in the first experiment, except that instead of matching the colour of one of the side lights to the sample light, the monkey had to choose the odd colour[8]. The oddity test was attached to a 'Plexiglas' panel covering a 14 inch square opening on one side of each monkey's 36 inch cubic home cage. On each trial one of five delay conditions was used: simultaneous, 0, 2, 8 and 32 s. They were presented in randomized order such that each animal received 960 trials (192 for each delay) during an 8 h session (starting at 1000 h). For the simultaneous presentation a red or a green light (the sample stimulus) was presented on the central display unit at which time the animal was required to touch a centre tube (under the sample light) within 3 s. A response to the sample stimulus immediately resulted in presentation of one red light and one green light (the comparison stimuli) on each of the two side units, respectively, for up to 8 s; the sample stimulus remained on with the comparison stimuli. The monkey then had to non-match (oddity) one of the side comparison stimuli with the colour of the sample stimulus by touching the correct tube in order to receive a water reward (1 ml.). The procedure for the real delay conditions was the same, except that the sample stimulus appeared alone and terminated some time before the comparison stimuli appeared. The monkeys were tested 7 days a week and received all their water in the test conditions.

The THC (supplied by NIMH) was diluted in 10 ml. of apple juice and administered orally. On control days 10 ml. of apple juice alone was administered. The monkeys drank the juice from a small plastic bottle through the front of their cages. The juice or juice–drug mixture was administered 15 min before testing. Drug days were Tuesdays and Fridays; six drug administrations were given, one at 0.5 mg/kg, three at 1 mg/kg and two at 2 mg/kg. The accuracy (% correct) and rate (% responses made out of possible total) data recorded on punched tapes were sorted and summed for drug and control days according to delay conditions. Again, the day before the drug day was considered the matched control day. Chi-square tests were used for data analysis.

The accuracy results (data for four monkeys combined) are presented in Fig. 1. Accuracy was significantly impaired by 0.5 mg/kg of THC on the 2 s and 32 s delays ($P < 0.05$ in both cases); by 1.0 mg/kg on the 32 s delay ($P < 0.05$); and by 2.0 mg/kg on the 0, 2, 8 and 32 s delays ($P < 0.05$, 0.05, 0.05 and 0.01, respectively). Accuracy was never impaired when sample and comparison stimuli were simultaneous. The rate of responding was significantly decreased by all doses of the drug in all testing conditions ($P < 0.01$ in all cases). The percentage of responses, taken out of the total offered, was approximately 60% on control days, 48% for 0.5 mg/kg THC, 39% for 1.0 mg/kg THC and 27% for 2.0 mg/kg THC.

In both the marihuana and THC studies, both the rate of responding and the accuracy were affected. The rate effects possibly confirm the conclusion[4] that THC induces a "loss of ability or motivations to perform complex tasks". But our results also strongly suggest that marihuana and THC have a definite effect on short term retention processes *per se*. In the smoking–matching test, marihuana lowered accuracy only when a 30 s delay was used. In the oddity test, THC impaired accuracy only in delay conditions and never in the simultaneous condition. This type of impairment is different from that shown with amphetamine, scopolamine, chlorpromazine, pentobarbital, LSD[7,9–11] or mescaline (unpublished data) on a similar task. By comparison with these other drugs, the specificity of THC is indeed remarkable.

Unlike Scheckel *et al.*[4] we obtained no evidence of long term effects. Performance on days immediately following drug administrations was always similar to that on days preceding them. Scheckel *et al.*[4] used squirrel monkeys and we used rhesus monkeys; this species difference may explain the variant results. We also failed to find objective criteria which would warrant the assumption of hallucinations[4] in our monkeys; their behaviour may have changed quantitatively, but not qualitatively. Scheckel *et al.*[4] also reported a biphasic effect of THC on response rate, with depression occurring at low doses and stimulation at high doses (16, 32 and 64 mg/kg). As more than half of their monkeys died after being administered these high doses, we were unwilling to attempt a confirmation of possible excitation effects.

This work was supported by grants from the US National Institute of Mental Health and the American Cancer Society, and a postdoctoral fellowship from the US National Institutes of Health.

B. ZIMMERBERG
S. D. GLICK
M. E. JARVIK

Department of Pharmacology and Psychiatry,
Albert Einstein College of Medicine,
1300 *Morris Park Avenue,*
Bronx, New York 10461

Received February 22; revised June 1, 1971.

[1] Tinklenberg, J. R., Melges, F. T., Hollister, L. E., and Gillespie, H. K., *Nature*, **226**, 1171 (1970).
[2] Melges, F. T., Tinklenberg, J. R., Hollister, L. E., and Gillespie, H. K., *Science*, **168**, 1118 (1970).
[3] Weil, A. T., and Zinberg, N. E., *Nature*, **222**, 434 (1969).
[4] Scheckel, C. L., Boff, E., Dahlen, P., and Smart, T., *Science*, **160**, 1467 (1968).
[5] Pybus, R., Goldfarb, T., and Jarvik, M. E., *J. Exp. Anal. Behav.*, **12**, 88 (1969).
[6] Glick, S. D., Canfield, J. L., and Jarvik, M. E., *Psychol. Rep.*, **26**, 707 (1970).
[7] Glick, S. D., and Jarvik, M. E., *J. Pharmacol. Exp. Ther.*, **169**, 1 (1969).
[8] Glick, S. D., Nakamura, R. K., and Jarvik, M. E., *Commun. Behav. Biol.*, **5**, 165 (1970).
[9] Robustelli, F., Glick, S. D., Goldfarb, T. L., Geller, A., and Jarvik, M. E., *Commun. Behav. Biol.*, **3**, 101 (1969).
[10] Glick, S. D., Goldfarb, T. L., Robustelli, F., Geller, A., and Jarvik, M. E., *Psychopharmacologia*, **15**, 125 (1969).
[11] Glick, S. D., and Jarvik, M. E., *Fed. Proc.*, Atlantic City, New Jersey (1970).

Oddity and Specific Searching Image more Important than Conspicuousness in Prey Selection

IN an earlier report[1] I presented results from a pilot experiment which suggested that conspicuousness of prey was the most important determinant in prey selection. I now have results from 1,600 laboratory experiments performed with two species of hawks in rigorous experimental conditions, which suggest that oddity and the specific searching image are more important. Many observations and experimental results fit well into the generalization that predators have a tendency to select odd prey, animals that differ in some way from most prey[2,3], although unequivocal evidence is lacking. Predators also show a tendency to continue to select a given type of prey, even though other types may be as readily, or even more easily available, a phenomenon that L. Tinbergen[4] has termed the "specific searching image". My experiments are a simultaneous test of three factors which influence prey selection: conspicuousness, oddity, and the specific searching image.

Six tamed American kestrels (*Falco sparverius*) and two broad-winged hawks (*Buteo platypterus*) were allowed to prey on white laboratory mice, some of which had been coloured grey with food dye. In each experiment, nine mice of one colour and one of the other were presented on a substrate which was painted to match one or the other colours of mice. Each of the two colour combinations of mice was replicated fifty times on each of the two colours of substrate, giving a total of 200 experiments for each bird (Table 1). The sequence in which given mouse and substrate combinations (*A*, *B*, *C*, *D*) were presented to the hawk was randomized. The hawk was allowed to select only one mouse per experiment; the expected number of captures of each colour of mouse, assuming no (or random) selection is given in Table 1. The mice were presented individually on pedestals about 10 cm square and 15 cm high which were arranged about 10 cm apart on an arc of a circle of approximately 2 m radius. The hawk was placed on a perch at the centre of the circle. Laboratory mice rarely leave such a pedestal and the distribution of mice can thus be controlled. The position of the odd mouse in each experiment was randomized.

All eight hawks showed a statistically significant preference for odd mice, but only of one colour; four for white, odd mice and four for grey, odd mice (Table 2). In two cases the preference for white was sufficiently strong that no grey (and odd) mice were taken from a grey substrate. Three birds took significantly more odd mice of the preferred colour when these differed in colour from the substrate than when the mice matched the substrate. Thus mice of a particular colour were preferred, but only when they were odd, and the conspicuousness of the mouse had relatively little influence on the results.

My results thus suggest that the specific searching image may be the major determinant in prey selection in hawks although oddity is very nearly as important. I am conducting

Table 1 Experimental Design

Series	No. of experiments	Substrate	Mice presented in each experiment		Expected total catch*	
			Grey	White	Grey	White
A	50	Grey	9	1	45	5
B	50	Grey	1	9	5	45
C	50	White	9	1	45	5
D	50	White	1	9	5	45

* If there is no, or random, selection.

Table 2 Number of Odd Mice Taken

Hawk	Sex	Experiment series A	B	C	D
Lucy	♀	4	16*	2	16*
Gimpy	♀	25*	6	21*	6
Bilbo	♂	31*	0*	18*	4
Gandalf	♂	48*	0*	31*	1
Frodo	♂	10*	4	15*	9
Arwen	♀	4	16*	2	21*
Baby	♂	5	14*	3	20*
Ignatz	♂	6	22*	2	37*

* Departs significantly from the expected value, chisquare test, $P < 0.05$. Baby and Ignatz are *B. platypterus;* all other individuals are *F. sparverius.*

further experiments in an attempt to separate, and assess, the relative importance of these two factors.

I thank D. G. Clark, A. Dawson, E. Moore, N. S. Mueller, W. Schwenn, H. Sears, S. Treistman and P. Young for technical assistance. The research was supported by the US National Science Foundation and the University of North Carolina Research Council.

HELMUT C. MUELLER

Department of Zoology,
University of North Carolina,
Chapel Hill,
North Carolina

Received May 25; revised June 14, 1971.

[1] Mueller, H. C., *Nature*, **217**, 92 (1968).
[2] Salt, G. W., *Ecol. Monogr.*, **37**, 113 (1967).
[3] Pielowski, Z., *Ekologia Polska* (A) Tom IX No. 11 (1961).
[4] Tinbergen, L., *Arch. Neer. Zool.*, **13**, 265 (1960).

CO_2-dependent Oxygen Evolution by Envelope-free Chloroplasts

THE chloroplasts isolated by Hill[1] in the 1930s were capable of rapid rates of oxygen evolution when provided with an artificial electron acceptor (oxidant) but did not retain the ability to assimilate CO_2 at rates detectable by the methods then available. Nearly 20 yr later the ability of isolated chloroplasts to fix CO_2 was demonstrated by Arnon, Allen and Whatley[2] but even then the rates were only 3–6% of those supported by the intact plant. By 1966, when the rates of fixation by chloroplasts isolated in sugar media first matched those of intact leaves[3,4], it had been established[5,6] that high rates of carbon assimilation were only achieved by chloroplasts with intact envelopes. It is unlikely that all the earlier difficulties were caused by the unintentional isolation of damaged chloroplasts but, except for some early work by Arnon[7] (in which chloroplasts were supplemented with cytoplasmic malic enzyme), it has certainly not been possible to show CO_2-dependent oxygen evolution by envelope-free chloroplasts[8] and rapid rates (comparable with the Hill reaction) have not been previously reported. Such high rates have now been observed in a reconstituted system containing ATP, ferredoxin, NADP (nicotinamide adenine dinucleotide phosphate), R5P (ribose-5-phosphate), CE (soluble components released from osmotically shocked chloroplasts) and envelope-free chloroplasts.

Fig. 1 shows that oxygen was evolved immediately after illumination of a mixture from which PGA (3-phosphoglycerate) and R5P were initially omitted. This evolution is attributable to the reduction of NADP and it ceased (or slowed, depending on the activity and content of the chloroplast extract) according to the stoichiometry of equation (1).

$$H_2O + NADP \rightarrow NADPH_2 + \tfrac{1}{2}O_2 \qquad (1)$$

At this point evolution can be restarted by the addition of PGA[9,10] or by the addition of R5P (if CO_2/bicarbonate is present), and it can be assumed that both of these compounds lead (by the appropriate reactions of the Benson–Calvin cycle) to the formation of DPGA (1,3-diphosphoglycerate) which then regenerates NADP in the oxidized form according to equation (2).

$$NADPH_2 + DPGA \rightarrow \text{glyceraldehyde-3-phosphate} + NADP + \text{orthophosphate} \qquad (2)$$

The response to the addition of PGA is very rapid (Fig. 1*A*) and the maximum rate is attained within seconds. The response to R5P depends to some extent on the activity of the preparation but it is always detectably slower (Fig. 1*B*) than the response to PGA and in some experiments the rate was not maximal for 2–3 min.

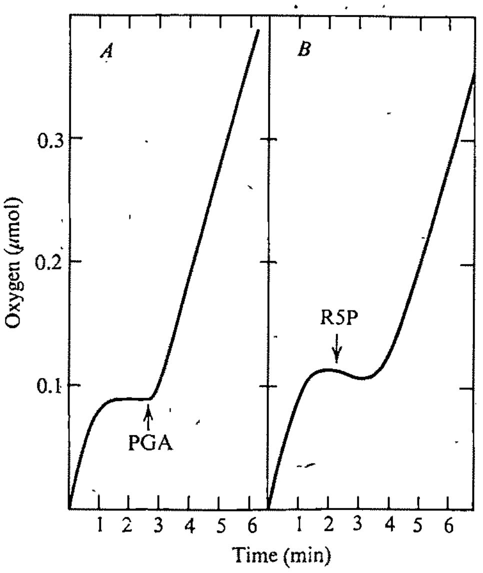


Fig. 1 Oxygen evolution by envelope-free chloroplasts initiated (*A*) by adding PGA or (*B*) by adding R5P. Only reaction mixture *B* contained added CO_2/bicarbonate. Intact chloroplasts were prepared in sorbitol medium[6] from 80 g pre-illuminated spinach leaf and freed of their envelopes by resuspending in 10 ml. assay medium (AM) at 1/25 full strength. NaCl was then added to a final concentration of 0.1 M. After a second centrifugation the envelope-free chloroplasts[6] were resuspended in AM and the supernatant retained and designated CE[9,10]. The chloroplasts from which the fraction CE was derived contained a total of 4 mg chlorophyll. The protein content of CE was 12 mg/ml. Each 2 ml. reaction mixture contained CE (0.9 ml.) envelope-free chloroplasts (100 µg chlorophyll) double strength AM (1 ml.) ferredoxin (3 nmol) and NADP (0.25 µmol). AM contained HEPES[17] (50 mM at *p*H 7.6), sorbitol (0.33 M), EDTA (2.0 mM) $MgCl_2$ (1.0 mM), $MnCl_2$ (1.0 mM). In *A*, the reaction mixture also contained 0.4 µmol of ATP and 3 µmol of PGA were added as indicated. In *B*, 4 µmol of ATP and 200 µmol of $NaHCO_3$ were added at the outset and 3 µmol of R5P as indicated. Oxygen evolution was measured in twin oxygen electrodes at 20° C in saturating red light[9].

Oxygen evolution could be initiated by PGA in the absence of added CO_2 (Fig. 1*A*) whereas with R5P (Fig. 1*B*) CO_2/bicarbonate was also needed. This requirement for bicarbonate is illustrated in Fig. 2. In this experiment, as in Fig. 1, the $MgCl_2$ content of the reaction mixtures was the same as that used in earlier experiments with intact chloroplasts (for example, ref. 6). At this concentration of $MgCl_2$, however, it is necessary to add CO_2/bicarbonate in ten-fold excess of that needed to saturate intact chloroplasts. The addition (Fig. 2) of the necessarily large volume (0.2 ml.) of bicarbonate solution produces a dip in the oxygen electrode trace (owing to the problem of matching the oxygen concentration of the reaction mixture with that of the added bicarbonate solution). This causes some difficulty in interpreting the kinetics of response but many experiments of this sort have led us to the conclusion that there is again a finite lag between the addition of bicarbonate and the subsequent attainment of the maximum rate (compare the addition of R5P in Fig. 1). If the concentration of $MgCl_2$ is raised by 4 mM there may be measurable oxygen evolution in the absence of added bicarbonate and saturation is reached at bicarbonate concentration closer to 10 mM (compare refs 11–14).

The CO_2-dependent oxygen evolution was observed only in the presence of added ATP. The response to added ATP was immediate (unlike that to R5P or CO_2/bicarbonate) but the immediacy of this reaction may be partly related to the further conversion of small quantities of PGA (newly formed at the expense of endogenous ATP) rather than to an excep-

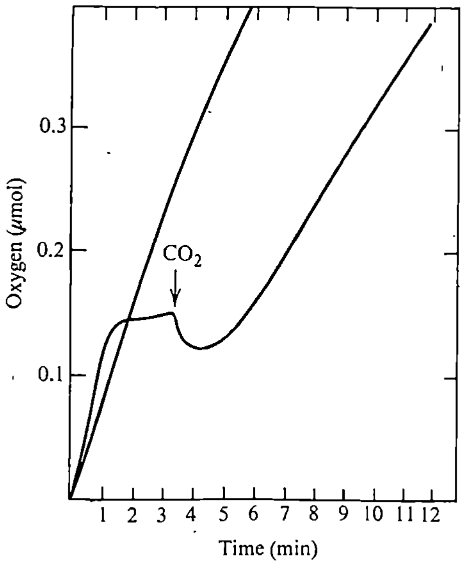


Fig. 2 CO_2-dependent oxygen by envelope-free chloroplasts. As for Fig. 1 except that both reaction mixtures contained ATP (5 μmol) and R5P (3 μmol) from the outset. CO_2/bicarbonate (200 μmol) was added before illumination or as indicated.

tionally rapid formation of triose phosphate from R5P. In most experiments there was no CO_2-dependent oxygen evolution unless ATP was added in excess of that required to saturate PGA-dependent oxygen evolution. Following the addition of ATP to reaction mixtures containing limiting quantities of chlorophyll (20 μg) which have been pre-illuminated (2 min) we have observed rates of evolution as high as 175 μmol/mg chlorophyll/h. When there has been no possibility of prior accumulation of PGA from R5P and CO_2 (for example, when R5P and $NADPH_2$ have been added simultaneously at the onset of illumination) the maximum rates were nearer 80 μmol/mg/h but under these conditions it was often impossible to obtain much faster rates with added PGA as substrate (Fig. 1) and it is probable that accumulation of PGA is not the only factor which contributes to the highest rates observed.

Our results show that it is possible to reconstitute a system in which completely envelope-free chloroplasts will support a carbon dioxide-dependent oxygen evolution and that this system requires added ATP and R5P. For maximal rates it is necessary to increase either the bicarbonate concentration or the Mg^{2+} concentration substantially beyond the respective optima for intact chloroplasts. Indirectly, our observations confirm and extends those of Whatley *et al.*[15] who first demonstrated CO_2 fixation by broken chloroplasts fortified with CE. Our approach is essentially similar but in the light of recent knowledge we have also been able to vary the nature and concentration of additives in such a way that we were able to obtain better rates (assuming an incubation period of 1 h, the figure given by Whatley *et al.* for CO_2 fixed with R5P corresponds to a rate of 8 μmol/mg chlorophyll/h).

Without the constraints imposed by physical compartmentation and biochemical regulation it is improbable that it will ever be possible to reconstitute, in one simple suspension of illuminated envelope-free chloroplasts, a system capable of operating the entire Benson–Calvin cycle, as a cycle. That long sequences of the complete system can be made operable, however, indicates that these are not (in themselves) dependent on an intact structure, except to the extent that structure defines the environment within the chloroplast in which the reactions normally occur. Further investigation of the reconstituted system could clearly lead to a better understanding of these environmental requirements including those which govern the affinity of the principal carboxylating enzyme for its substrate[16].

This work was supported by the Science Research Council and the Royal Society. We thank Dr David Hall for ferredoxin.

D. A. Walker
Ann V. McCormick

Department of Botany,
The University,
*Sheffield S*10 2*TN*

D. M. Stokes

Department of Botany,
University of Melbourne,
Parkville, Victoria

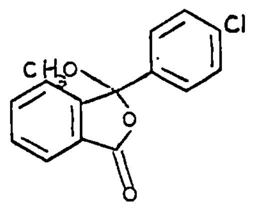
Received June 1, 1971.

[1] Hill, R., *Nature*, **139**, 881 (1937).
[2] Arnon, D. I., Allen, M. B., and Whatley, F. R., *Nature*, **174**, 394 (1954).
[3] Bucke, C., Walker, D. A., and Baldry, C. W., *Biochem. J.*, **101**, 636 (1966).
[4] Jensen, R. G., and Bassham, J. A., *Proc. US Nat. Acad. Sci.*, **56**, 1095 (1966).
[5] Walker, D. A., *Plant Physiol.*, **40**, 1157 (1965).
[6] Walker, D. A., in *Biochemistry of Chloroplasts* (edit. by Goodwin, T. W.), Proc. NATO Adv. Study Inst., Aberystwyth, 1965, **2**, 53 (Academic ,New York, 1966).
[7] Arnon, D. I., *Nature*, **167**, 1008 (1951).
[8] Walker, D. A., and Crofts, A. R., *Ann. Rev. Biochem.*, **36**, 389 (1970).
[9] Stokes, D. M., and Walker, D. A., *Plant Physiol.* (in the press).
[10] Stokes, D. M., and Walker, D. A., *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.) (Wiley Interscience, New York, 1971).
[11] Sugiyama, T., Matsumoto, C., and Akazawa, T., *Biochem. Biophys. Res. Commun.*, **30**, 118 (1968).
[12] Sugiyama, T., Nakayama, N., and Akazawa, T., *Arch. Biochem. Biophys.*, **126**, 737 (1968).
[13] Bassham, J. A., *Science*, **172**, 526 (1971).
[14] Preiss, J., and Kosuge, T., *Ann. Rev. Plant Physiol.*, **21**, 433 (1970).
[15] Whatley, F. R., Allen, M. B., Rosenberg, L. L., Capindale, J. B., and Arnon, D. I., *Biochim. Biophys. Acta*, **20**, 462 (1956).
[16] Walker, D. A., *Nature*, **226**, 1204 (1970).
[17] Good, N. E., Winget, G. D., Winter, W., Connelly, T. N., Isawa, S., and Singh, R. M. M., *Biochemistry*, **5**, 467 (1966).

Elimination of the Response to Gravity of Lettuce Seedlings

While investigating the effects of certain lactones on the germination of lettuce seeds I have found that 3-4′ chlorophenyl 3-methoxy phthalide (I) modifies the growth of seedlings. Jones *et al.* found that this compound and other derivatives of 2-benzoylbenzoic acids—either as the free acid or as lactones—affected the growth, geotropic and phototropic behaviour of seedlings.

Radicles and hypocotyls of most seedlings are strongly orthogeotropic[2,3]. Synthetic growth regulators with physiological action like that of IAA (β indol-3-yl acetic acid) affect root and shoot growth, sometimes making these organs grow in an unexpected direction. Antitropistic effects can also be induced by morphactins (derivatives of 9-hydroxy-9-carboxylic acid fluorene)[4], N-α-naphthyl phthalamic acid (NAP)[5], and tri-iodo benzoic acid (TIBA)[6].



(I)

Table 1 Orientation of Radicles and Hypocotyls of Lettuce Seedlings Treated with 3-4′ Chlorophenyl 3-Methoxy Phthalide (I) (Effective Concentration Saturated Aqueous Solution)

	Degrees from horizontal (negative upwards) Radicle	(positive downwards) Hypocotyl
Control	$+74.06 \pm 4.79$	-94.94 ± 1.52
Treated	-20.50 ± 5.18	-0.44 ± 2.57

Compound (I) eliminates the geotropic response of the seedling, and the direction of growth is then determined by the initial orientation of the embryonic axis. Lettuce, cultivar Grand Rapids, or mustard seedlings were grown either on seed test paper which was loaded with (I), or from seed which had been soaked in a saturated aqueous solution of (I) at 4° C for 48 h. (Seed test paper 90 mm by 0.4 mm thick can be loaded with a given weight of physiologically active material in an organic solvent. After this has evaporated water is added to provide an aqueous milieu equivalent to a given molarity. Although this may seem to be greater than saturation concentrations the fat content of the seed ensures that the active material is present at an endogenous level corresponding to the stated molar concentrations. Control substratum was treated with the solvents used.)

Seeds were placed on the paper so that the radicle pointed in the desired direction. Because small seeds adhere readily to seed test paper, I usually positioned seeds on the paper lining the base of the Petri dish and then set the paper vertically. Using another method, I folded seed test paper (90 mm × 0.4 mm thick) to give a centre upright fold secured by two paper clips. Seeds were inserted into the apex of the fold and held in any desired position. These assemblies were placed in plastic trays (15 cm × 20 cm) containing about 50 ml. of deionized water. The seeds germinated and the seedlings developed at 20° C in darkness. Phototropic curvature was induced in 72 h dark grown seedlings derived from vertically oriented embryos, radicle down, by irradiating unilaterally with a 40 W tungsten bulb, run at mains voltage (240) 75 cm from the seedlings. In all experiments on phototropism at least nine, and usually sixteen, individuals were measured. Each of these experiments was performed at least twice. Observations on the statoliths of the hypocotyl of mustard were made on freshly cut sections stained in aqueous iodine.

Elimination of geotropism by compound (I) was demonstrated by placing sixteen lettuce seeds at appropriate compass points on vertically positioned Petri dishes, with radicles facing outwards. The paper was loaded with (I) to a final concentration equivalent to 10^{-4} M. Fig. 1 shows these plates after 72 h at 20° C. Clearly (I) affects seedlings so that the direction of growth of the axis is determined by the original orientation. Fig. 1 also shows that the plumular hook does not form in treated material, and is only manifest in control material when the axis is off centre or inverted—note the appearance of the lowermost seedling in the control dish.

A better measure of the response of the seedlings to (I) was obtained by placing seeds horizontally and measuring the angle between the radicle or hypocotyl and the horizontal axis. Above horizontal attitudes are prefixed by a negative sign to agree with conventional nomenclature of orthogeotropic behaviour. Table 1 lists one set of results.

To compare growth by radicles and hypocotyls of treated seed, seeds were distributed on seed test paper as described, the paper being loaded with appropriate mixtures.

Table 2 shows that the growth of radicles of treated seeds was reduced by about 20% while the growth of hypocotyls was reduced by about 40%. As IAA is involved in tropic responses and gibberellic acid (GA) in extension growth, the effect of coapplication of each of these with (I) was examined. Table 2 shows that 10^{-4} M GA overcomes the slight inhibition of growth caused by (I) without any effect on the elimination of the geotrophic response. IAA severely decreases the extension of the radicle, as expected, but there is evidence that at 10^{-5} M it can re-establish normal geotrophic behaviour at the expense of growth in some radicles.

Phototrophic behaviour is affected less by (I) (Table 3). The slower response of the treated material may be a result of the reduction in extension growth that (I) causes, and it is possible that the compound was used in a concentration less than that which would have eliminated phototrophism. The results of Jones *et al.*[1], while not strictly quantitative, indicate that these compounds affect phototropism less than geotropism.

A possible point of action of (I) on the geotropic response is the statolith, which many people think is a central feature in the perception of gravity[2,3]. If statolith starch were lost,

Table 2 Growth, Geotropic Response and Development of Plumular Hook in the Young Axis of Lettuce Seedlings Treated with (I), GA, and IAA Singly and in Combination

	Radicle			Hypocotyl			
	Geotropic response		Length	Geotropic response		Length	Plumular
	+ve	none	(mm)	−ve	none	(mm)	hook
Control	30	0	20.10 ± 0.916	30	0	5.00 ± 0.352	+
10^{-4} M (I)	0	31	16.13 ± 0.897	0	31	2.87 ± 0.225	−
10^{-4} M (I)+ 10^{-5} M GA	0	32	16.03 ± 0.823	0	32	4.09 ± 0.341	−
10^{-4} M (I)+ 10^{-4} M GA	0	31	20.19 ± 0.500	0	31	4.97 ± 0.359	−
10^{-4} M (I)+ 10^{-3} M GA	0	32	18.09 ± 0.792	0	32	4.53 ± 0.327	−
10^{-4} M (I)+ 10^{-7} M IAA	0	32	9.41 ± 0.609	0	32	2.13 ± 0.257	−
10^{-4} M (I)+ 10^{-6} M IAA	2	30	5.91 ± 0.500	†	†	1.66 ± 0.122	−
10^{-4} M (I)+ 10^{-5} M IAA	15	17	2.37 ± 0.396	†	†	†	†

† Not measurable.

Table 3 Phototropic Curvature of Lettuce Seedling Hypocotyls grown in the Presence of a Saturated Solution of (I)

Length of exposure to light (h)	Degrees curvature from vertical Treated	Control
7.5	5.8 ± 2.46	29.3 ± 1.53
24.0	41.1 ± 7.27	51.5 ± 2.85

then the axis could not perceive the stimulus[7], or if the starch grains could not move under gravity than it might be expected that there would be no curvature when the axis is placed off vertical. Fig. 2 shows that the starch grains in the endodermis of mustard hypocotyl, often considered to be statoliths, are present in treated material, and move in response to gravity. They can be seen congregated along the basal wall of the cell in vertically grown hypocotyls, but are less distinctly oriented in seedlings which were growing horizontally.

According to the Cholodny–Went hypothesis, curvatures result from the misorientation of an organ in the gravitational field, or from unilateral illumination, because the unequal distribution of auxin across the width of the organ gives unequal bilateral growth in the region of cell elongation[8,9]. At least for lettuce seedlings (I) can prevent curvature in a gravitational field but not in the case of unidirectional illumination. If the Cholodny–Went hypothesis is accepted, and the phototropic curvatures of seedlings treated with (I) result from unequal bilateral distribution of auxin, then it might be argued that the establishment of this bilateral asymmetry in auxin concentration is *per se* not affected by (I). The effect of (I) on geotropic sensitivity of seedlings must be sought elsewhere.

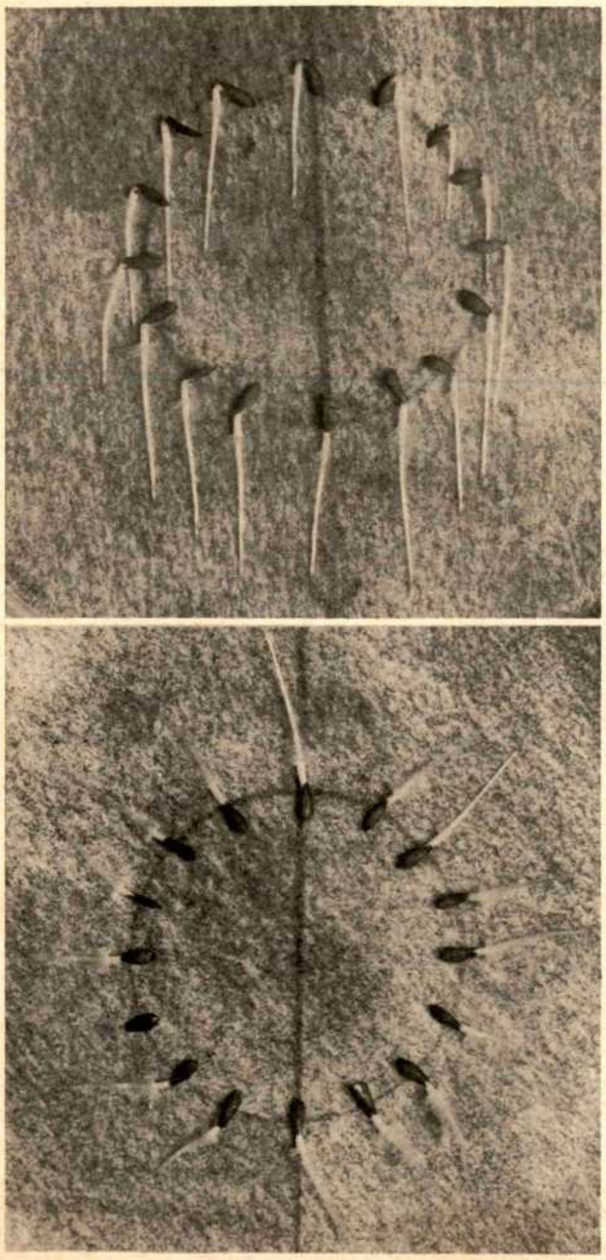


Fig. 1 Seedlings from lettuce seed in which embryonic axis is positioned with respect to given compass points. Upper—control. Lower—treated with 3-4′ chlorophenyl 3-methoxy phthalide.

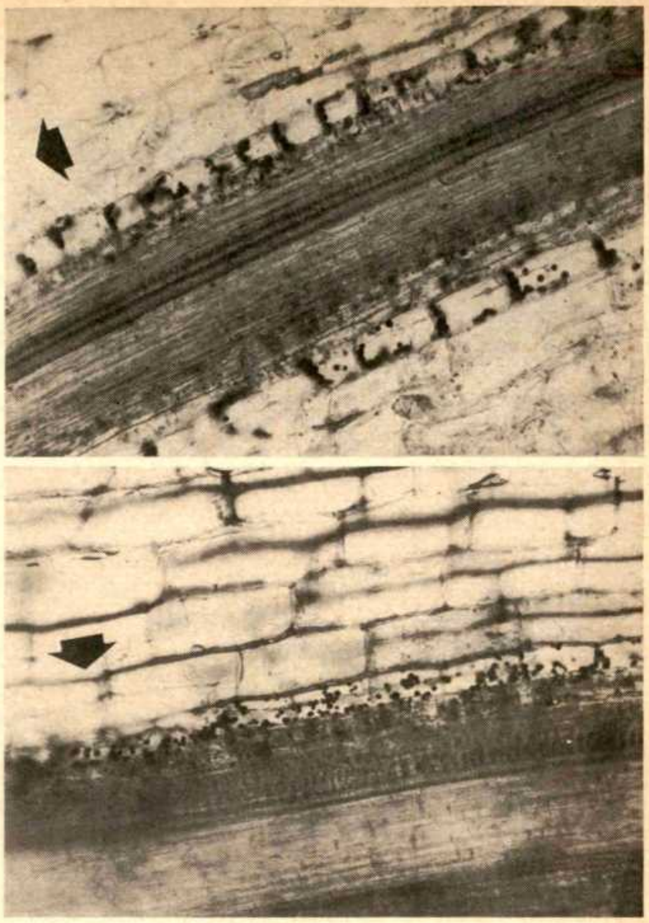


Fig. 2 Statolith orientation in hypocotyl endodermis of mustard. Arrows indicate direction of gravity.

Audus suggests that geotropism is a catenary process with several interconnected links, each requiring to be completed before the phenomenon can be expressed properly[2]. The first link relates to the site of perception, and if this is the statolith then (I), which has no observable effect on the starch grains of the endodermal cell of the hypocotyl of mustard, is unlikely to act directly on the statolith. I do not know which link in Audus's chain of events is affected by (I), but this compound clearly provides a useful tool for the study of this phenomenon.

The reversal by GA of the slight growth inhibition caused by (I) in the roots and hypocotyls suggests that (I) competes with endogenous GA for the site of action of this hormone. This is in agreement with the idea that candidate antigibberellins should occur among unsaturated lactones[10], which include coumarin[11], ramulosin[12] and patulin[13]. It is well known that these and other unsaturated lactones are potent physiologically active compounds in many classes or organism[14]. So far, however, such lactones seem to be restricted in their antigibberellin activity, being most noticeably active in the lettuce seed germination assay. Inhibition of GA-induced elongation of organs is less common. Perhaps the inhibition of elongation by (I) is sufficient to mask the growth differential across the elongating organ in geotropism but not in phototropism, though the less rapid attainment of the new position in unilaterally illuminated hypocotyls is possibly a reflexion of the reduced rate of growth, and if the inhibition were greater then phototropic curvature might not occur. Even in untreated material phototropic curvature supervenes on geotropic response of hypocotyls suggesting that light is much more potent in establishing unequal distribution of auxin than gravity, and it is possible that (I) is unable to maintain equality of auxin concentration in a unilateral light field.

The material used in this investigation was a gift from Dr A. H. Berrie, ICI Dyestuffs Division, Manchester.

A. BERRIE

Department of Botany,
University of Glasgow

Received October 12; revised November 17, 1970.

[1] Jones, R. L., Metcalfe, T. P., and Sexton, W. A., *J. Sci. Food and Agric.*, **5**, 32 (1954).
[2] Audus, L. J., in *Physiology of Plant Growth and Development*, 205 (edit. by Wilkins, M. B., Publisher, 1969).
[3] Wilkins, M. B., *Arm. Rev. Plant. Physiol.*, **17**, 379 (1966).
[4] Khan, A. A., *Physiol. Plant.*, **20**, 306 (1967).
[5] Mentzer, C., Molho, D., and Pacheco, H., *Bull. Soc. Chim. Biol.*, **32**, 572 (1950).
[6] Vaníček, V., *Ann. Acad. Tchecoasl. Agric.*, **22**, 337 (1950).
[7] Iversen, T.-H., *Physiol. Plant.*, **22**, 1251 (1969).
[8] Cholodny, N., *Ber. Deutch. Bot. Ges.*, **42**, 356 (1924).
[9] Went, F. W., *Proc. Koninkl. Neo. Akad. Wetenschap. Amsterdam*, **34**, No. 8, 1 (1925).
[10] Berrie, A. M. M., Parker, W., Knights, B. A., and Hendrie, M. R., *Phytochemistry*, **7**, 567 (1968).
[11] Nutile, G. E., *Plant Physiol.*, **20**, 433 (1945).
[12] Mayer, A. M., *Israel. J. Bot.*, **13**, 41 (1964).
[13] Berrie, A. M. M., Hendrie, M. R., Parker, W., and Knights, B. A., *Plant Physiol.*, **42**, 889 (1967).
[14] Veldstra, H., and Havinga, E., *Enzymologia*, **11**, 373 (1943/45).

Mitotic Cycles in Dicotyledons and Monocotyledons

THE duration of the mitotic cycle in comparable tissues of different plant species is closely correlated with the amount of nuclear DNA[1,2]. In a survey of seven species Van't Hof[2] showed that the average duration of the mitotic cycle in root tip meristems increases at the rate of 0.3 h/pg of DNA. Our own data agree in general with these results, but we have found certain exceptions. For example, where the increase in DNA is brought about by the addition of B chromosomes the duration of mitotis is increased to a disproportionate extent[3]. We have also found an unexpected difference in the relation between nuclear DNA and the duration of mitosis in monocotyledons as compared with dicotyledons.

Table 1 and Fig. 1 show, as expected, the increasing duration of the mitotic cycles with increasing DNA. They also show that the rate of increase is of the same order in both monocotyledons and dicotyledons. There is in one respect, however, a marked difference between monocotyledons and dicotyledons. For the range of DNA values covered by this survey the complete mitotic cycle is, on average, almost 4 h longer in dicotyledons.

It is pertinent to inquire whether this consistently large difference between the two classes of angiosperms is attributable to any particular phase of mitosis or whether all phases are equally affected. From Table 1 and Fig. 1 it is evident that there is no difference in the duration of the synthesis (S) phase. Neither were there significant differences in the G2 and division phases. From Table 1 it is clear that the difference between monocotyledons and dicotyledons is accounted for largely by the variation in G1, that part of interphase preceding synthesis.

There is evidence that the extension of G1 in dicotyledons may be associated with the degree of chromosome coiling during division. Table 1 shows that the DNA density of chromosomes at metaphase of mitosis in the dicotyledons is almost twice that in monocotyledons, the chromosomes being more highly coiled. It therefore seems reasonable to suggest that in dicotyledons the uncoiling of chromosomes before synthesis would require a longer time and a longer G1 than in monocotyledons. The difference in chromosome organization which distinguishes the two groups may, in turn, stem from one of two genetic causes. The first is a difference in the basic archi-

Table 1 Amount of DNA per Nucleus, Duration of Mitotic Cycles, and G1 and the DNA per Unit Volume of Metaphase Chromosomes in Root Tip Meristems of Diploid Dicotyledons and Monocotyledons

	DNA (2C value) [$\times 10^{-12}$ g]	Mitotic cycle (h)	Synthesis (h)	G1 (h)	DNA per unit volume of chromosomes [pg/μm^3] at metaphase
Dicotyledons					
Lycopersicum esculentum	3.9	10.6[2]	4.3[2]		
Crepis capillaris	4.2	10.8[2]	3.25[2]		
Linum usitatissimum	1.4	11.2	4.1		
Lathyrus angulatus	8.9	12.25	3.9	4.4	0.400
Lathyrus articulatus	12.4	14.25	4.3	3.9	0.508
Lathyrus tingitanus	18.1	16.75	5.3	6.3	0.574
Lathyrus hirsutus	20.1	18.0	5.0	7.8	0.454
Nigella damascena	21.1	16.5	10.5	1.5	
Vica faba	23.9	19.3[5]	7.3[5]	4.9[5]	0.430
					Mean 0.473
Monocotyledons					
Lolium perenne	9.9	8.6	4.2	0.5	0.352
Zea mays	11.0	10.5	4.25	0.5	0.364
Secale cereale	18.9	11.7	6.0	1.0	0.178
Allium fistulosum	26.3	15.1	7.1	<0.5	0.194
Allium cepa	33.5	17.8	9.6	<0.5	0.222
A. cepa × *A. fistulosum*	29.9	16.6	8.6		
Tradescantia paludosa	38.8	20.0[4]	10.8[4]		
Hyacinthus orientalis	49.7	24.0	13.6	0.8	0.234
					Mean 0.257

2C DNA values were obtained by Feulgen photometry. Conversion from arbitrary to absolute units was achieved using Van't Hof's 2C value for *Allium cepa*—33.5×10^{-12} g. The duration of mitotic cycles and of synthesis were estimated by the pulse labelling technique of Quastler and Sherman[6].

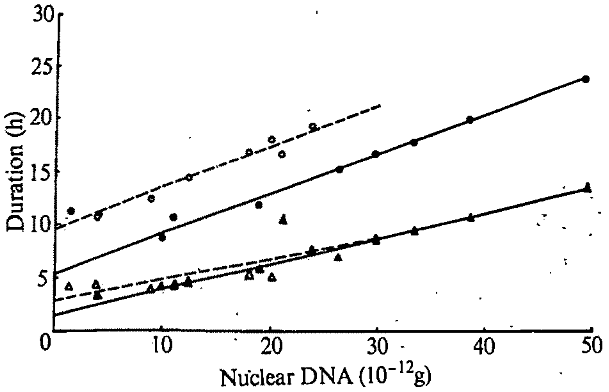


Fig. 1 The duration of the complete mitotic cycle and of the synthesis phase in root tip meristems plotted against the 2C nuclear DNA amount in diploid monocotyledons (———) and dicotyledons (- - -). ●, ○, Complete cycle; ▲, △, S.

tecture of the DNA–protein complex. The second is a genotypic control imposed by the specific activity of one or more genes which regulate the extent of chromosome coiling. There is no means at present of distinguishing between the two possibilities.

We thank S. Sun, U. A. U. Ayonoadu, P. Williams and C. L. Snell for data on mitotic cycles.

G. M. Evans
H. Rees

Department of Agricultural Botany,
University College of Wales,
Institute of Rural Science,
Penglais,
Aberystwyth

Received March 26; revised May 17, 1971.

[1] Van't Hof, J., and Sparrow, A. H., *Proc. US Nat. Acad. Sci.*, **49**, 897 (1963).
[2] Van't Hof, J., *Exp. Cell. Res.*, **39**, 48 (1965).
[3] Ayonoadu, U. A. U., and Rees, H., *Exp. Cell. Res.*, **52**, 285 (1968).
[4] Wimber, D. E., *Amer. J. of Bot.*, **47**, 828 (1960).
[5] Evans, H. J., and Scott, D., *Genetics*, **49**, 17 (1964).
[6] Quastler, H., and Sherman, F. G., *Exp. Cell. Res.*, **17**, 420 (1959).

Importance of Synchrony of Ear Emergence in Plant Breeding Programmes

In plant breeding, there is a general trend to apply the term "synchrony" to intra-plant variance or inter-tiller variance of a character like ear emergence. Thus, plants with the least intra-plant variance are considered more synchronous. The importance of synchrony of tillering in breeding programmes and the procedure for studying it is not yet well defined. To investigate this topic further I have studied six genetically distinct varieties of spring barley which have been bred in the past with little or no evidence of conscious selection for synchrony.

Table 1 Correlation Coefficients between Grain Yield per Plant and the Intra-plant Variance (Synchrony) of the Four Component Characters Studied

Variety	Correlation coefficients between grain yield per plant and Synchrony of ear emergence	Synchrony of height	Synchrony of the number of grains per ear	Synchrony of weight of grains per ear
'Archer'	−0.14	−0.39	−0.59	−0.71 *
'Impala'	0.79 †	0.17	0.51	0.60
'Proctor'	0.21	−0.07	−0.46	0.43
'Rika'	−0.49	−0.52	−0.69 *	−0.21
'Spratt'	0.46	−0.24	−0.20	0.21
'Sultan'	−0.18	−0.17	−0.22	−0.10

* Significant at the 5% level.
† Significant at the 1% level.

A randomized block design comprising five replications was used for laying out the field experiment in 1969. Observations on grain yield per plant, days to ear emergence, plant height, the number of grains per ear and weight of grains per ear were recorded for each individual tiller of a plant. Individual plants were spaced 5 cm apart in normal field conditions. Two approaches were used to study synchrony, one using intra-plant variance and the other using regression coefficients of the mean value of a particular tiller on its own tiller number. The tiller numbers were given in the order of their ear emergence. In the absence of a more appropriate term, synchrony has been used to describe characters other than ear emergence to reflect intra-plant variance, the higher synchrony denoting least intra-plant variance.

The correlation coefficients between grain yield per plant and the intra-plant variance used as synchrony of the four characters studied are given in Table 1 for each variety. If the synchrony of ear emergence was negatively correlated with grain yield (as would be desirable if the genotypes were higher yielding and had least intra-plant variance for ear emergence), then the association was also negative between the synchrony of other characters and grain yield per plant. Of the six varieties studied only 'Rika', 'Sultan' and 'Archer' have shown this trend, and the intra-plant variance for all the characters is also low. Moreover, the synchrony of all the characters in three of the six varieties seems to be positively associated with the synchrony of ear emergence.

The regression slopes obtained by regressing the mean value of a particular tiller on its own tiller number can be used to judge the synchrony of a variety for the character in question. The idea is that the subsequent tillers of a synchronous variety should show phenotypic expression of a particular character which is similar, if not identical, to that of the first few tillers. Thus the genotype showing the least or no regression slope is

Table 2 Regression of the Mean Value of each Individual Tiller on its Number for Different Characters estimated for Six Varieties of Spring Barley

Variety	Ear emergence (days) $b \pm S_b$	Height (cm) $b \pm S_b$	No. of grains/ear $b \pm S_b$	Weight of grains/ear $b \pm S_b$
'Archer'	1.301 ± 0.111	−1.769 ± 0.345	−0.654 ± 0.144	−0.068 ± 0.012
'Impala'	1.461 ± 0.025	−2.190 ± 0.128	−1.100 ± 0.045	−0.069 ± 0.004
'Proctor'	1.510 ± 0.043	−1.883 ± 0.209	−0.912 ± 0.097	−0.066 ± 0.005
'Rika'	0.698 ± 0.061	−0.717 ± 0.144	−0.388 ± 0.065	−0.043 ± 0.004
'Spratt'	1.429 ± 0.057	−3.425 ± 0.318	−1.106 ± 0.145	−0.099 ± 0.011
'Sultan'	1.095 ± 0.085	−1.752 ± 0.120	−0.795 ± 0.076	−0.061 ± 0.004

b, Regression coefficient; S_b, standard error of b.

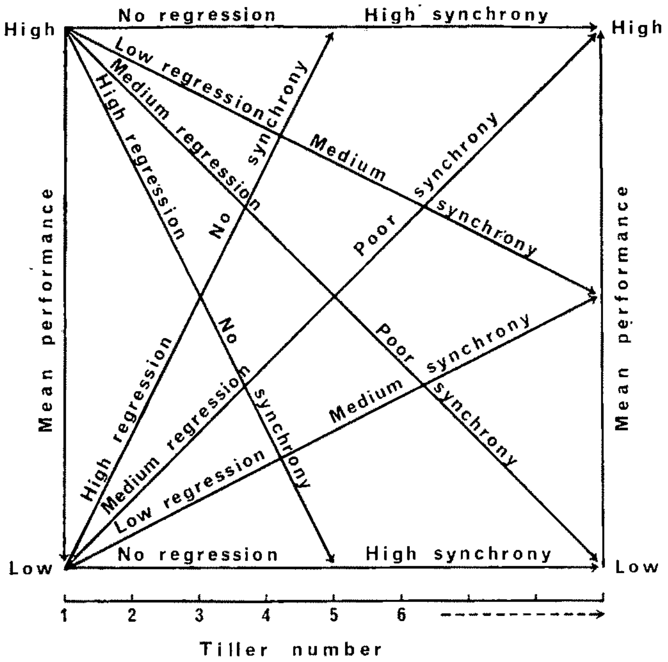


Fig. 1 A generalized scheme for studying synchrony.

the most synchronous. Accordingly, a generalized scheme for studying synchrony is presented in Fig. 1. A two-way approach is presented, where characters like ear emergence and height are recorded in ascending and descending order, respectively. Thus, the sign of the regression slope is not relevant, and it is the magnitude of the slope which determines synchrony. As Table 2 shows, 'Rika', 'Sultan' and 'Archer' expressed least regression slopes for the four characters studied. 'Rika' had the lowest regression slopes and was the most synchronous, whereas 'Sultan' and 'Archer' expressed medium synchrony. 'Spratt' was the most non-synchronous variety.

'Rika', 'Sultan' and 'Archer', which showed synchrony of ear emergence and other characters, might well be exploited. It would be desirable, however, to confirm the trend in a wide range of environments and the genetic basis of synchrony needs careful investigation. Of the two approaches used for studying synchrony, that involving regression coefficients is simpler and more informative and could be used in the future.

Reports of the effectiveness of the component approach to selective breeding are becoming available for cereal crops such as wheat[1-4] and barley[5,6]. These studies have provided considerable evidence in favour of component breeding and it has been established that selection on the basis of grain weight without ignoring grain yield would lead to improvements. I have now found that synchrony of tillering, which can be judged at the stage of ear emergence or even earlier in plant development, could also be used as a selection criterion for improving yield in plant breeding programmes.

I thank Professor H. Rees for support and for reading this manuscript. I also acknowledge financial assistance from the Commonwealth Scholarship Commission in the United Kingdom.

R. S. Paroda*

Department of Agricultural Botany,
University College of Wales,
Aberystwyth

Received November 2, 1970; revised March 15, 1971.

*Present address: Department of Plant Breeding, Haryana Agricultural University, Hissar, New Delhi 22, India.

[1] Borojevic, S., and Cupina, T., *Proc. Third Intern. Wheat Genet. Symp.*, Canberra, 388 (1968).
[2] Borojevic, S., and Cupina, T., *Contemporary Agriculture-Novi Sad*, **17**, 5 (1969).
[3] Paroda, R. S., and Joshi, A. B., *Heredity*, **25**, 383 (1970).
[4] Paroda, R. S., and Joshi, A. B., *Indian J. Genet.*, **30**, 298 (1970).
[5] Rasmusson, D. C., and Cannell, R. Q., *Crop Sci.*, **10**, 51 (1970).
[6] Borthakur, D. N., and Poehlman, J. M., *Crop. Sci.*, **10**, 452 (1970).

Evaluation of Bicuculline as a GABA Antagonist

THERE has been controversy recently about the reliability of bicuculline as an antagonist of the putative inhibitory transmitter γ-aminobutyric acid (GABA) in the brain[1-3]. Although the effects of bicuculline have so far been explained in terms of its combination with the GABA receptor, bicuculline might also affect the removal processes for GABA and the synthesis and release of endogenous GABA. We have therefore made quantitative analyses of the effects of iontophoretically applied bicuculline on the responses of neurones to GABA and have also estimated the effects of bicuculline on the concentration and turnover of GABA in the brain, GABA uptake and the synthetic and degradative enzymes glutamate decarboxylase (GAD) and 4-aminobutyrate : 2-oxoglutarate aminotransferase (GABA-T).

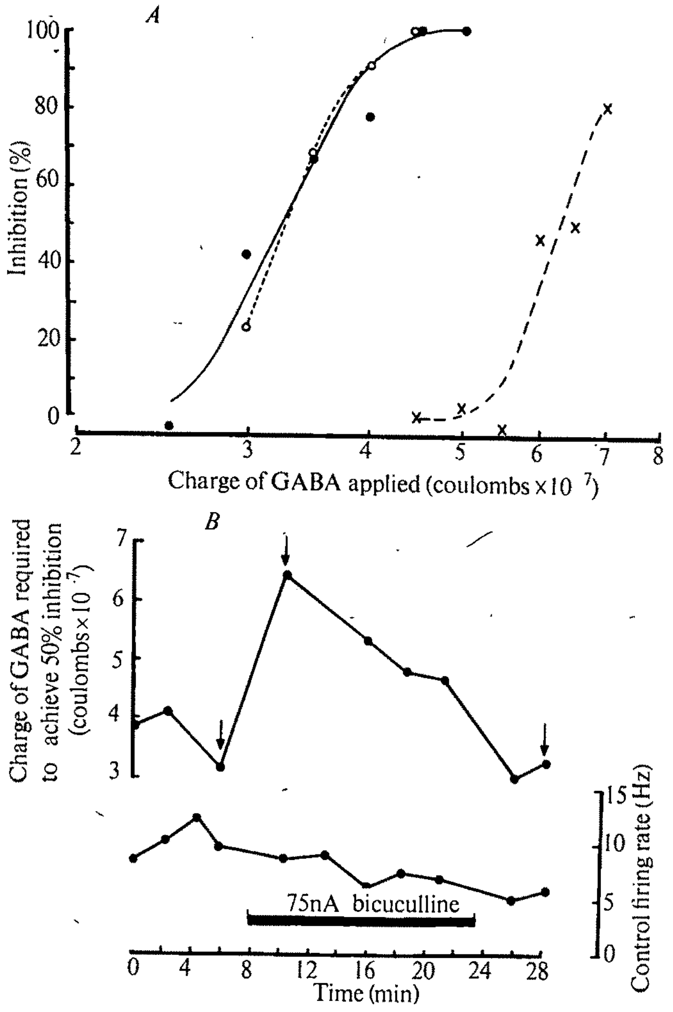


Fig. 1 *A*, Dose-response curves of percentage inhibition of cell firing against coulombs of GABA applied at a constant current of 10 nA. The cell was 1.33 mm deep in the suprasylvian cortex of a cat and was driven by continuous application of 75 nA glutamate. ●, Control response; ×, response during continuous application of 75 nA bicuculline; ○, recovery response. *B*, Plot of coulombs of GABA required to achieve 50% inhibition of the firing rate of the same cell as above during repeated 10 nA applications of GABA before, during and after a 75 nA application of bicuculline. The arrows indicate the values taken from the curves in *A*. Bicuculline clearly antagonized the action of GABA but had no effect on the control firing rate of the cell.

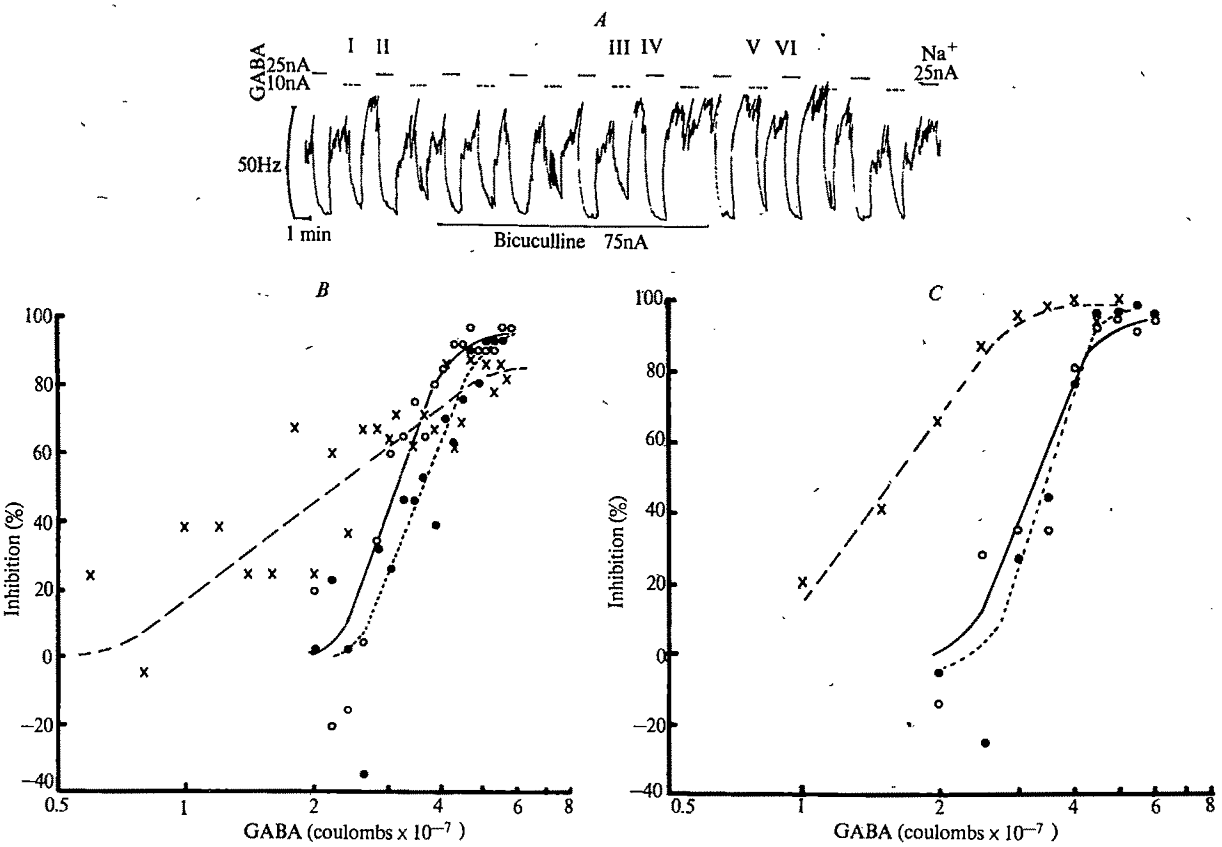


Fig. 2 *A*, Analogue rate-meter record of the firing of a cell driven by continuous application of 25 nA glutamate at a depth of 1.82 mm in the suprasylvian cortex of the cat. GABA 10 nA and 25 nA was applied as indicated by the bars above the trace. Bicuculline 75 nA was applied continuously during the period indicated by the bar below the trace. Responses labelled I–VI are plotted in the form of dose-response curves in *B* and *C*. *B*, Responses I (—○—), III (- -×- -) and V (. . .●. . .) plotted as percentage inhibition against coulombs of GABA applied at a constant current of 10 nA. Responses were calculated from the interval-time spike counter record (not shown) which was obtained simultaneously with the rate-meter trace shown above. *C*, Responses II (—○—), IV (- -×- -) and VI (. . .●. . .) to a constant current application of 25 nA GABA plotted as in *B* above.

In iontophoretic experiments, sixty cortical cells have been studied in twelve cats anaesthetized with 50% nitrous oxide in oxygen and 1% halothane. Drugs were applied and extracellular spike potentials recorded through standard five and seven-barrelled glass micropipettes with tip diameters 5–7 μm as in previous studies[4]. The extracellular spike potentials were counted with an interval-time spike counter and also displayed on an analogue rate meter. Cells which did not fire spontaneously were driven by continuous application of glutamate or DL-homocysteate. The inhibitory effects of GABA were analysed by constructing dose-response curves of percentage inhibition of cell firing against the coulombs of GABA passed.

We have confirmed the experience of Curtis *et al.*[1] that bicuculline can reversibly antagonize the depressant effect of GABA on cortical neurones (for example, Fig. 1). This was not a consistent finding, however, for, in common with Godfraind *et al.*[2] we sometimes found that the response to GABA was potentiated in the presence of bicuculline. For example, in the experiment shown in Fig. 2, we applied two currents of GABA alternately. Inspection of the analogue rate-meter trace (Fig. 2*a*) shows that the response to 10 nA GABA was eventually blocked after about 15 min continuous application of bicuculline, whereas the response to 25 nA GABA was not blocked. Closer inspection of the trace, however, reveals that the onset of the response to both currents of GABA was accelerated throughout the application of bicuculline. This potentiation is more clearly shown in the dose-response curves plotted for certain of the GABA applications in Figs. 2*b* and *c*. The effects of bicuculline were completely reversible and indicate that it can both potentiate and antagonize GABA on the same cell, depending on the relative concentrations of the two substances.

In ten cells (five spontaneous, five driven), the responses to

Table 1 Effect of Bicuculline on the Rate of Disappearance of ^{3}H-GABA from the Cerebellum, Cerebral Cortex and Whole Brain of the Rat

Area	Route	Dose	$t_{½}$ h between 2–8 h (95% confidence limits) Control	Bicuculline	P value
Cerebellum	i.c.	12.5 μg	1.25 (1.00–1.50)	2.95 (2.49–3.41)	<0.05
Cerebral cortex	i.c.	12.5 μg	3.21 (2.75–3.71)	8.40 (7.62–8.98)	<0.02
Whole brain	i.p.	2.5 mg/kg	2.88 (2.57–3.19)	4.30 (4.09–4.59)	<0.05
	i.c.	12.5 μg		3.68 (3.36–4.00)	<0.02

^{3}H-GABA (10 μCi) was injected intracisternally (i.c.) immediately after the injection of bicuculline. The animals were killed at times between 2 and 8 h after injection and the ^{3}H-GABA remaining in the brain was measured. From the regression of log. ^{3}H-GABA concentration on time, $t_{½}$ was calculated by the formula: $t_{½}$=(log 2)/slope. In whole brain of rats injected with bicuculline 2.5 mg/kg intraperitoneally (i.p.) 2 h after injection, the total ^{3}H-GABA content was 78,260 ± 6,382 d.p.m. (mean ± s.e.) which was not significantly different from control rats at 2 h, 62,320 ± 5,820 d.p.m. Controls were injected with an appropriate volume of solvent.

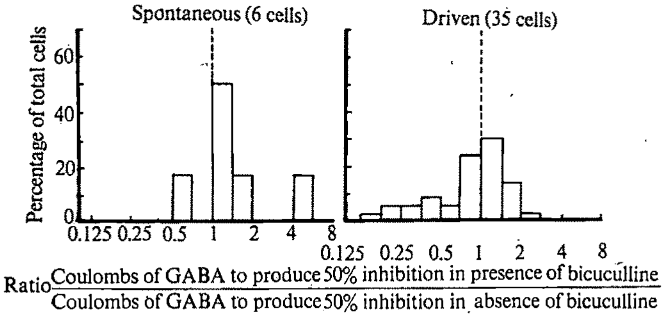


Fig. 3 Frequency distribution of the effect of bicuculline on the responses of cat cerebral cortical cells to GABA. A quantitative estimate of the effect of bicuculline was obtained from the ratio of the coulombs of GABA required to give 50% inhibition of the firing rate of a cell in the presence and absence of bicuculline. A ratio of more than 1 indicates antagonism of GABA and a ratio of less than 1 indicates potentiation.

GABA were sufficiently antagonized by bicuculline such that the inhibition due to the selected current of GABA failed to reach more than 80% in the presence of bicuculline. In a further forty-one cells, more quantitative estimates of the effects of bicuculline were made. In these cells, lateral shifts in the dose-response curves could be measured since the applied current of GABA was sufficient eventually to cause complete inhibition of firing in the presence of bicuculline. An analysis of the shifts in the dose-response curves (Fig. 3) reveals that bicuculline had a greater tendency to antagonize the effects of GABA than to potentiate them in cells which were spontaneously active. In cells driven by glutamate or DL-homocysteate, there seemed to be an approximately equal occurrence of potentiation and antagonism of the action of GABA in the presence of bicuculline.

The effects of bicuculline on the enzymes responsible for the synthesis (GAD) and degradation (GABA-T) of GABA were studied in rats, using the assay techniques of Roberts and Simonsen[5] and Hall and Kravitz[6], respectively. There was no significant effect of bicuculline on the activity of glutamic acid decarboxylase (GAD) in brain homogenates exposed to 1 mM bicuculline *in vitro* and no effect was seen in brain homogenates prepared from rats which had been previously injected with bicuculline, 2.5 mg/kg intraperitoneally or 12.5 µg intracisternally. There was a small inhibitory effect of bicuculline on the activity of GABA-T in brain homogenates exposed to bicuculline (1 mM) *in vitro*, the activity of the enzyme being 82% ($P<0.02$) of the control value. It was not possible, however, to show any inhibition of GABA-T in brain homogenates prepared from rats which had been previously injected with subconvulsive doses of bicuculline (2.5 mg/kg intraperitoneally or 12.5 µg intracisternally) nor did these doses of bicuculline have any significant effect on the endogenous GABA concentration in the brain, as measured by the method of Jakoby and Scott[7].

Since brain tissue has a powerful uptake mechanism for GABA[8], the action of GABA released from nerve terminals or applied iontophoretically onto neurones may be terminated by re-uptake into nervous tissue. Bicuculline appears to have no effect on this mechanism since the uptake of ^{3}H-GABA by cortical slices from rat brain exposed to bicuculline (0.14 mM) was not significantly different from that in controls.

To obtain some idea of the effect of bicuculline on GABA turnover, a tracer dose of ^{3}H-GABA was injected intracisternally into the rat and the rate of disappearance of ^{3}H-GABA from the brain was followed[9]. Subconvulsant doses of bicuculline significantly increased the half-life of ^{3}H-GABA in the brain between 2 and 8 h after injection (Table 1). The total amount of ^{3}H-GABA in the brains of the bicuculline treated animals 2 h after injection was not significantly different from controls, suggesting that the half-life of ^{3}H-GABA during the first 2 h was not altered. The interpretation of these results is difficult at present, as the functional significance of the two phases of ^{3}H-GABA disappearance from the brain is not yet understood. The increased half-life of ^{3}H-GABA between 2 and 8 h is unlikely to be due to inhibition of GABA-T since this effect was not seen in animals treated with AOAA (Collins and Neal, unpublished results). It is possible that bicuculline affects the neurally evoked release of GABA from nervous tissue *in vivo*, although the spontaneous efflux of ^{3}H-GABA from slices of rat cortex is unaffected by incubation with bicuculline (1 mM) for periods up to 1 h.

We have shown that iontophoretically applied bicuculline has more than one effect on the responses of cortical neurones to GABA. The potentiating effect of bicuculline is unlikely to be due to its weak inhibitory action on GABA-T or to inhibition of uptake. Other workers[10], however, have found that bicuculline can potentiate the effect of stimulating inhibitory pathways and have explained this as antagonism of an inhibitory influence on the inhibitory pathway being stimulated. A similar explanation for the potentiation of iontophoretically applied GABA does not seem to be feasible and the possibility remains therefore that this could be due to a direct non-specific effect of bicuculline on the post-synaptic membrane.

The antagonistic effects of bicuculline on the responses to GABA are probably due to a post-synaptic action, but a pre-synaptic component cannot yet be completely excluded in view of our results on GABA turnover. The potency of bicuculline as an antagonist is low, less than a doubling of the amount of GABA applied being required to overcome the antagonism. This should be compared with a ratio of 3.4 in the case of glycine and strychnine[11] and a very much larger ratio still in the case of acetylcholine and atropine (Clarke, Davies and Straughan, unpublished results). Thus, it appears that locally applied bicuculline is not a potent or reliable enough antagonist of GABA in the cerebral cortex to be used for confident identification of GABA-operated synapses or evaluation of the physiological role of GABA in that part of the brain.

This work was supported by the Medical Research Council through a grant to D. W. S. A grant for equipment from the Wellcome Foundation is acknowledged as is a grant for equipment from the SKF Foundation to M. J. N. R. G. H. is a Wellcome research student.

D. W. Straughan
M. J. Neal
M. A. Simmonds
G. G. S. Collins
R. G. Hill

Department of Pharmacology,
School of Pharmacy,
University of London,
29/39 Brunswick Square,
London, WC1N 1AX

Received May 21; revised June 29, 1970.

[1] Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222 (1970).
[2] Godfraind, J. M., Krnjević, K., and Pumain, R., *Nature*, **228**, 675 (1970).
[3] Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **228**, 675 (1970).
[4] Johnson, E. S., Roberts, M. H. T., and Straughan, D. W., *J. Physiol.*, **203**, 261 (1969).
[5] Roberts, E., and Simonsen, D. G., *Biochem. Pharmacol.*, **12**, 113 (1963).
[6] Hall, Z. W., and Kravitz, E. A., *J. Neurochem.*, **14**, 45 (1967).
[7] Jakoby, W. B., and Scott, E. M., *J. Biol. Chem.*, **234**, 937 (1959).
[8] Iversen, L. L., and Neal, M. J., *J. Neurochem.*, **15**, 1141 (1968).
[9] Collins, G. G. S., and Neal, M. J., *Brit. J. Pharmacol.*, **41**, 396 (1971).
[10] Duggan, A. W., and McLennan, H., *Brain. Res.*, **25**, 188 (1971); Felix, D., and McLennan, H., *Brain Res.*, **25**, 661 (1971).
[11] Johnson, E. S., Roberts, M. H. T., and Straughan, D. W., *Brit. J. Pharmacol.*, **38**, 659 (1970).

BOOK REVIEWS

Berzelius: His Life

Jacc Berzelius: His Life and Work. By Erik J. Jorpes. Translated from the Swedish by Barbara Steele. Pp. 156. (University of California: Berkeley and Los Angeles, 1970.) n.p.

THIS is an unimaginative, but useful, book which will be of value principally as a summary of what is already familiar to students of the chemistry of the first half of the nineteenth century, but is not readily available in a single small volume. The author describes Berzelius's life as a succession of exercises, with little attempt to put any particular piece of work into a historical context, so that one is left with a feeling of: "So that is how it looked to Berzelius day by day, but what was his life really all about?"

The story of any man's life is always a story about something else as well, and Berzelius's life was really all about a great revolutionary movement in human thought (roughly contemporary with the French Revolution and its aftermath, but more far reaching in its effects on mankind as a whole). This was the great advance in human thought which brought quantitative considerations into man's knowledge and control of the material substances of his environment. But Dr Jorpes is no philosopher of chemistry. (Indeed, do we have one today? How useful for us all if there were.) He prefers to present lists of Berzelius's work in each of his several practical fields. His theoretical work and the important controversies it aroused get short treatment and even that without much comment, so we get very little justification for Berzelius's reputation in the eyes either of his contemporaries or of his modern critics.

Perhaps Dr Jorpes, being a countryman of Berzelius, sees him close to, as a fellow medical man, as a fellow member of Swedish institutions, and so finds no need to draw a heroic portrait. Here we see the main value of this book. Most studies of Berzelius in languages other than Swedish have necessarily concentrated on his great influence, and, seeing this to be very great, have given us small flat portraits of a genius. Dr Jorpes gives a larger, rounder picture of a plain, likable, modest, determined, sensible man, sometimes witty, sometimes preoccupied, sometimes sick and sad, magnanimous in his private life even if he could be, on uncharacteristic occasions, grudging and resentful in his public scientific life. In his middle age he was man enough to attract as his wife a much younger woman above his station, and weak enough to be swayed by her to dismiss the phenomenal Anna, cook, housekeeper and laboratory assistant.

Dr Jorpes hints here and there at aspects of Berzelius's life which would be worth extended study. He was very conscious of the problems of communication. He knew the linguistic obstacles for even a widely travelled Swede, yet he was aware that this was far less of an obstacle to scientific development than the insularity of attitude of which he accused English chemists, not excluding Davy.

In Berzelius's works we can see the dual role of mineralogy both pulling chemistry along the road of quantitative systematics, and yet hampering its progress towards a grasp of the importance of internal structure. Someone might investigate this. And we have not yet discerned all the differences between what electricity meant to chemists and to physicists in Berzelius's time.

To some extent he was the man of a book, spending a great deal of time that might have been spent in the laboratory in writing and revising a compendious textbook. Although much translated and widely used, it seems to have had far less influence than his novel yearbooks, which set the pattern for those progress reports which kept scientific controversy active and wide ranging when communication was slow.

His epitaph could be written in a few technical terms like isomerism and catalysis, but any chemical formula would serve just as well, for it was he who devised the literal notation which gave chemistry H_2O and $CaCO_3$ and all the intelligibility such formulae imply.

As a supplement there is reprinted (in German) the summary of Berzelius's analytical work during 1808–21, which H. G. Söderbaum originally gave in his Kahlbaum Monograph, *Jac. Berzelius' Werden und Wachsen* (Leipzig, 1899). This, more than any mere description, shows Berzelius's enormous productivity in his best years.

The book is aptly illustrated and well indexed. The translator seems to be as competent in the subject matter as she is in the language of the original.

F. GREENAWAY

Chemical Tribute

Chemical Dynamics: Papers in Honor of Henry Eyring. Edited by Joseph O. Hirschfelder and Douglas Henderson. (Advances in Chemical Physics, Vol. 21.) Pp. xxxiii+816. (Wiley–Interscience: New York and London, April 1971.) £10.75.

THE reviewer of this volume is in something of a quandary. Should he review Henry Eyring the man, Henry Eyring the scientist, the contributions of Henry Eyring or the contributions to the volume under review? Convention would dictate the last of these four possibilities. But the reviewer craves the indulgence of the readers while he says a word about the other three. In an age when society, and in particular the young, have shown a degree of revulsion from science, the question might well be asked whether or not this is due to the manner in which it is often presented—cut and dried, pre-packaged and apparently untouched by human hands. One speculates whether science should not be presented as a living thing, made by human beings subject to all the frailties of the species. Rarely is a paper presented to show the often haphazard lines along which the work developed. Only the crystal clear logic of the final analysis is presented, cold and remorseless. This is not science as most practitioners know it. A book like the present volume goes a long way to restore humanity to science, in the many glimpses we are given by the various contributions of Henry Eyring the man, the teacher and the scientist. And the picture that emerges is not only that of a great scientist, but of a great human being.

It would be wrong to suggest that all the papers will be of interest to everyone. Indeed, the wide spectrum covered is yet another tribute to the catholicity of interest of Henry Eyring. The papers are divided into six sections: molecular quantum mechanics (edited by A. A. Frost); theory of reaction rates (edited by K. J. Laidler); properties of molecules (edited by W. Kauzmann); theory of liquids (edited by D. Henderson); biological applications (edited by F. H. Johnson), and engineering applications (edited by C. J. Christensen). As might be imagined, the largest section is that dealing with the theory of reaction rates, which consists of fourteen varied contributions spanning a wide area from the mechanism of electron energy transfer from excited mercury atoms to paraffins, to the theory of non-Newtonian flow. The second largest is the theory of liquids, which ranges from molecular beam studies (as a means of evaluating intermolecular potentials) to the theory of plastic crystals. The sections on molecular quantum mechanics and properties of molecules overlap somewhat, as might be expected. Biological applications also cover a wide area, from protein conformations to the dynamics of life. No less varied are the engineering applications, which include the synthesis of diamond and the theory of detonation.

As will have been observed from what I have said, the volume accurately mirrors the scientist in honour of whose seventieth birthday it was produced. The man-made terms, mathematician, physicist, chemist, biologist, are too narrow to describe Henry Eyring. In browsing through this volume, the reader is brought face to face with a scientist, a man dedicated to enlarging the understanding of the working of nature, using techniques drawn from many disciplines. And this may well prove an inspiration to the young worker who feels that in the past he has been confined within a rigid but arbitrary discipline. Few scientists, whatever their interest, could fail to benefit from recourse to this volume.

ALLAN MACCOLL

Science and Indian Development

Science in India: Institution-Building and the Organizational Systems for Research and Development. By Ward Morehouse. Pp. xvi+144. (Popular Prakashan: Bombay, 1971.) Rs 25.

Physics in India: Challenges and Opportunities. (Proceedings of the Conference on Physics, Education and Research, June 1970.) Pp. iv+323. (National Council for Science Education: New Delhi, 1971.) Rs 10; $4; £1.25.

THE recent interest in the social implications (both good and bad) of science and technology has reached its culmination in what may well be the most significant social problem of all—overshadowing such currently fashionable fields as environmental pollution, the arms race and technological unemployment—that is, the possible use of Western techniques in helping to resolve the problem of chronic poverty endemic in well over two-thirds of the world's population. The argument runs as follows: since one of the principal factors influencing the very rapid rise in the standard of living of the Western "developed" nations has been the effective application of techniques derived from advances in the natural sciences to the productive process, it is necessary for the underdeveloped world to make every effort to foster science so as to achieve the same ends. Couched in such simplistic terms the process seems easy, but in practice the transplantation of what is to a large extent an alien social process, in spite of colonialism, has proved fraught with difficulties and has in some cases helped to exacerbate those symptoms commonly associated with underdevelopment, for example open and disguised unemployment, urban squalor and neglect of the land. Clearly the field is ripe for research.

Ward Morehouse's *Science in India* is a preliminary account arising out of a research programme currently being carried out at the Administrative Staff College, Hyderabad, on the organization of science and technology in India. The book has two principal aims. First, to provide the beginnings of a theoretical framework which will act as a basis for assessing the impact of scientific organizations on Indian development. Second, to provide an empirical account of the organizational structure of Indian science.

Taking the second aim first, Dr Morehouse has given us a valuable summary of the various bodies connected with Indian science, their respective functions, history, modes of finance, links with the socio-economic environment and relationships with the political power structure. For anyone intending to work in this field, his account must be necessary reading, drawing together, as it does, a wide variety of material from disparate sources. The author's attempts to provide a theoretical framework, however, are rather weaker not so much for what is said but for what is omitted. According to Dr Morehouse a scientific "organization" will only be effective in contributing to development once it has become an "institution". How shall we know when it becomes an institution? Well, when it begins to interact meaningfully with the socio-economic environment and evolves a cohesive dynamic of its own. This type of functionalism, besides evincing partial circularity of reasoning, does not seem to hold much promise for the resolution of the many problems which characterize Indian science today. In particular it does not give us criteria for deciding when a scientific "institution" is not helping development. Nor is there more than a cursory attempt made to define what is meant by "development".

These criticisms do not attach so much to the proceedings of a recent conference on physics education in India (*Physics in India: Challenges and Opportunities.* This conference, sponsored by Indian and American governmental and educational institutions at Srinagar, set out to discuss the principal problems connected with physics teaching and research in India and how these might be resolved. The report consists of a series of papers which stress the need for curricular reform in schools and universities, improved laboratory and library facilities, and a more rational use of teaching staff. In addition the need for education to be more broadly based and more intimately related to developmental objectives is emphasized and there are a number of interesting papers on the brain drain and unemployment of graduates, especially that by Parthasarathi. Again this volume is probably of chief interest to the specialist but could usefully be consulted by those concerned with wider aspects of social and economic development.

NORMAN CLARK

Swansea Survey

Swansea and Its Region. Edited by W. G. V. Balchin. Pp. xx+391+58 plates. (Swansea University: Swansea, 1971.) £2.50.

THIS is the traditional volume prepared on the occasion of the annual meeting of the British Association for the Advancement of Science, in this case at Swansea. A similar volume was prepared for the meeting at Cardiff in 1960 and the two together, although a decade apart in time, provide a South Wales survey.

During the past decade the BA handbooks have grown and the present volume is about twice the size of that prepared for the Cardiff meeting. It also has had the advantage of a geographer editor who is experienced in thinking in regional terms. In a review of this length it is impossible to give attention to each of the twenty-five chapters, even if any one person could be competent to assess them individually. It is to the general structure of the volume, therefore, that these comments must be directed although this means that many excellent, individual contributions may appear to be ignored.

In its general make-up the volume suffers from the nature of its origins. Presumably the earliest productions were meant to be simple guide books to the host cities and their environs, but they have evolved into full scale regional surveys. Unfortunately the handbooks fail to throw off the old role as guides and also a commitment to represent most of the sections of the association. The result is that the volume is not a true regional survey but a hybrid product. The contributions to this volume fall into three categories. The first reflects the old guide book character where local institutions are enumerated and described. Perhaps the chapter on the education service is the best example; for it is a straightforward account of facilities, but with no mention of problems. Have the new comprehensives avoided becoming one-class neighbourhood schools? What are the problems posed by the Welsh language? Indeed, is it taught at all, for it is not mentioned? Is it compulsory and to what level? Also in this same category is the account of the University College, and that of the City of Swansea, which is very far removed from modern urban studies. The second category is the "review of research" approach which devotes its space not to the region *per se* but to the research carried on in the locality. No chapter falls completely into this category, although that on zoology comes nearest to it. One could also place within it the very oddly located part five of the book, which deals with the Lower Swansea Valley Project. This important piece of work certainly merits discussion but it appears as an apparent climax at the end of the volume whereas it might have been more appropriately linked with "conservation". The third type of chapter is that of regional description and interpretation, directed to the local area itself. This quite properly forms the bulk of the book and is admirably done. Even so there is a lack of balance in that social structures are dealt with most briefly in some twelve pages as against some one hundred and twenty on the natural environment.

It is in this general area that I would make my basic criticism. The anthracite area of the coalfield, although not the whole of the Swansea region, has a very distinctive "character". Perhaps the basic feature is that it is the only part of Wales which is both industrialized and substantially Welsh speaking, a point which is never clearly made. It is all the greater pity that no attempt was made to demonstrate these characteristics when the person who could do it best, Professor T. J. Morgan, contributes but a brief chapter on place names, although according to the programme he presented a paper to section H of the association on the peasant culture of the Swansea Valley. This is, after all, to Welshman the land of Gwenallt, but none of that seeps into the book.

There are two further points which are disconcerting. The first is that no attempt is made to define the Swansea region, particularly in terms of its metropolitan structure. It is understandable that, for example, geologist and economic geographer might not be content with the same bounds, but it is a little odd to find the chapter on climate, exclusively concerned with Swansea itself, while that on botany includes the whole of Carmarthenshire and south-west Breconshire. This at a point where interrelations might be expected. The other disconcerting feature is that there is no direct urban survey of Swansea itself, no growth plan, no land use map, no study of "social areas". This survey of the city has been a conventional feature of nearly all the handbooks and it is certainly not lack of ready material which is the reason.

But perhaps my strictures are unfair. Critics too frequently demand a volume different from the one before them or ask for extensive and, for practical reasons of cost and bulk, impossible additions to the existing volume. If this were a coherent regional survey there is much that one could cut out from this volume and much that would need to be added: but of course it is not, it is a British Association handbook and is thereby committed. Perhaps a conclusion is that the purpose of the BA handbook be reviewed. I conclude, therefore, by reiterating my comments on the high quality of the work that has gone into this book. The editor writes in his introduction that no recent survey of the Swansea area exists and I am sure that many people, for many different purposes, will have recourse to the material contained in *Swansea and Its Region*.

H. CARTER

Caloric Theory

The Caloric Theory of Gases from Lavoisier to Regnault. By Robert Fox. Pp. xiv+378. (Clarendon: Oxford; Oxford University: London, May 1971.) £5.

EDWARD FRANKLAND'S comment that "it is by no means necessary that a theory should be absolutely true in order to be a great help to the progress of science" is now a commonplace among historians of science. It is one of the chief purposes of this detailed and technical study to demonstrate the sophistication and complexity of the caloric theory, at least as far as the gaseous state was concerned, and to reveal how successful its conceptual framework proved. The notion that caloricists were wrong headed and obscurantist was a late-Victorian view developed, understandably enough, in the wake of thermodynamic triumphs. In part, it was a Royal Institution saga devised by John Tyndall in praise of its founder Count Rumford. But, as Dr Fox cogently argues, Rumford appears in the history of caloric theory only by default since his experiments were unoriginal and "off the point", for they were directed only against the weaker Irvinist model of heat rather than against the stronger continental theory of Lavoisier and Laplace. Indeed, the latter's correction of Newton, and absorption of adiabatic phenomena in the derivation of the velocity of sound in 1816, two years after Rumford's death, was a major triumph for the caloric theory of gases.

The basic assumption of the caloricists from the 1770s onwards was that gas particles were held in stationary (not vibrationary) positions by repulsive forces between them which were produced by the presence of an imponderable fluid of heat which either surrounded, or fitted between, the gas particles. Heat itself, like ponderable matter, was conserved. This model was basically Newtonian, modified by the discoveries of pneumatic chemists, and fused with eighteenth century views on fire. However, Dr Fox's book is more than an internalist study of an outmoded theory and its experimental justifications. His own interest is obviously in the character of nineteenth century French science, particularly in the general scheme of scientific investigation he calls "Laplacian", and whose institutional basis at Arcueil was recently explored by Dr Crosland. According to this research programme, all physical phenomena were to be explained in terms of short range attractive and repulsive forces. This general interpretation enables the author to highlight and contrast the activities of the mathematical Laplace, the engineering-orientated Carnot, and the arch-calorist Avogadro who, in Italian isolation, remained tragically unaware of his work's discredibility to both physicist and chemist.

The author attributes the downfall of the caloric theory to a complex of causes, including the rise of Berzelian electrochemistry and the Fresnelian wave theory of light. Readers will find the pertinence of these new theories are too briefly analysed in contrast to the main contention that the most significant cause was philosophical and institutional: "an overall change of the style of French science between 1815 and 1830" which was anti-Laplacian. Comte's positivism reflected this change of mood, while the failure of Dulong and others to find viable alternative theories in the 1820s and 1830s led to widespread agnosticism towards theories of heat and atomism except as pedagogic aids. Regnault's brilliant, but tedious, experimental work is seen as a symbol of the decline of French theoretical physics. It follows, as Dr Fox plausibly argues, that the rise of the kinetic theory of gases in Britain and Germany during the 1850s was almost completely unrelated to the downfall of caloric theory which had effectively occurred two decades before.

This is an important monograph and deserves to be read for the historical questions it raises about nineteenth century science as much as for its definitive account of a forgotten physical theory. W. H. BROCK

Analytical Geochemistry

Analytical Geochemistry. By Lord Energlyn and L. Brealey. (Methods in Geochemistry and Geophysics, Vol. 5.) Pp. xv+426. (Elsevier: Amsterdam, London and New York, 1971.) £9.25.

THIS book attempts to describe a wide variety of chemical and physical analytical procedures of use to geochemists. While chemical methods are described in some detail, the treatment of physical methods is disappointing, and out of date in many instances.

The introduction contains a curious selection of geochemical topics. Igneous and metamorphic geochemistry are discussed only in terms of classification, with details of the well established "CIPW norm" and "Niggli equivalent norm". Although hydrothermal processes are briefly mentioned there is virtually no reference to modern work, even to such excellent texts as *Geochemistry of Hydrothermal Ore Deposits* edited by H. L. Barnes. A very brief, dated section on chemical solubility products and an introduction to fluidization phenomena are followed by interesting observations on the role of organic solvents in sedimentary geochemistry. The latter topic reflects the senior author's interests, and includes considerable reference to his own work.

The second chapter outlines the principles of qualitative analysis of rocks and minerals and is particularly noteworthy for the detailed description of the little known technique of membrane colorimetry. This technique incorporates a variety of methods for the transfer of chemical images from flat surfaces of metal, rocks and plants.

Quantitative chemical analysis is covered by the third and fourth chapters. The third chapter describes the main rapid methods of analyses of silicates, carbonates and phosphates in sufficient detail for the book to serve as a bench manual. The fourth chapter is a lengthy account of the chemical determination of minor elements in which the occurrence, detection and estimation of over fifty elements are described element by element.

The fifth to ninth chapters cover emission spectrography, flame photometry, X-ray spectrography and diffraction and fluorimetry. In each case the brief description covers basic principles, equipment and procedure. As an introduction for geologists with a limited chemical background knowledge these chapters may serve some purpose. However, even in this context it would have been useful to outline the relative speed, precision and general applicability of each technique rather than cite limited, specific examples of their application. Some of the pitfalls of these techniques are understated, particularly matrix effects which are of prime importance in optical and X-ray spectrography and in X-ray diffractometry. A fuller description of matrix effects would have been of benefit to the beginner, particularly if ways of circumventing or overcoming such problems were outlined or, at least, referenced. There is, indeed, little reference to modern literature; nothing more recent than 1962 in relation to emission spectrography, or 1961 for X-ray spectrography! The latter is a technique in which enormous advances have been made in the last decade, for example the equipment shown in Figs. 70 and 71 is now essentially obsolete.

The final chapter, on chromatography, is more thorough and includes an outline of the basic principles, followed by detailed descriptions of paper and thin-layer chromatography, gas or vapour-phase chromatography and pyrochromatography. The latter technique, developed by Lord Energlyn (W. D. Evans), includes an interesting account of geochemical applications.

In spite of the wide variety of techniques discussed in this book there are notable omissions, particularly important being atomic absorption spectrometry, which is rapidly gaining status as a major analytical technique in geochemistry. The lack of reference to modern work reduces the value of this book as an introductory text, since many physical methods of analysis have changed to such an extent that the methods and hardware of ten years ago are essentially redundant. Priced at £9.25, this book cannot be considered value for money. D. M. HIRST

Maths and Music

The Acoustical Foundations of Music. By John Backus. Pp. xiv+312. (John Murray: London, March 1971.) £3.50.

MANY physicists have been interested in the physical principles of music, in how sounds and the particular quality of sounds are produced by musical instruments, in the admixture of sounds and the effect of the surroundings on what one hears, and in the many other problems connected with music, including the physiological questions about hearing and the voice. It is a fascinating subject for physicists who are interested in music or musicians who want to have some idea of "how things work".

This book is directed to musicians and particularly to those "with no scientific background and no mathematical equipment past the high school algebra they have already forgotten". The author goes on to say "thus they are representative of musicians generally". Is this so? One hopes not, but even if it is, twenty pages of elementary mechanics, including careful definitions of length, time and mass, seem rather unnecessary. This determination to keep mathematics to a minimum precludes the use of trigonometric functions and logs (although logs are explained in an appendix) and this leads to rather cumbersome explanations of several topics, for example, the various musical scales.

Given this limitation, the author describes the main features of the subject very well, but this has been done before by a number of authors. It is in the discussion and explanation of finer details that both musicians and physicists find most interest, because the fundamentals are in most cases fairly easy, but the exact description of what happens is often extremely complicated. Here the author is concerned to show, without too much complication, how some of these problems can be tackled and the results that have been obtained. He is also concerned to refute the "fallacies and superstitions that music could better do without". Most of these onslaughts seem to be fair comment but in the case of piano touch there is a point which seems not to have been made. The physicists' argument, strongly expressed by the author, is that when the hammer strikes the string it is "completely separate from the impelling mechanism attached to the key" and that "it follows that the pianist cannot independently control the quality of a single note on the piano by the manner in which he strikes the keys". The first statement is true, but the second does not follow because the stem carrying the hammer will bend and the bending is proportional to the acceleration while the final velocity depends on the integral of the acceleration. It is doubtful whether the experimental work done in the 1920s was of sufficient accuracy to resolve this point and it should be added to the very many unsolved problems which the author points to as requiring further investigation.

This constant reminder of how much work is waiting to be done is one of the very good features of this book, as is the treatment of wind instruments, so often neglected in books of this sort (the author plays the bassoon), and also the extensive bibliography, much of it of recent work, which will so much ease the path of those interested in the pursuit of some of these topics.

J. H. E. GRIFFITHS

NATURE Volume 233 OCTOBER 8 1971

Research Councils on the Brink

EVIDENTLY the research councils which administer government expenditure on basic research in Britain are becoming edgy. In the past few days, both the Natural Environment Research Council (see page 364) and the Science Research Council have taken the publication of their annual reports as opportunities to complain that the Dainton report on the organization of civil scientific expenditure should be made public. What the research councils are afraid of is that the supposedly moderate recommendations of the Dainton committee will be replaced by more radical proposals when the review being carried out by Lord Rothschild is completed. The issue is important not merely because the research councils between them spend roughly one eighth of the money spent on research and development in Britain but also because their effectiveness depends on the intimacy of their connexion with their constituents—the large company of professional scientists who work in British universities, research institutes and in industry at large. If the government hopes to carry with it the scientific profession, in the reorganization which is now impending, it must do everything it can to let the people concerned appreciate the reasoning behind its decisions To be sure, it may in the end be decided that the Dainton recommendations are not acceptable to the government. They may not be radical enough, for example. In those circumstances, however, it will be much better for everybody concerned, the government included, if the constituents can see the differences between the government's arguments and those of the Dainton committee. And if this proposition is accepted, there is of course no reason why the Dainton report should not be published immediately, without waiting for the Rothschild review. To that extent, the research councils are right to complain.

Whether they have prepared themselves sufficiently for the reorganization that lies ahead is unfortunately a different matter. Several developments in the past few weeks suggest that the councils are a long way from a strategy for the administration of government expenditure on basic science in Britain.. To be sure, the Science Research Council a week ago was talking of closer collaboration between the councils but there is very little to boast of yet. Each of the four research councils has, for example, an interest in supporting graduate students at British universities. In the year just past, for example, the Science Research Council had 5,700 graduate students on its books, the Medical Research Council had 600 pensioners on various kinds of courses, the Natural Environment Research Council had 633 graduate students on its books and the Agricultural Research Council followed its traditional policy of spending very little on studentships. In this pattern, of course, the Science Research Council has a dominating position. Although the other councils tend to take the view that one of the most effective ways of influencing the development of university science is to set up research units and institutes of various kinds, studentships are also powerful levers. Many heads of university departments are prepared to jump through hoops they would have avoided for the sake of providing places in their departments for graduate students. But is it sensible that the process of awarding these grants should be divided for practical purposes between three of the councils in circumstances in which there must plainly be a great deal of overlap? Is this not a field in which the best way to collaborate is to centralize?

In the spending of government money on basic research, there is also a need for the research councils to strike a better balance between research grants and other forms of expenditure. In the year just past, the Science Research Council spent a total of £15.7 million on research grants and studentships, roughly 30 per cent of its total expenditure. The rest of its money went on research institutes which provide the universities with research facilities of various kinds, some of them abroad. The Natural Environment Research Council seems less able to part with money—in the year just past, just under 12 per cent of its much smaller budget was spent in this way. Twenty-five per cent of the council's whole expenditure went on research grants, fellowships and research units in the universities. The proportion of the agricultural money spent in universities, harder to identify, is more like that spared from the budget of the Natural Environment Research Council. Is it really necessary that so much of the money set aside by the government for basic research should be spent outside the universities? Is it really wise that all the councils should be busily giving hostages to fortune by taking on their books research institutes and laboratories which could just as well be operated through and by the universities? In the flush of pleasure which there has been in the past few years at the increasing rationality with which the research councils make decisions, the question has been overlooked whether they have struck the right balance between the roles of sponsor of university research and midwife to it. It would be a reasonable intermediate goal to ask that the research councils between them should spend half of their budgets as cheques to universities or to students.

One obvious difficulty is that this concept of how basic research should be organized will not suit all of the research councils. The Agricultural Research Council will no doubt be the first to protest that basic research in agriculture cannot always be carried out in a university atmosphere. To be sure, if the history of agricultural research in Britain were reconstructed, it is entirely

Foreigners at CNRS

THE leading article in *Nature* on September 17 (**223**, 152; 1971) implied that the Centre Nationale de la Recherche Scientifique does not employ scientists from outside France. In fact a substantial number of the scientists on the CNRS payroll come from outside France. The Editor of *Nature* apologizes for this unintended and unjustifiable slur.

possible that spendid establishments like that at Rothamsted would have grown up more closely linked with some university department or another, but nobody at this stage would wish to unscramble that omelet. Whether there is a strong case for the formal independence of laboratories such as the Institute of Animal Physiology from their nearby universities is much less certain. Much the same arguments would no doubt be advanced by the Medical Research Council on behalf of some of the institutes which it controls—here again only a particular analysis of the present functions and future potentialities of the several institutions can inform a sensible decision. There is an urgent need for a clearer distinction between the basic research, in agriculture, medicine or the natural environment, which should be carried out by universities and that which must of necessity be carried out in separate research institutes. Three important questions of organization stem from this question, and perhaps the most difficult and interesting is that of how to decide the function of universities and similar institutions in the pattern of basic research. In the past few years in Britain, it has been customary and even justifiable to say that university research has been too uninterested in the practical consequences that may result. This is why the Science Research Council has been trying to persuade university departments and their graduate students to take a greater interest in research with an industrial slant. In doing so, however, it has also helped to confirm the impression that the universities are not particularly interested, and thus to justify the claims of those who say that money for basic research relevant to the long-term needs of industry should best be channelled to other kinds of institutions than the universities. Is it not time for a reappraisal of this view? Should not the universities now be regarded as potential administrators of relevant research? After all, the government (or its predecessor) has willed the universities; is it not sensible to try to make the fullest use of them? No harm would be done if the Rothschild review were to suggest a scheme for giving the universities much more money and a much greater responsibility for the health of British science.

Another question concerns the relationship between the research councils. There is an important distinction between the technical research on which a part of the energy of the Agricultural Research Council and the Medical Research Council is spent and the long-term work which they support in the universities. On the other hand, where the sponsorship of university work is concerned, the four research councils have even more in common than they are prepared to admit when boasting of their fondness for collaboration. In the circumstances there would be a great deal to be said for some kind of concentration. After all, the four research councils between them spend more than £4 million a year on headquarters administration, and even if much of this goes on the kinds of committee meetings which give working scientists a sense of participation in the decisions which affect them, this is a large part of what the councils as a whole have to spend. At worst, a reorganization of the research councils which reduced their number from, say, four to three would save a certain amount of public money, would make possible a clearer separation between the work that can be done in universities and that which must be done outside and would at the same time provide a framework for better collaboration with other government departments. In all the circumstances, this is a line that Lord Rothschild should be prepared to follow in his review.

Phosphates are Free Again

THE pantomime two weeks ago in Washington when the Administration's three high priests of the environment—Mr Russell E. Train, the chairman of the Council on Environmental Policy, Mr William D. Ruckelshaus, the director of the Environmental Protection Agency, and Dr Jesse L. Steinfeld, the Surgeon-General—gathered to announce that detergents containing phosphates might be lesser evils than some of the materials which have appeared on the market in the past few months on the environmental ticket should be a warning to all who leap before they look. Especially with Dr Charles Edwards, the head of the Food and Drug Administration, in attendance, it must have been painfully apparent to all concerned that the United States is rapidly becoming a prisoner of its own whims in dealing with the environment. After all, Dr Steinfeld was by all accounts the one who helped, in the days of Mr Robert Finch, to persuade the then head of the Food and Drug Administration, Dr Herbert J. Ley, that cyclamates should be taken off the market in a trice. Later that year, the Food and Drug Administration was playing its own politics by agreeing that cyclamates might be sold over the counter as non-prescription drugs. It was Dr Edwards who in the end had to attempt making sense out of nonsense when Congress eventually caught up with the scandal.

Why is the United States Administration so prone to errors of this kind? The haste with which herbicides such as 2,4,5-T were restricted just over a year ago was entirely unseemly—at that point, the experiments on which were based the claim of teratogenicity were about as convincing as would be a demonstration of spontaneous generation in a high school laboratory. To be sure, the FDA and everybody else has the Delaney amendment hanging round its neck like an albatross, although there is a case for saying that the Administration could shed its curse if only somebody would stand up and say that the Delaney amendment is altogether too crude a guide to good practice. After all, why should materials be entirely prohibited once it has been shown, in feeding experiments with laboratory animals of any kind—even cancer-prone hamsters—that there are carcinogenic possibilities? Surely, enough has been learned since the fifties for more sophisticated judgments to be possible.

Unfortunately, however, there is no compulsion to explain why the Administration appears to be prepared to act forcefully against the persistent pesticides, DDT and things like that. The report by the DDT advisory committee to the Environmental Protection Agency, published last week (see *Nature*, **233**, 299; 1971), was reasoned but incomplete. What the committee did was to add to the conclusions of the Mrak Commission, published at the end of 1969. Its general conclusion was that the amounts of DDT and other pesticides now found in soil are unlikely to be removed by natural processes in the immediate future. It follows that there is no immediate prospect of a rapid decrease of the amount of these materials in the standard American diet and therefore very little chance that concentrations of pesticides in fatty tissues will quickly decline. In short, persistent pesticides are likely to persist for the next twenty years or so. What seems to be inferred from this is that while there cannot in the circumstances be an urgent need to stop the use of DDT and similar materials, this is a goal at which to aim. The weakness in the argument is that very few of the advisory committee's data have been collected from outside the United States. This in turn implies that there are at present no means of knowing what would be the condition of American soil a decade or so from now if the immoderate use of persistent pesticides which characterized the fifties were in the seventies to give way to a discriminating but potentially valuable use of a family of pesticides which is, after all, one of the most effective known. In short, here again is a field in which the Administration may act too soon and too fiercely.

Why should this be? The case of the phosphate detergents contains an explanation. For close on a decade, phosphates have been pilloried as if they were endowed with all the malevolence of the organisms of infectious disease—plague, for example. This point of view stems from the recognition that a great many lakes in North America which had been severely damaged by insupportable quantities of organic waste material were nevertheless allowed to grow algae in vast amounts because of the phosphates and nitrates contained in sewage effluents, treated or otherwise. To be sure, nobody would seriously pretend that phosphates by themselves are a sufficient cause of what is called eutrophication. But the fact that phosphates (not nitrates) are often the limiting nutrients in algal growth has given phosphates a bad name, and on the principle that honest taxpayers unwilling to pay for proper sewage treatment plants or sewerage systems may wish to salve their consciences by doing something for the common cause against pollution, there has been a great outcry against detergents which contain phosphates. Several states have already banned the sale of detergents containing phosphates. Several manufacturers have made small fortunes by marketing alternatives in the name of what they call ecology. Congress was about to gird its loins for a full-scale attack on them. Only sheepishly did the environmental high priests step in with a belated warning that the fire could easily be more uncomfortable than the frying pan.

The moral is simpler than most. In the past year, the Environmental Protection Agency has disappointed some of its wilder supporters by cultivating a proper sense of pragmatism. As things have turned out, the agency has become an empirical amalgam of those parts of the Department of Health, Education and Welfare and the Department of the Interior which used to worry about air and water pollution. Nobody knows how soon it will be strong enough or eager enough to take the Atomic Energy Commission by the scruff of the neck. Nobody knows how soon the Administration will give it responsibility for the Food and Drug Administration. To tell from the agency's second annual report, published a few weeks ago, with its careful attempt to demonstrate that getting rid of pollution will cost money, the agency has determined to fight a discreet battle, keeping in the process what is called a low profile. The trouble is, of course, that such a strategy leaves the initiative to others. On this occasion, the agency has been able to divert Congress from an expensive blunder only in the nick of time. What it needs to become is a much more active agency able on occasion to say that the devil you know may be better than the devil that comes in a well-designed packet marketed by people who know that the environment is now the most popular cause of all. In other words, the agency must try to become one for saying that it may often cost very large amounts of money to win small and even imagined benefits, and that it may often be wiser to live with some minor sources of pollution than to seek to abolish them. Mr Ruckelshaus could save himself a lot of time at press conferences like that two weeks ago if he would follow such a policy.

---

100 Years Ago

NATURE

RECENT UTTERANCES

THE Oracle has spoken. In fact several Oracles have spoken. Let us take them seriatim. From the lips of two of the most enlightened members of the Cabinet we have had at last an authoritative expression of the desirability — nay more, of the absolute necessity — of scientific education for the country at large. Addressing his constituents at Bradford on Monday the 2nd inst. in a speech to which we have already alluded, on the occasion of the opening of the new Mechanics' Institute for that town, Mr. W. E. Forster, the Minister for Education, as he ought to be styled, made use of the following emphatic language :—" The old grammar-school teaching was almost framed upon the advantage that Latin and Greek well taught gave to the boys ; now, we find that the boys cannot do without the use of more general knowledge than is given by Latin and Greek ; that there must be a knowledge of modern languages. But there may be also a feeling that we ought to know something of the daily facts of life, and the rudiments of Science. There, again, I speak from a sense of my own want, and I have often thought how much more useful I might have been —at any rate, how much stronger I might have been—if I had had given to me a 'scientific education, such as I think we may now hope that our children will attain." And again : " We now believe that we have taken measures by which we may secure elementary education to all children of all classes in our borough, and throughout the country, and, consequently, those who attend this institution will have the foundation of a training that will enable them to fulfil the original idea of its promoters," that is, " to give mechanics scientific knowledge."

From Nature, 4, 461, *October* 5, 1871

OLD WORLD

ASTRONOMY

New Face at the RGO

SPECULATION about the next Director of the Royal Greenwich Observatory has been stilled by the announcement that Professor Margaret Burbidge has accepted the post. Reaction among staff members of the observatory is one of pleasure that an astronomer of such distinction will be directing the RGO in the near future.

Asked what it felt like to be the first woman director of the RGO, Professor Burbidge said on Monday "I find the prospect very interesting especially since there is a great move to do something about the position of women in science." But she also pointed out that there is a better tradition of accepting women in astronomy than in other physical sciences.

Before moving to the United States in 1951, Professor Burbidge was Assistant and Acting Director of the University of London Observatory. She is perhaps best known for her work on quasars, largely carried out in collaboration with her husand, Professor Geoffrey Burbidge. Much of the apparent tardiness of the Science Research Council in announcing the appointment may, indeed, be attributed to the difficulty of offering such a post to one half of such an eminent astronomical couple. Both Geoffrey and Margaret Burbidge have spent the past few summers in Cambridge (since the inception of the Institute of Theoretical Astronomy) and the new director says that her husband will probably divide his time between California and Cambridge after she takes up the post next summer. No formal decision on this can be made, however, until the future of astronomy in Cambridge is itself decided. The present uncertainty about the Institute and Cambridge Observatories will be resolved in discussions starting at Cambridge on Tuesday, October 12.

The future of the Royal Greenwich Observatory is also undecided. One vital question is whether the observatory will have direct control over the proposed British observatory to be established in the Mediterranean area (see *Nature*, **232**, 289; 1971). Because the new director is unable to take up her appointment immediately, it seems that it will be some months before decisions will be made known about this new observatory. But whatever developments there are in the observational plans for Greenwich, it seems certain that the close links forged recently with the group of theoretical astronomers at the University of Sussex will be maintained. Professor Roger Tayler, Director of the Astronomy Centre at Sussex, said this week that he hopes the present collaboration with the RGO will continue and expand in the future.

ATOMIC ENERGY

Swan Song?

THE annual report of the United Kingdom Atomic Energy Authority, published on Wednesday this week, shows an increased proportion of work done in collaboration with industry and government departments but carried out with a smaller staff (House of Commons Paper 552, HMSO, £0.85.)

On April 1, 1971, the day after the period covered by the report, the Production Group of UKAEA was transferred to British Nuclear Fuels Limited, the Radiochemical Centre at Amersham was converted into The Radiochemical Centre Limited and a newly-formed National Radiological Protection Board took over responsibilities previously held by the authority in the field of radiation hazards and their effect on health and twenty-nine members of its staff. Since then it has been announced that the Weapons Group, including the Atomic Weapons Research Establishment at Aldermaston, will be transferred to the care of the Ministry of Defence, probably by the summer of 1972 (see *Nature*, **232**, 434; 1971). This leaves the authority with the Reactor and Research Groups and 19,802 employees as at April 1, 1971, compared with 29,427 on March 31, 1971. This number will be reduced by a further 6,000 when the transfer of the Weapons Group is effected and the principal continuing function of the authority, according to the report, will then be "the further development of nuclear energy for the generation of electricity . . . and the continuation of the underlying work necessary to support this activity".

The research and development budget for the past year shows a decrease from £59.4 million in 1969–70 to £58.5 million. Money spent on the reactor programme decreased from £43.3 million to £41.9 million with a change of emphasis in the support for the gas cooled reactors—the Mark III reactor support being increased by £1.9 million to a total of £5.2 million with a corresponding decrease in the support for the Mark II system.

Support for other research remained substantially unchanged at £12 million compared with £12.8 million the previous year, nearly all the decrease originating from decreased support for back up nuclear projects. Expenditure on non-nuclear research, however, increased by £0.6 million to £3.6 million. The authority's net expenditure during 1970–71 was £37.1 million compared with an estimated £42 million this year. The gross expenditure of the authority during 1970–71 was £95.3 million, offset by £57.2 million in receipts—£20.5 million in defence settlement payments, £9 million in sales and £8.6 million in agency services and receipts under research agreements. Sales this year are estimated to bring in £6.9 million.

Success in the sale of nuclear fuel abroad is also reported, with three contracts for supplying uranium hexafluoride to the United States won during the year as well as two contracts for supplying the material to Sweden and Switzerland. Other overseas contracts were won in Canada, Germany and Japan. The upward trend in enriched uranium production in recent years is now bound to level off following delays and changes in the programme for commissioning nuclear power stations in Britain.

Support for the plasma research laboratory at Culham amounted to £4.1 million last year. Maybe it is in this laboratory that increased effort should be concentrated in future years to harness the power of nuclear fusion. It is reported that results along these lines are encouraging and that "continuing attention was given to the technical problems of envisaged generating systems using fusion reactors". It is possible that the revenue lost to the authority by the formation of the new companies will eventually be replaced by income arising from the commercial development of the work currently in progress at Culham.

RESEARCH COUNCILS

Rallying of the Clans

HARD on the heels of the Science Research Council's comments on the Dainton report (see *Nature*, **233**, 298; 1971) come those of the Natural Environment Research Council in its own annual report published last week (HMSO, £0.85). The council says it learned "with relief" that the Council for Scientific Policy had decided to conduct an inquiry under Sir Frederick Dainton into the organization of civil science as "the continual reviews and proposals for reorganization of science, of the kind that the council (NERC) has been subjected to for most of its existence, are necessarily disturbing". The council warns, however, that concepts and plans for the advance of scientific knowledge must not be "unduly dictated by the immediate practical problems". Like the Science Research Council, NERC believes in the "basic good sense of the Research Council system" and that "the principles on which decisions are eventually to be based ought first to be the subject of wide and free debate".

The council also speaks plainly on the matter of finance. The council's objective of bringing the environmental sciences to a position which, with only a modest growth rate, will allow it to keep pace with the demand for basic knowledge about the natural environment has not yet been achieved, and the council claims an average level of funding about one-third higher than last year is required (just under £14 million). With cuts in research expenditure in the offing the council also warns that too sudden a reduction of growth is likely not only to waste previous investment and cause a loss of confidence, but also to damage, possibly irreparably, the attainment of the balance between the demand for knowledge and the research capability to meet it.

The council's new committee structure is outlined in the report, the changes being made because the council felt it was becoming isolated from important issues and from the directors of its institutes. Five preparatory groups for the fields NERC covers have now been given the task of thoroughly investigating policy issues before they are put to the council; the groups bridge the gap between the various institutes, but they have no executive power, the actual implementation of these policies, once approved by council, being the province of the institute director and his advisory committee. NERC feels this structure is both more flexible and better able to clarify the issues involved than the old system of eight standing committees whose terms of reference were too narrow to consider interdisciplinary policy problems.

The council's working party on computers has concluded that the cost of hiring computer time for the council is approaching the cost of buying computers; NERC expects to spend £3 million on computers over the next five years, but no decision to buy has yet been taken, pending discussion with the Computer Board and the Inter-Research Council Computer Review Committee, set up to plan and coordinate computer use by the councils.

Of the council's £14 million expenditure, £975,000 went on research grants at universities and other institutions, and £1.52 million on grant-aided laboratories and units. Among the council's own establishments the Institute of Geological Sciences received the largest amount (£3.36 million), with the Nature Conservancy and British Antarctic Survey getting £1.96 million and £1.76 million respectively. The total spent on NERC's eleven establishments was £9.89 million, the field with the largest expenditure being Marine Sciences at £4.29 million.

CIVIL SERVICE

Fulton Fulfilled

THE announcement last week of the restructuring of the Scientific Civil Service has the full approval of the Institution of Professional Civil Servants. This step towards implementing the Fulton recommendation of a unified structure throughout the Civil Service has been long awaited by the IPCS and Mr Colin Waters, the Institution's Assistant Secretary, said this week that they were very much in favour of the reorganization and that they would like to see more of the Fulton recommendations on career management and prospects implemented as soon as possible.

The details of the merger of the Scientific Officer, Experimental Officer and Scientific Assistant Classes into the Science Category are given in Table 1. The old nine class structure is replaced by a streamlined five class structure that becomes retroactive to January 1, 1971. Of the other Civil Service categories the Administration Category has already been reorganized while discussions on the future of the Technology Category are still in progress with a decision expected at the latest by early 1972. The Civil Service Department stresses that the restructuring will increase efficiency and improve promotion prospects. Lord Jellicoe, minister in charge of the Civil Service, said that "the way is now open . . . for us to push on with improved career management to ensure that the individual scientist as well as the Service benefits from the full and timely development of talent". Mr William McCall, General Secretary of the IPCS, said last week that the new structure "represented worthwhile improvements over the arbitration tribunal's award" (see *Nature*, **232**, 594; 1971).

The IPCS is now keen to make further progress towards a unified grade structure within the Civil Service and is hoping that the Science and Technology categories can be merged in the near future. The Civil Service Department is not enthusiastic at present about further merging of these categories although it has agreed in principle to the Fulton recommendations of a unified structure. A spokesman for the CSD said this week that the department agreed to the principle of exploratory negotiations to examine the practicability and desirability of a unified grading below the under-secretary level. He also said that one of the problems associated with merging the present three categories would be the determining of salary scales by comparison with pay in outside organizations—a practice that the CSD is committed to. The IPCS, however, is now committed to changing the system following the recent pay tribunal. Mr Waters pointed out that the difference in salary between the Science and Technological Categories—a Principal Scientific Officer at the top of the scale earns £108 less than his equivalent in the Technology category, an inconsistency soon to be magnified in a pay review for the Technologists—cannot be perpetuated after the merger.

Table 1 New Civil Service Science Category

New grades	Existing grades		
Science Category	Scientific Officer Class	Experimental Officer Class	Scientific Assistant Class
Principal Scientific Officer (£3,100–£4,100)	Principal Scientific Officer	Chief Experimental Officer	
Senior Scientific Officer (£2,303–£3,255)	Senior Scientific Officer	Senior Experimental Officer	
Higher Scientific Officer (£1,810–£2,350)		Experimental Officer	
Scientific Officer £1,120–£1,900)	Scientific Officer	Assistant Experimental Officer (over 21)	Senior Scientific Assistant
Assistant Scientific Officer (£550–£1,395)		Assistant Experimental Officer (under 21)	Scientific Assistant

TRANSPORT

Wheels Keep on Turning

THE news that the Transport Committee of the Science Research Council has accepted the chief recommendations of the council's Transport Panel will be of little comfort to those academics who originally opposed the recommendations.

The transport panel was established in May 1968 to consider the role of the SRC in supporting university research in transport, to suggest the function of university research in the context of the national effort, and to develop for it a coherent policy. The report recommends that university transport research "should be treated as a priority area for finance and manpower", and proposes that the council's financial commitment should rise from £140,000 in 1968–9, to £550,000 by 1973–4. The transport panel says that universities already play a valuable role in transport research but their work is criticized for being too fragmented. The solution proposed is the SRC's standard one—concentration of effort.

The report suggests that three centres be established, one each for transport planning, transport management and control, and transport technology. A smaller marine transport centre is also an urgent requirement as "there is a clear lack of any university group studying the transport problems associated with ports and shipping". The centres will provide multi-disciplinary teams to study the problems of transport research on a theoretical rather than an experimental level but 25 per cent of SRC funds will still be available for research outside the centres.

The four centres have already been selected by the committee, but they are not being officially revealed until the Department of Education and Science has given approval. The proposal to create centres met with appreciable opposition from the Universities Transport Study Group (which claims members in about 35 universities) when the first draft of the report was offered to them and other interested bodies for comment. Many of the fears then expressed have been allayed by the council's assurances that researchers outside the centres will not become ineligible for support once the centres start operating.

In spite of these assurances a number of academics still believe transport research does not require concentration of the type proposed, but feel finance should simply be given to those with promising ideas, rather than to centres which it is piously hoped will then attract ideas. It is felt that if the wrong universities are made centres they will not attract the best people—good academics will not follow after bad.

The SRC's answer, based on experience in other fields, is that the centres will prove attractive, and will draw new high class brains into transport research —which is one of the panel's main hopes. It also hopes that the long term programmes of the centres will attract short term contract work from industry and government departments (as has happened in the control engineering centres), which will in turn help the long term work. The status of the centres, however, will not be permanent and new centres will arise and old ones fall as and when necessary.

To be sure, academics welcome the moves, feeling that rationalization is required to provide larger teams and to prevent duplication of effort. The reaction of bodies outside the academic world is enthusiastic—British Rail, the Port of London Authority, and the Ports Council, for example, all welcome the idea.

It will be interesting to see if the implementation of the report's proposals eventually silences the critics, and indeed to see if the SRC will be able to afford the increased support it proposes.

RESEARCH COUNCILS

Plans for Manpower

THE Science Research Council has it in mind to limit the number of new postgraduate studentships (for research and for the shorter advanced courses) available next year to 3,850, the same number as were taken up at the beginning of this month. This is spelt out in the SRC report for 1970–71 (HMSO, £0.65), in which the present financial and employment situations are cited as the chief factors that have to be taken into account when the council makes its final decision. What are the likely effects of this and how will the composition of the postgraduate research population be altered?

Table 1 Numbers of SRC Research Studentships

	Chemistry	Total
1967–68	1,516	5,058
1968–69	1,529	5,322
1969–70	1,480	5,498
1970–71	1,515	5,727
1971–72	476*	2,230*

*New studentships commencing October 1971.

The number of research studentships in some subjects has remained static or has even been reduced during the past few years, in spite of increases in the total number of awards. In chemistry, for example, the number of SRC studentships held during the past few years has fluctuated and the number in 1967–68 is the same as in 1970–71—although about thirty studentships are now separately awarded in enzyme chemistry and polymer science. (The statistics in Table 1 include the cooperative awards in pure science (CAPS) which are expected to number 210 for all subjects in 1971–72.) The recent SRC chemistry report showed, however, that university chemistry departments have about 1.9 research students and graduate assistants for each staff member compared with about 1.2 per staff member in other subjects; the report goes on to recommend that the total number of SRC research studentships in chemistry should not be changed substantially in the near future.

An increase in the number of research studentships in computing science—from 101 to 110 between October 1969 and October 1970—is recorded in the annual report and the new computing science awards taken up this month number more than 50. The total number of studentships held in nuclear physics has remained almost constant at about 200 for the past two years but the total in other branches of physics has dropped slightly from 594 in 1969–70 to 582 in 1970–71; total numbers in the biological sciences have increased from 836 to 909 in the same two years.

The proportion of British research students funded by the SRC is about 50 per cent overall and the remainder are supported chiefly from university, industrial and private sources; the SRC's relative contribution does not appear to fluctuate very much—although there is a slight upward trend —probably because financial pressures usually bear on industry and on the SRC at the same time. Any levelling off in the availability of SRC training awards will probably mean that the number available in some subjects will not grow as quickly as in the past and that other subjects will suffer decreases in the numbers of awards allocated to them.

CONCORDE

Industrial Innovation

ONE third of the sub-contractors and suppliers to the British Aircraft Corporation for the Concorde project obtained spin-off benefits as a result, according to a report from the Centre for the Study of Industrial Innovation (*Aspects of Spin-off*, £0.50).

The report is the result of a survey among 161 of the 600 companies engaged on work for Concorde. Spin-off is taken to be not only the transfer of products developed for Concorde into other fields, but also the less tangible benefits such as the introduction of more effective quality control, or of new processes which have wider applications.

Spin-off in the tangible sense of new products was limited to only 14 per cent of the companies involved. The report also says that several companies, particularly when referring to the less tangible effects of spin-off pointed out that the benefits are likely to disappear if the project does not go into full production. Companies involved in the Advanced Passenger Train development, which the centre also consulted in the course of the survey, expressed similar views.

The report attacks the assumption that spin-off could be used in part as justification for projects that otherwise are uneconomical, and points out that "for the majority of companies supplying the Concorde programmes the work was of a straightforward type not involving commercial or technical conditions any more strenuous than those normally met".

The dependence of supply firms on Concorde varied considerably, contracts ranging from £3.7 million to £320, and the report also found that the economic dependence of companies on Concorde work was surprisingly low—only one quarter of firms had order-values of more than one-sixth of their annual turnover, and only six firms of more than half. A further quarter had order-values of only one per cent of their annual turnover.

The survey was only concerned with assessing how many companies benefited from spin-off as a result of work on Concorde, and in the types of spin-off experienced—no attempt being made to assess the economic value of the spin-off. The centre refused to hazard a guess at the percentage value of the spin-off from the £500 million spent on Concorde to date, and without any guide to this the figures in the report are less meaningful. Its conclusions are in the main only those expected, some of them so obvious as to be barely worth making—for instance that sub-contractors who supply specifically designed non-standard items benefit more from spin-off than suppliers who provide standard items. The centre says that more studies are needed to assess the economic value of spin-off, at the same time suggesting that it may never be quantifiable—because so much of it is intangible—in terms that can be taken into account in the cost/benefit analysis of large programmes. The centre hopes, however, that its report will provide a yardstick against which some sort of assessment of the economic value of spin-off can be made.

EDUCATION

No A-level Electronics

THE study of electronic systems in school sixth forms is apparently regarded by the Schools Council on the Curriculum and Examinations as excessively vocational and specialized. As a consequence, the council has not allowed the Associated Examining Board to set an experimental A-level examination based on a syllabus devised by Professor G. B. B. Chaplin and his colleagues of the Department of Electrical Engineering Science at the University of Essex.

Work on the syllabus started in 1966 and the project came to fruition when members of the Electrical Engineering Science Department began to teach a sixth-form course at Colchester Royal Grammar School in 1970. The Associated Examining Board considered that the syllabus would provide a reasonable basis for an A-level examination and duly sought permission from the Schools Council to set an examination for the eight boys in the first year sixth form who have been taking the course as an extra subject. The Schools Council handed down its decision on the grounds that the course did not satisfy the criteria by which the suitability of A-level courses is judged; one of these is that courses "should not be largely or wholly of a narrowly vocational character".

Professor Chaplin emphasized last week, however, that the electronic systems course is by no means a pre-university training for electronic engineers but that it is a non-specialist course which would be equally useful to those not going to university. He also pointed out that the syllabus had been enthusiastically received by teachers attending vacation courses within his department.

The syllabus is divided into three parts—communication systems, control systems and computer systems—each of which is prefaced by some consideration of the relevant human functions, such as hearing, seeing, feedback and problem solving. Although the course concentrates on the underlying principles of electronic systems, the characteristics of electronic devices themselves can be examined in more detail as appropriate. Experimental work is an integral part of the course and includes, for example, measurement of the frequency response of the human ear and simple experiments with servomechanisms.

Professor Chaplin is not unduly discouraged by the attitude of the Schools Council and it is planned that the second half of the syllabus will be taught to another group of boys in the first year sixth form during the coming academic year. Meanwhile it seems likely either that another approach will be made to the council in the near future or that efforts will first be made to interest more and more teachers in the idea of a course on electronic systems—possily through the Association for Science Education and the National Electronics Council.

Appointment

MRS MARGARET THATCHER, Secretary of State for Education and Technology, announced this week the appointment of Professor R. C. O. Matthews as chairman of the Social Science Research Council in succession to Mr Andrew Shonfield. Professor Matthews, Drummond Professor of Political Economy at Oxford, will take over from Mr Shonfield on January 1, 1972. He is at present a member of the SSRC and is chairman of the council's Economics Committee as well as being a member of the Central Advisory Council for Science and Technology, the Council of the Royal Economic Society and the Executive Committee of the National Institute of Economic and Social Research.

Miscellaneous Intelligence

SWEDEN is reported to be the first West European country to introduce Chinese as an optional subject at the secondary school level. Twenty pupils at a Stockholm school are now studying Chinese as their third foreign language, after English and German or French. Most pupils at the equivalent level of education in Sweden study Spanish as their third language, and roughly 1,300 take Russian.

THE University of Leicester experiment to determine the precise position of the source GX3+1 was successfully flown from Woomera in Australia on September 26. It is hoped that the data obtained on this flight will lead to an improvement in the position known for this X-ray source from 2 arc min to better than 0.25 arc s in the coordinate measured. This is achieved by determining the exact time at which the source disappears when it is eclipsed by the Moon. The flight took place aboard a Skylark sounding rocket, which was stabilized and "locked on" to the strong X-ray source Sco X-1 during the four minutes of observing time. The experiment and the Scorpius sensor were provided by the group working under Dr K. Pounds at Leicester. The determination of a precise position for GX3+1 may lead to its identification with an optical object; first results indicate that the flight was a complete success.

NEW WORLD

Safeguard Survives Another Attack

by our Washington Correspondent

THE Safeguard anti-ballistic missile system (ABM) seems to be rapidly losing its vulnerability to attack, at least from forces in the Senate which are ranged each year against the project's budget. Its vulnerability to Soviet missiles is still, however, open to question. Two years ago, the crucial decision on whether to provide funding for the first phase of deployment of Safeguard was decided by a casting vote from Vice-President Spiro T. Agnew; last year, supporters of the ABM defeated another attack on Safeguard's funding by a vote of 52 to 47, and last week the Administration mustered 64 senators against an amendment seeking to prevent any more money being spent on the ABM system. The amendment gained only 21 supporters.

Safeguard has been hardened to attack in the Senate not because it is thought to be any more capable of providing effective defence of selected missile silos in the United States, but because it is claimed to be essential to the negotiation of an agreement in the Strategic Arms Limitation Talks, the fifth round of which has recently ended in Helsinki. A lesser reason for the ease with which Safeguard gained Senate approval last week was that the Senate Armed Services Committee had already cut the Administration's budget request by $161 million and restricted full-scale deployment of the system in 1972 to the two sites where preparations are already most advanced—at Grand Forks in North Dakota and at Malmstrom in Montana. In addition, the Whiteman Air Force Base in Missouri and the Warren Air Force Base in Wyoming are to be prepared to receive the Safeguard system at a later, so far undetermined, date. The Armed Services Committee also blocked funds designed for deploying ABMs around Washington.

The effect of the Senate's adoption of the Armed Services Committee's bill is to increase the authorization for Safeguard from the $3,700 million that had been approved up to May 31, 1971, to $4,800 million. (The latest estimate of the full cost of setting up ABM defences around Grand Forks, Malmstrom, Whiteman and Warren is now reckoned to be about $7,100 million.) Four of the twelve sites originally planned for ABM defence have been given the go-ahead by Congress, with full scale deployment limited to only two, and the immediate purpose of the system has been restricted to the defence of Minuteman missile silos against Soviet attack.

Since September 1967, when the Sentinel system—the forerunner to Safeguard—was first announced by the Pentagon, the objects of and justification for the ABM have constantly shifted. First designed to provide a light area defence against the possibility of attack by Chinese missiles, the system was later reworked to provide protection for US missiles against Soviet attack, the object being to provide sufficient defence against a first strike to preserve intact enough offensive missiles to cause "unacceptable damage" to the Soviet Union. The Pentagon maintained, however, the option of using Safeguard to provide a light area defence against missiles directed at centres of population if the need arises.

Whatever its proposed role, however, supporters of the system have effectively argued that it is an essential bargaining chip at the Strategic Arms Limitation Talks (SALT), to be used as leverage for concluding an agreement on the limitation of offensive weapons. That argument even won over Senator John Sherman Cooper, a vigorous critic of the ABM and co-sponsor of the amendment that failed by only one vote to stop the project in 1969. Cooper this year decided to vote against the amendment offered by Senator Harold E. Hughes, seeking to block funds for Safeguard, because although Safeguard "remains a system inadequate to defending the Minuteman missile force, the SALT talks are at a stage now where an agreement on the limitation of ABM is attainable".

Critics of the ABM system have in the past based their opposition on three chief propositions: that it will not work, that it is unnecessary and that it is a destabilizing factor in the arms race. Supporters have countered that the Soviet Union's offensive missiles are reaching the stage at which they have the capability of destroying a large proportion of the United States' missiles on the ground by a surprise first strike. Strengthened by the fact that the Soviet Union is also deploying an ABM system around Moscow, the Pentagon argues that if numbers of US missiles are substantially reduced by a first strike, the Moscow ABM defences may be able to cope with the few Minuteman missiles left for retaliation, and the United States' offensive weapon therefore ceases

Nuclear Maginot Line

"IN the course of hearings and debate on this purposed system, except for the claim that it might cure cancer, I have heard almost every conceivable argument for spending billions of the taxpayers' dollars on this nuclear maginot line.

"First, we were told this system was needed to defend the United States against a possible attack from the Chinese. At the same time we were also told that it would provide little if any protection against the obviously far greater missile threat from the Soviet Union.

"The absurdity of this argument became apparent and the proponents of the ABM then switched to a new rationale to attempt to justify its existence.

"The name of the system was changed from Sentinel to Safeguard and its primary mission was changed from area defense to defense of the deterrent; that is, maintaining our second strike capacity. It was argued that its deployment was vitally necessary to protect our Minutemen from the ever-growing Soviet arsenal of SS-9s.

"Anyone familiar with how small indeed is the number of defensive missiles in this system could not help but question the degree of protection it might provide against a possible nuclear attack from the other superpower. Unfortunately, however, the Defense Department refuses to declassify the number of Sprints and Spartans planned for each complex.

"It would appear these officials realize that if they do release even approximate numbers, they will substantiate the claims of those who say the system can be easily overwhelmed through very little additional effort on the part of the Soviet Union; hence the entire system is unprecedented waste of money."

—Extract from a speech by Senator Stuart Symington in the Senate debate on the amendment to block funds for Safeguard.

to become a deterrent. So, the argument goes, the ABM is needed to preserve the balance of power.

The chief flaw in that argument, according to many independent scientists, is that the Safeguard system simply will not be capable of protecting missile silos against massive attack by Soviet weapons. The original Nike-Sentinel project was abandoned essentially because it was thought to be too vulnerable to attack, yet the Safeguard system is based on similar hardware. Moreover, several witnesses before the Armed Services Committee brought up the possibility that if a sufficient number of Soviet missiles were unleashed at silos defended by Safeguard, all the anti-ballistic missiles would be used up and the silo would be left vulnerable. That argument depends in large measure on the ability of the Soviet Union to deploy sufficient numbers of offensive weapons not only to destroy defended missile silos, but also all the other silos operated by the United States.

The scientists' handling of the past debate on the effectiveness of the Safeguard system has, however, recently been called into question in a report published by the Operations Research Society of America (a report on which will be published in next week's issue of *Nature*), but in any case, the ABM was sold to the Senate this year chiefly as a bargaining chip in the SALT talks. The Armed Services Committee argued that "unilateral termination of the Safeguard programme would undermine the American negotiating posture and diminish rather than increase the likelihood of a successful and stabilizing agreement". The suggestion that the negotiating parties in Helsinki were close to agreement on anti-ballistic missiles before the session broke up last month gave more weight to the theory that to abandon construction of Safeguard at this stage would cut the ground from under the feet of Ambassador Gerald Smith, and, on the floor of the Senate at least, the viability of Safeguard took a back seat.

The negotiators in Helsinki were reportedly close to an agreement which would limit ABM deployment to 100 missiles for defence of either capital or of 300 ABMs to defend missile silos. The Soviet Union would be expected under the terms of such an agreement to complete its defence of Moscow by extending the Galosh system, and the United States would almost certainly extend the Safeguard defence of minuteman silos. The chief stumbling blocks, however, seem to be the number of ABMs to be allowed under the agreement, and also the fact that the United States has declared its unwillingness to enter into an agreement applying only to ABMs. The chief US objective at the talks is to use an agreement on ABMs as a lever to halt development of the SS-9 and sea-launched MIRV missiles. Accord is reported to be close on the ABM agreement, and hopes are high that some agreement will be struck in the next phase of the talks to take place in Vienna.

SALT apart, however, the Administration seems to share some of the scepticism of critics of the Safeguard system, for in testimony before the armed services committee, Dr Foster admitted that "if enough SS-11s were sent against a defended Minuteman field, the interceptor supply could be exhausted and the radar could be destroyed". If the deployment of Soviet missiles continues at the rate of the past year, Pentagon officials admit that Safeguard will have to be augmented by an ABM system known as Hardsite. This system relies on a greater number of smaller radars and fast Sprint rockets than Safeguard, and is thought to be less susceptible to destruction. Final cost of Hardsite, although unknown, may turn out to be rather less than that of Safeguard (see box). With serious, unanswered questions about the technical viability of Safeguard, the Department of Defense is probably as anxious as anybody for an agreement at SALT that would stop the system from being fully deployed.

MANPOWER

Jobless Engineers

by our Washington Correspondent

ONE of the first studies of the job situation among scientists and engineers since this year's crop of graduates left the universities has given little room for optimism. The study, carried out by the National Science Foundation, has found that 3.0 per cent of the engineers in the United States are out of work, and the foundation believes that the correct figure could be nearer 3.4 per cent. Derived from a questionnaire mailed to 100,000 engineers in June, this estimate compares with a figure of 1.6 per cent in the summer of 1970, and with a national unemployment rate of 5.8 per cent.

The survey found that holders of master's degrees were the worst hit among those who had been through the universities, with 3.2 per cent of their number out of a job. Holders of the bachelor's degree reported a 2.8 per cent unemployment rate, while 1.9 per cent of PhDs were out of work. A slight consolation for those engineers who had been to university and yet found themselves in the dole queues is the fact that 4.4 per cent of the engineers who do not possess a degree of any kind were jobless in the summer.

An indication of the difficulties experienced by new graduates in finding a job after graduation is the fact that 5.5 per cent of the engineers under 25 years old were out of work—not far short of the national average—while those in the age group 55–64 fared only marginally better with 4.4 per cent of their number jobless.

Other findings of the survey include:

- 78 per cent of the out-of-work engineers replying to the questionnaire gave industry and business as their last employer.
- The unemployment rate for engineers employed in space research was running at 6.3 per cent and defence at 4.8 per cent, while those previously employed in public works only experienced an unemployment rate of 1.3 per cent.
- An indication that the situation seems unlikely to get much better in the near future is given by the finding that more than two per cent of the engineers currently employed said that they had been given notice that their jobs would come to an end before July 1, 1972.

The estimates in the NSF survey should be regarded as minima because they apply only to the engineers belonging to major engineering societies, who could be reached. The response rate to the questionnaire was 65 per cent.

Short Note

Detergents

WITNESSES ranging from William D. Ruckelshaus, Administrator of the Environmental Protection Agency, to Mayor Richard Daley of Chicago shared the billing for one day of hearings held by the Senate Subcommittee on the Environment on the Administration's recent announcement on phosphate detergents. The subcommittee was told that the Administration had not, in fact, given a blanket recommendation to the housewife to switch to high-phosphate detergents, but simply that she should examine the label on the detergent packaging and purchase the product that does not require a toxicity warning. Surgeon-General Jesse L. Steinfeld said, in answer to a question from William B. Spong, the committee chairman, that he had not considered submitting an environmental impact statement to the President's Council on Environmental Quality before making his recommendations concerning phosphate detergents. The National Environment Policy Act requires that all agencies of the federal government submit an environmental impact statement on any project likely to alter the environment in any way.

NEWS AND VIEWS

Transduction of Human Cells by Lambda

THE experiments reported by Merril, Geier and Petricciani on page 398 of this issue of *Nature* will be of the greatest and most startling interest to all biologists, and if they can be independently confirmed they will have very far reaching implications indeed. For what Merril and his associates claim is nothing less than that bacteriophage lambda particles, or naked phage DNA genomes, can infect human fibroblasts, can be transcribed and translated in the milieu of eukaryotic cells and are stably inherited as the fibroblasts multiply through many generations. In short, they believe they have detected the transduction of human cells by transducing derivatives of bacteriophage lambda and fully appreciate all that that implies. This is, of course, a claim little short of the revolutionary. It is inevitable therefore that virtually all readers, having seen the title of this report, will probably find their minds flooding with *a priori* scepticism and prejudice as they begin to read the text. And as Merril and his colleagues no doubt realize and must accept, everybody will be out to find flaws in their work.

What have Merril *et al.* actually done? They have set out to test whether or not bacterial genes work in human cells. A formidable taks indeed; but to make things easier for themselves they have exploited a particular sort of human cell and two transducing viruses derived from phage lambda. The human cells were fibroblasts adapted for growth in culture and derived from a patient with galactosaemia, the inborn error of metabolism which is characterized by an absence of the enzyme galactose-1-phosphate uridyl transferase (GPU transferase) and lack of the ability to take up and metabolize galactose. The two transducing phages derived from lambda carry the *Escherichia coli* galactose operon; in one of these phages the operon is wild type and specifies an active GPU transferase (λ pgal T^+), whereas in the other it carries a mutation which inactivates the transferase enzyme (λ pgal T^-). The experiment is to infect cultures of the galactosaemic fibroblasts growing in flasks with either the particles or the naked DNA genomes of one or other of the transducing phages or with wild-type lambda phage and then assay for the synthesis in the cells of phage specific RNAs and for the appearance of GPU transferase activity, without worrying at all about how the phage particles or their DNA manages to infect the cells or about the fate of the infecting phage genomes.

The assay for phage specific RNA involves, of course, a hybridization experiment. Lambda phage DNA is denatured and immobilized on nitrocellulose filters and is then challenged with RNA extracted from the cultures which have been exposed to phage and labelled with ^{3}H-uridine or from control cultures which have not been infected. As Merril *et al.* describe, the results of such experiments indicate that by 4.5 days after infection with the phage, as much as 0.2 per cent of the total labelled RNA in the cells will hybridize with lambda DNA. By contrast less than 0.005 per cent of the RNA extracted from the controls will hybridize, and less than 0.005 per cent of the RNA from both controls and infected cells hybridizes with *E. coli* DNA. Merril and his colleagues conclude, not unreasonably, that the phage DNA is transcribed in these mammalian cells and, as critics will no doubt be quick to notice, to a perhaps surprisingly great extent if 0.2 per cent of the total labelled cellular RNA is really phage specific. But that is not all; Merril *et al.* assert, although they do not present numerical data, that this expression of the phage genome is not transient but persists at much the same level during some 40 days of culturing which included twice splitting the cultures 1 in 3.

The changes in the activity of GPU transferase, which were assayed by measuring the conversion of galactose-1-phosphate to UDP-galactose in the infected cultures, are equally startling. Extracts made at 4 days and subsequently after subculturing from eight cultures of cells infected with λ pgal T^+, which specifies an active transferase, contain from ten to seventy-five-fold more activity than extracts of uninfected cells or cells infected with λ pgal T^-, which cannot specify an active transferase. The fact that cells infected with the transducing phage λ pgal T^- do not gain transferase activity is a crucial and particularly telling control. This result makes it hard to deny the conclusion that the appearance of transferase in the cells infected with λ pgal T^+ is a direct result of the expression of the galactose operon of the infecting phage genome.

The possibility that the cell cultures might be contaminated with some microorganism is very real and cannot be lightly dismissed but several observations make it extremely unlikely. First, the appearance of transferase activity is blocked by cycloheximide which implies that the enzyme is being made by eukaryotic translation machinery. Second, the addition of penicillin, streptomycin and mycostatin does not affect the appearance of the transferase. And what is perhaps the best argument of all, Merril *et al.* failed to detect bacterial fungal or mycoplasmal contamination in their cultures when they were monitored. As they comment, if the transferase was being made in such amounts by contaminating microorganisms they should have been easy to detect but no contamination was found. One other finding which supports the case that transferase is being made and promotes cell survival is that cells infected with λ pgal T^+ survive 1–3 weeks longer in a medium in which galactose is the sole source of hexose sugars than do cells infected with λ pgal T^- phage.

Although several quibbles can be raised after a close reading of what Merril *et al.* have to say, even the most dyed-in-the-wool sceptics will be very hard put to it to find anything seriously wrong with the design and execution of these experiments. How can the conclusion that the phage genome is not only expressed but maintained for several weeks at least in the human cells be avoided? Merril and his colleagues have not yet made any attempt to determine where in the cell, in the nucleus, the cytoplasm or in the mitochondria, the phage genomes reside but no doubt more than one research group will earn its living trying to answer that and other such questions if these experiments can be confirmed. And assuming that is the case, a great deal of hard thinking will have to be put into the related questions of how the introduction of selected bacterial genes into mammalian cells, so as to alter the recipient's metabolic pathways, can usefully be applied and what hazards might arise from too indiscriminate experimentation with this system. Merril's group have thrown down the gauntlet; those biologists, who through intuition or prejudice disbelieve these results, know how they can accept the challenge.

Anomalous Redshifts Explained?

THE article by Ferencz and Tarcsai on page 404 of this issue of *Nature* will be a relief to orthodox workers in general relativity because it provides an explanation, simple in principle, for certain puzzling phenomena for which very speculative explanations have hitherto had to be constructed.

Three years ago, Sadeh, Knowles and Yaplee reported (*Science*, **159**, 307; 1968) an anomalous redshift in the 21 cm radiation from Taurus A as it passed near the Sun. The extent of the shift was 150 Hz—about one part in 10^7—but it was too great, they claimed, to be explained either by the interaction of the signal with the Sun's atmosphere, or by any effect attributable to the curvature of space time around the Sun according to the equations of general relativity and similar theories of gravitation. For two reasons this anomaly was either worrying or exciting. First, relativistic theories of gravitation are so elegant and illuminating that theorists would be more loath to part with them than with other esoteric theories. Second, the two sources of redshift in well-understood situations, recessional velocity of the source and gravitational fields, provide the best means of measuring masses and velocities of very distant objects. The first mentioned is the best reason for believing that the universe expands. It is suspected that other causes of redshift exist because it is difficult to explain the huge redshifts of quasi-stellar objects; but to find inexplicable redshifts near to the Sun was most perplexing.

Later in 1968, Sadeh, Knowles and Au reported (*Science*, **161**, 567; 1968) a repetition of this experiment and also another phenomenon of similar type. In this experiment a caesium clock was carried about on the Earth's surface and exhibited a decrease in frequency proportional to the distance moved. Again, a timing system, this time a clock instead of a photon, was showing an irreversible decrease in frequency on being moved through a gravitational field. If such a phenomenon were general it would cause the frequency of photons to diminish as they moved through space and would account for 10 per cent of the observed redshift.

Doubts were cast on the validity of this experiment by Markowitz (*Science*, **162**, 1387; 1968). Sadeh and Knowles, in collaboration with Hollinger and Youmans, had also studied the most precise clock in the sky, the pulsar CP 0950, as it was eclipsed by the Sun (*Science*. **162**, 897; 1968). By now a diminution in the frequency of its flashes was expected as its light grazed the solar limb, but none was observed.

At least three radical theories were invoked to explain the measurements. Sadeh, Knowles and Yaplee in their first article had put forward the hypothesis that photons might resemble electrons in losing the energy that they gain by falling into gravitational fields but requiring energy to climb out of them. Such a picture exaggerates the effect of the first experiment by a factor which they calculate to be about 15, explains the small result of the third but provides no clue to the disputed second.

Szekeres explained (*Nature*, **220**, 1116; 1968) the results by a unified field theory. Theories of this type ascribe the electromagnetic field as well as the gravitational field to curvature in the space-time continuum. Szekeres pointed out that if the electromagnetic field was simply proportional to the difference between the "connexions" (geometrical quantities describing displacements between parallel lines)—one for world structure, the other for the actual displacements of light waves—then frequency changes of the type observed in the first and the second experiments could be ascribed to travel through the magnetic fields of the Sun and of the Earth. Laboratory experiments reported later by Shamir (*Nature*, **222**, 362; 1969) did not support this hypothesis, however.

A third theory was that of Woodward and Yourgrau, who pointed out last year (*Nature*, **226**, 619; 1970) that the crucial point is whether or not the relative frequency shifts due to the gravitational doppler effect is frequency dependent. A comparison between the numerical magnitudes of the results of the first two experiments suggests that it is. If this is so, however, it contradicts general relativity, according to which acceleration cannot normally be distinguished from gravity. Accordingly, although Woodward and Yourgrau's model of a frequency dependent interaction fitted all the experimental data, it gave rise to much controversy.

All would be explained if the first experiment was understandable. The second experiment has not been successfully repeated and the third gave a negative result. Even the first is partially contradicted by the fact that experiments with radar pulses grazing the Sun as they echo from the Earth to the planet Mercury give results in accordance with relativity.

Exactly such an understanding is claimed in the article by Ferencz and Tarcsai in this week's *Nature*. The authors cite detailed calculations which for the first time take into account the fact that the Sun's plasma is not at rest nor homogeneous. They account for shifts in frequency well within the limits of experimental error of the first experiment. General relativity is thus vindicated.

But Ferencz and Tarcsai have not simply laid a ghost. Their technique of calculating redshifts in inhomogeneous plasmas may explain in the future the large redshifts of quasi-stellar objects. The original statement about the plasma is beginning to look like a fruitful error.

HAEMOGLOBIN

Down Among the Dimers

from our Molecular Biology Correspondent

Two steps forward to one step back is perhaps the best rate of advance that can be hoped for in a complex field. Following the recent work by Kellett on the dissociation of liganded and unliganded haemoglobin, which I discussed a few weeks ago, the situation—the dust of conflict having apparently settled—appeared to be as follows: oxyhaemoglobin exists under normal conditions of salt and *p*H in an equilibrium state between tetramers and dimers, the concentration of the latter becoming significant only at extremely low protein concentrations. There are no monomers. Deoxygenated haemoglobin is tetrameric and does not dissociate to dimers even at the lowest measurable concentrations and at high ionic strength. What has also long been accepted, and successively confirmed by a series of workers, is that oxyhaemoglobin (or carboxyhaemoglobin and other liganded forms), even at high protein concentrations, dissociates in media of high ionic strength to dimers. Norén, Ho and Casassa (*Biochemistry*, **10**, 3222; 1971), however, say not so, and are prepared to take on the world.

The method which they use is light scattering with a laser source, the long wavelength of emission averting the difficulties caused by haem absorption. The results show that down to a concentration of less than 10^{-4} M in haem, 2 M sodium chloride has essentially no effect on the molecular weight. From the invariance of molecular weight with protein concentration over the measured range it follows that the tetramer–dimer equilibrium constant must certainly be lower than about 10^{-6} mole/l. Norén *et al.* then take a hatchet to earlier work, based variously on light scattering, osmometry, sedimentation equilibrium and sedimentation velocity. Earlier light scattering results, for example, stand condemned by internally inconsistent refractive index increment measurements, which failed to reveal the considerable dependence of this parameter on salt concentration. Other evidence is likewise revealed as flawed. The moral again is that work on nonideal polydisperse systems in mixed solvents is fraught with hazards.

The notion that lamprey haemoglobin, being apparently a monomer, would present a simpler system for study has also turned out to be risible. The oxygenation properties, it was soon established, are concentration dependent, and, worse yet, there is a Bohr effect. Briehl then found that the deoxygenated form is not predominantly monomeric. A re-examination of this haemoglobin is now to be found in an article by Andersen (*J. Biol. Chem.*, **246**, 4800; 1971).

In the deoxyhaemoglobin the sedimentation equilibrium distributions can be fitted by equilibrium constants for association of monomers to dimers, and dimers to tetramers. The first process is *p*H-dependent and seems to involve a single charged group on each monomer. This at once explains the Bohr effect. The liganded haemoglobin also forms dimers at sufficiently high concentration, and apparently ultimately tetramers as well. At the haemoglobin concentration of 25 per cent in the red cell, the equilibrium constants as determined dictate that the deoxygenated pigment will be largely dimeric and the oxygenated form monomeric. Oxygenation will thus lead to dissociation of the protein. This will generate a measure of cooperativity at these physiological protein concentrations—a mechanism which may be seen as a precursor in this evolutionary prototype to that in more advanced models. In an accompanying article, Andersen and Gibson (*ibid.*, 4790) examine the kinetics of ligand binding by lamprey haemoglobin, and show that they can now be more or less quantitatively explained, assuming only that the monomer has a high and the dimer a low ligand affinity.

The cause of the earlier, and erroneous, impression that human oxyhaemoglobin would dissociate at sufficiently low concentrations to the monomeric state proved to be the ease with which, under these conditions, the iron is oxidized to the ferric state. The methaemoglobin so formed has always been recognized as being unstable.

Bucci and Fronticelli (*Biochim. Biophys. Acta*, **243**, 170; 1971) have now examined the behaviour of isolated α-chains in the ferric state. The monomers are prepared by treatment of native haemoglobin with a thiol reagent, which can be stripped off after fractionation of the two chains. When the isolated chains were converted into the ferric state, the result was not in fact methaemoglobin, but a type with the characteristic absorption spectrum of a ferric haemochromogen, in which it is supposed that the iron is linked on both sides of the haem plane to a nitrogenous base. Judged by optical activity there is some loss of ordered structure, rather on the lines found in haem-free globin. In the ferric β-chains the effects are even more drastic. Reduction of the new type to the ferrous state failed to regenerate the native structure. The ferric chains evidently exist only in this modified and monomeric condition.

ADENOVIRUS

Hybridizers Beware

from our Cell Biology Correspondent

ANYBODY who has recourse to DNA-RNA hybridization techniques, and in particular competition hybridization, would be well advised to cast more than

---

Mouse Haemoglobin made *in vitro*

IN *Nature New Biology* next Wednesday two groups, Lockard and Lingrel and Mathews, Osborn and Lingrel, describe the synthesis *in vitro* of mouse globin and haemoglobin and conclude that the 9S murine globin messenger RNAs can be translated in cell free systems derived from ascites cells or reticulocytes of species other than the mouse. At least for this messenger neither tissue specific nor species specific initiation factors seem to be required for translation.

Lockard and Lingrel describe the translation of mouse reticulocyte 9S messenger RNA in a rabbit reticulocyte cell-free system and also mention that this messenger is translated in guinea-pig and duck reticulocyte systems. By fingerprinting the product of translation and by studying its behaviour on chromatography and gel filtration they conclude that both mouse α and β globin chains are being made and therefore the 9S RNA must contain messengers for both these molecules.

Mathews, Osborn and Lingrel have used this 9S RNA to programme a cell-free system obtained from mouse Krebs ascites cells, which were originally derived from a mouse mammary carcinoma. They find that without the addition of any extract from reticulocytes both α and β globin chains are made. In short, the Krebs ascites cells contain all the factors required for the accurate initiation, elongation and termination of globin chains.

Recently Stavnezer and Huang reported in *Nature* (**230**, 172; 1971) that mouse messenger RNAs specifying the light chain of immunoglobulin are translated in the rabbit reticulocyte cell-free system *à la* Lingrel and Lockard. This result, taken together with those published in next Wednesday's *Nature New Biology*, suggests that the hypothesis that the translation of particular messengers depends on the existence of tissue-specification initiation factors may not rigorously apply to eukaryotic cells. Alternatively it can be suggested that tumour cells such as Krebs ascites cells, because they are cancerous, retain the capacity to translate a wide range of messengers and, further, that the factors in reticulocytes which initiate translation of globin messengers work also with immunoglobulin messengers.

just a glance at the contents list of the current number of the *Journal of Virology* because it contains a report on the synthesis of adenovirus 2 RNA in infected KB cells by Lucas and Ginsberg (*J. Virol.*, **8**, 203; 1971). This is also a cautionary tale with a moral extending far beyond the biology of adenovirus replication. Lucas and Ginsberg show that the results obtained from competition hybridization experiments depend greatly on the precise technique which is used.

Lucas and Ginsberg apparently began their experiments with two objects in mind; first, a re-analysis of the regulation of adenovirus 2 RNA synthesis in permissive KB cells and, second, a comparison of the current competition, or to be more accurate, presaturation hybridization techniques. They first showed by simple RNA.DNA hybridization that by 6 hours after infection in the presence of FUDR, which blocks DNA synthesis, adenovirus RNA can be detected in extracts of total cytoplasmic RNA and that if polysomal RNA is isolated virus specific sequences can be detected as early as 3 hours after infection, which is long before the replication of adenovirus genomes begins. Furthermore, in the absence of FUDR the amount of ^{3}H-uridine incorporated into viral RNA continues to increase for 20 hours after infection, by which time about 40 per cent of the total label entering the cells ends up in viral RNA.

By first prehybridizing the viral DNA with RNA made at late times and then measuring the extent to which labelled RNA made at early times can hybridize, Lucas and Ginsberg attempted to determine whether there exists a class of early RNA which is not made at late times. And by varying the experimental procedure they determined how results of hybridization experiments are influenced by differences in manipulation. The first procedure they used, which they believe is rigorous, involved treating the filters with ribonuclease after the prehybridization step as well as after the challenge step with early RNA. This procedure revealed three classes of adenovirus RNA; a class of early RNA (70 per cent of the total RNA made at early times), the synthesis of which begins before DNA replication starts, and ceases some time after such that by 18 hours this RNA can no longer be detected; a class of early RNA which continues to be synthesized at increasing rates throughout the infection; and a class of late RNA made only after DNA replication has begun.

With the two other procedures, in which the first ribonuclease digestion is omitted and the prehybridized filters are simply washed in saline, results are obtained which indicate that from 40 per cent to 98 per cent of the RNA sequences made at early times are made throughout the infection. In other words these less rigorous hybridization procedures fail to reveal the class of true early RNA which is made before DNA replicates and then switched off as DNA replication gets under way. As Lucas and Ginsberg remark, in the light of these observations there is a strong possibility that differences in experimental technique rather than biology may account for the conflicting data in the literature concerning the patterns of transcription of numerous animal viruses.

The early adenovirus 2 RNA made in KB cells has been analysed on polyacrylamide gels by Parsons and Green (*Virology*, **45**, 154; 1971) and compared with the viral RNA made in nonpermissive rat cells transformed by this virus. They have resolved the 2–6 hour cytoplasmic RNA from KB cells into two chief components (23S and 16S), which are made in the same relative amounts and to an amount five-fold greater in the presence of cycloheximide, which blocks cell and viral protein synthesis. The parallels between the pattern of transcription of adenovirus 2 and a phage like T4 are clear. By contrast in transformed rat cells adenovirus 2 RNAs in both nucleus and cytoplasm are heterogeneous in size and at least some of these RNAs are so large that they may well comprise molecules with both viral and host sequences.

PARASITOLOGY

Plasmodium Problems

from a Correspondent

Of the many species of malaria parasites available to research workers, perhaps the most widely used is the rodent species *Plasmodium berghei*. Although the species was first isolated in 1948, it was not until 1964 that its complete life cycle was elucidated and methods for routine laboratory maintenance developed. In spite of these achievements, many laboratories using the *Plasmodium berghei–Anopheles stephensi* system still experience difficulties in cyclical transmission. Frequently these difficulties seem to be attributable to concomitant infections with other organisms, in both rodents and mos-

Filament Arrays in Mammalian Smooth Muscle

During the past few years, the structure of the myosin-containing elements in mammalian smooth muscle has been providing a fertile field of research for electron microscopists, and several different types of structures have been reported. The chief protagonists in the field are Lowy and his colleagues at Aarhus University who see ribbon-like structures, and two groups in Pennsylvania headed by Rice and Somlyo, who see roughly circular filaments similar to the thick filaments in striated muscle. The discrepancy probably arises because vertebrate smooth muscle is a spontaneously active muscle and different techniques have been used to try to relax it and to preserve the structure as it exists *in vivo*.

Lowy *et al.* (*Nature*, **227**, 46; 1970) observed ribbons in muscles which had been equilibrated in Krebs solution for 3–4 hours at 0° C supporting a 10 g weight (which produced an extension of 3–10 per cent). The Pennsylvania groups, however, observed fairly regular arrays of circular thick filaments in muscles which had been stretched to 1.5 times excised length, incubated for 30 minutes in a Krebs solution and fixed either in this condition or with a relaxant added (*Nature New Biology*, **231**, 242 and 243; 1971; *J. Cell Biol.*, **49**, 636; 1971).

Furthermore, the Pennsylvania groups found that if the muscles were excessively stretched (2.1–2.6 times excised length) or incubated in hypertonic media, then ribbons were seen. They therefore suggested that arrays of circular filaments are the normal structure existing *in vivo*, and that the filaments only aggregate to form ribbons under non-physiological conditions which abnormally decrease the inter-filament spacing.

Because there were indications that the circular filaments themselves could only be seen in any numbers when stretch (up to 1.5 times excised length) was applied, Somlyo *et al.* have now investigated this problem further (see next Wednesday's *Nature New Biology*), and obtained micrographs showing arrays of circular filaments in completely unstretched muscles. Somlyo *et al.* suggest that the reason why filaments are seen in stretched and incubated preparations is simply because of better orientation along the fibre axis, and recovery from dissection trauma respectively.

The electron micrographs of Somlyo and his colleagues clearly show arrays of circular thick filaments in mammalian smooth muscles at all physiological lengths. It would be interesting to know whether the X-ray pattern *in vivo* bears out their observations: is there an equatorial reflexion arising from these filament arrays, and can the shape of the 144 Å meridional myosin reflexion be accounted for by such arrays?

quitoes. It was timely therefore that the first European multicolloquy of parasitology, which was held at Rennes from September 1 to 4, should include a section devoted exclusively to the interaction of *P. berghei* with other organisms.

Problems concerned with blood infections were covered by Professor W. Peters (University of Liverpool). Both *Eperythrozoon coccoides* and *Haemobartonella muris*, themselves parasites of red cells, may severely limit the development of *P. berghei* and other blood protozoa. Their presence, either in recipient laboratory rodents or in wild rodents, may interfere with the growth of protozoa during the attempted isolation of new strains, and may give the false impression that either the donor is uninfected or the recipient insusceptible. Both organisms can be eradicated by treatment with neoarsphenamine, which has no effect on *P. berghei*.

Infections of mosquitoes which appear to suppress development of oocysts and sporozoites were discussed by several contributors. Drs C. Draper and R. Bird (London School of Hygiene and Tropical Medicine) described a cytoplasmic polyhedrosis virus in the midgut cells of *A. stephensi* from a colony in which *P. berghei* transmissions were proving difficult. Elaboration of viruses was seen within oocysts, and some were observed inside malformed sporozoites. Drs R. Howells and E. Davies (University of Liverpool) have also observed viral particles in midgut cells and oocysts, as well as rickettsia-like organisms which seemed to interfere with *P. berghei* development because they were never seen in mosquitoes containing oocysts. Dr R. Hulls (Imperial College, London) showed that the microsporidan *Nosema algeri* can reduce the numbers of developing oocysts, and cause oocyst degeneration resulting in a severe depletion in the number of sporozoites reaching the salivary glands.

But not all concomitant infections are harmful. Dr F. E. G. Cox (King's College, London) pointed out that mice which have recovered from infections with the piroplasms *Babesia microti* and *B. rodhaini* and the dactylosome *Anthemosoma garnhami* are strongly resistant to challenge with *Plasmodium vinckei*, but not to challenge with *P. berghei*. Professor J. Jadin and his colleagues (Institute of Tropical Medicine, Antwerp) showed that the bacterial fauna of the mosquito gut could play an important part in the nutrition of oocysts. By labelling *Pseudomonas* with carbon-14 and then feeding them to mosquito larvae, they were able to demonstrate the uptake of the label into oocysts and sporozoites. Perhaps the most unusual example of interaction between parasites was that described by Dr S. Rădulescu and his colleagues ("Dr I. Cantacuzino" Institute, Bucharest), who have studied the effect of *P. berghei* on infections of *Giardia muris*. *G. muris* is a protozoan which is normally restricted to the intestine of mice, but in heavy infections can invade other tissues. A much higher incidence of tissue invasion was found in mice infected with *P. berghei*.

MAGNETIC RESONANCE

Discovery Celebrated

from a Correspondent

THE celebration of the twenty-fifth anniversary of the discovery of nuclear magnetic resonance (NMR) was a special feature of the fourth international symposium on magnetic resonance held in Israel at Rehovot and Jerusalem from August 24 to 31.

Professor Felix Bloch (Stanford University) gave an entertaining account of the thoughts which led to his part in the discovery of NMR, and of the first experiments. Hansen and Packard built the electronic apparatus and Bloch's experimental assignment was the calibration by ballistic galvanometer of the magnetic field of a borrowed lecture-room electromagnet. This calibration was evidently not quite perfectly done, with the result that efforts to see the resonance were made in vain. Finally, on switching off the magnet current to make some adjustments for the next attempt, they saw the signal drift across the oscilloscope screen. This experiment had cost only a few hundred dollars, most of which was spent on the oscilloscope. Their announcement in *Physical Review* appeared one issue later than the letter by Purcell, Pound and Torrey announcing their independent discovery. The first mutual discussion in the spring of 1946 called for integration of two different viewpoints of the same phenomenon: on the west coast they spoke of nuclear induction and precession, but in the east the terminology was of transitions and resonance absorption. Was it curiously coincidental that such a discovery should be made at just the same time by unrelated groups of workers? Professor Bloch thought not, and he instanced another striking example from the early days of quantum mechanics.

The growth of the subject from these early beginnings was illustrated by the great diversity of applications covered in the 170 contributions presented by participants from some thirty countries. High-resolution NMR spectroscopy has become an instrument that no reputable

---

Specificity of Non-histone Protein-DNA Complexes

IT is known that the histones are non-specific gene repressors and that they do not vary from tissue to tissue. Because DNA is also not tissue specific the important problem remaining is what type of macromolecule, or macromolecular complex, is capable of specific interaction with chromatin to maintain or change the pattern of active and inactive genes. John Paul's "chromatin" group at the Beatson Hospital in Glasgow has come down in favour of the non-histone chromatin proteins for this role, whereas James Bonner at the California Institute of Technology feels that an RNA complex is involved.

In next Wednesday's *Nature New Biology*, Chytil and Spelsberg add more weight to the non-histone protein side by showing that some of the antigenic properties of non-histone protein-DNA complexes isolated from chromatin are tissue specific.

The non-histone protein-DNA complexes were prepared from various chick organs by a mild procedure which did not result in their dissociation. They were then all tested against antisera produced by a similar complex isolated from the oviduct of chicks after treatment with diethylstilboestrol. In brief, the only reaction obtained was that from the homologous non-histone protein-DNA complex.

A similar result was obtained with the intact chromatin of the various organs. Using the native chromatin from the chick oviduct and comparing it with the results from the chick oviduct non-histone protein-DNA complex, Chytil and Spelsberg estimate that at least 20 per cent of the antigenic sites located on the non-histone protein-DNA complex remain accessible in the native chromatin.

Chytil and Spelsberg tentatively suggest that some of the non-histone protein is associated with the transcribable regions of DNA, and show, in support of this, that there is a substantial lowering of transcription when specific antibody is added to the homologous antigen (chromatin). This is an interesting demonstration of tissue specificity of a complex, isolated from chromatin, but as Chytil and Spelsberg mention, the specificity may not arise from specific proteins, but from a specific structural relationship between similar proteins and DNA. The unambiguous demonstration of tissue specificity of non-histone chromatin proteins remains a challenging problem.

chemistry laboratory can do without, and several contributions demonstrated the latest developments, which notably included Fourier transform spectroscopy—giving greatly enhanced sensitivity especially for ^{13}C and ^{15}N, and for protons in weak concentration. The fortunate owners of spectrometers based on superconducting magnets presented proton spectra with impressive resolution at frequencies greater than 200 MHz.

Wide-line NMR was strongly represented with many contributions on topics ranging from metals and magnetic materials to ferroelectrics and polymers. Of special interest was the report by Dr M. Goldman (Saclay Laboratory) of the discovery of nuclear antiferromagnetism of the ^{19}F nuclei in calcium fluoride when cooled to a spin temperature below 10^{-6} K by nuclear adiabatic demagnetization. No longer is nuclear magnetism merely a branch of paramagnetism, and no longer is the study of ordered magnetic materials the prerogative of experimenters working with materials containing unpaired electrons.

The audience had glimpses of high-resolution NMR in solids too as the broadening interactions were stripped away in copper and copper compounds by high-speed magic-axis rotation (Professor E. R. Andrew, University of Nottingham), by special pulse sequences (Dr U. Haeberlen, University of Heidelberg) and by magic sandwiches (Professor J. S. Waugh, Massachusetts Institute of Technology).

A feature of the conference was the growth of the applications of magnetic resonance to systems of biological interest. Altogether there were twenty-five contributions on enzymes, membranes, cells and cell constituents, and without doubt the next international conference on magnetic resonance (in Bombay, 1974) will see further development in this important area.

PROSTHESES

Plastics in Medicine

from a Correspondent

THE symposium on plastics in medicine and surgery which was held at the University of Newcastle upon Tyne on September 15 and 16 was jointly organized by the north-east section of the Plastics Institute and the University of Newcastle upon Tyne Medical School with the object of bringing together the medical users of plastics and the producers.

A great deal of stress was laid on the proper selection of materials which are compatible with the internal environment of the body and the questions of toxicity, chemical stability, initiation of clot formation, allergy and carcinogenesis were discussed. It was also suggested that the physical form of the implanted material may in some circumstances be as important as its chemical structure. One conclusion was that guide lines for the testing of plastics for medical application are urgently required. There seemed some doubt, for example, as to whether testing the toxicity of a plastic to a tissue culture medium is a realistic measure of its likely performance in the body.

One session was spent discussing some of the technical problems of designing membrane oxygenations and dialysing equipment for patients with chronic renal disease. Professor R. A. M. Kenedi (University of Strathclyde), together with several other speakers, stressed the importance of mechanical design and testing of prostheses which were going to be implanted into the human body. Several surgeons recounted their clinical experiences using plastic materials for reconstructive surgery and for joint replacement.

There is no doubt that this first confrontation between members of the plastics industry and the medical profession was extremely valuable. It was clear to all the participants that, generally speaking, the plastics at present used in medicine have been drawn from the commonly available materials which were not designed with such uses in mind and that therefore much requires to be done in determining the ideal specifications that will meet the doctor's requirements. Unfortunately, just formulating a requirement is not sufficient for, as Sir Harry W. Melville, president of the Plastics Institute, said, the plastics industry thinks in terms of producing 100,000 tons of plastics at a time. Thus there is a financial difficulty because not only will a great deal of money have to be spent on development in producing special materials but also the total national demand may only be for a kilogram or two of the product.

Finally, there seemed to be general agreement that a national body should be set up to collect information from both the plastics industry and the medical profession with the object of aiding the future development of the use of plastics in medicine and surgery.

Changes in Membranes during the Cell Cycle

A DIVERSITY of functions is believed to be carried on at the membranes which bound all living cells and the organelles within them. Correspondingly, electron microscopy shows a variety of membrane contours, and the constituent proteins and lipids occur in all proportions. (The rolled-up membrane forming myelin is 75 per cent lipid whereas the membrane surrounding the tiny gas bubbles in some forms of alga is entirely protein.)

Another side to this diversity is the possibility that a membrane changes during the life of the cell. Evidence that the structure so varies is reported by R. E. Scott, R. C. Carter and W. R. Kidwell of the National Institutes of Health, Bethesda, in next Wednesday's *Nature New Biology*. Known techniques are used to synchronize the cells in a culture so that, in step, the cells divide into two daughter cells, grow and duplicate essential molecules, and divide again. The cells are examined at various times after the initial division, and the structure of the membrane is seen to vary in a regular way.

The cells are examined by the method of freeze cleavage. A suspension of the cells in buffered glycerol-saline is frozen in liquid nitrogen and is cleaved at low temperature and under vacuum. A replica of the newly exposed surface is made by shadowing it with platinum-carbon and the replica is cleaned of the organic material and viewed in the electron microscope. The question, what surface is exposed by cleaving, was previously answered by the striking micrographs of da Silva and Branton (*J. Cell Biol.*, **45**, 598 ; 1970) and of Tillack and Marchesi (*ibid.*, 649). Large molecules of known appearance (ferritin and F-actin) are put on the surface of red blood cells. These molecules are not seen on the cleaved surface; they appear only after ice is allowed to sublime, revealing the cell surface adjacent to the cleaved surface. Thus the surface of cleavage is within the membrane. The complementary surfaces made by the cleaving are not identical. Rather the inside half of the cell membrane shows many more particles than the extracellular half.

Scott *et al.* report a large variation in numbers of particles during the cycles of two kinds of cell. Shortly after division the daughter cells show the lowest number of the characteristic 85 Å diameter particles per square micron of membrane. Then the number gradually doubles to the level seen before division. The numbers of particles remain in proportion on the two surfaces. Similarly there is a cyclic variation in the number of the large pores in the membrane bounding the cell nucleus. The density of pores is low shortly after division and then quickly trebles during the following growth phase. Scott *et al.* believe that the 85 Å particles are glycoprotein and lipid.

[illegible] The Getty Library.

BACTERIOPHAGES

Lysogeny versus Lysis

from our Cell Biology Correspondent

THE infection of a susceptible bacterium by a temperate bacteriophage can result either in the lytic replication of the phage and the death of the host or lysogeny, the integration of the repressed phage genome into that of the host which survives the infection. But what determines in the first few minutes after infection which way the infection will go? The answer to this perennial question, according to Hong, Smith and Ames (*Proc. US Nat. Acad. Sci.*, **68**, 2258; 1971), is the intracellular concentration of cyclic AMP, that ubiquitous regulatory molecule. They reach this conclusion from investigations of the influence of the physiological state of *Salmonella typhimurium* on lysogenization by the temperate phage P22.

Mutants of this bacterium which have defective adenylate cyclase (the enzyme which catalyses cyclic AMP production) or defective cyclic AMP receptor protein (the protein which when active binds cyclic AMP and activates RNA polymerase) are lysogenized by P22 at considerably lower frequencies than wild type *S. typhimurium*. This is also true of *rif* mutants of this bacterium which have an altered β subunit of RNA polymerase. Hong *et al.* suggest that it is no coincidence that these three classes of mutants not only reduce the activity of RNA polymerase but also cause the infecting P22 phage to replicate and lyse the cells rather than to lysogenize them.

They believe that in wild type cells cyclic AMP, by binding to the cyclic AMP receptor protein, activates wild type RNA polymerase to transcribe the phage genes, the expression of which establishes lysogeny. The amount of cyclic AMP in the cells will determine between lysis and lysogeny. Because of catabolite repression when there is an abundant supply of energy and little cyclic AMP, lytic replication will occur, but when energy is in short supply and there is a high concentration of cyclic AMP, lysogeny will predominate. The selective advantage of such a regulatory system to the phage is obvious and, as Hong *et al.* comment, other groups have made, and are about to publish, similar observations and conclusions made with other host and phage combinations; cyclic AMP is therefore probably the deciding factor in all infections by temperate bacteriophages.

In the same issue of the *Proceedings* (*ibid.*, 2185, 2190), Reichardt and Kaiser and Echols and Green describe in detail the genetic elements that regulate the synthesis of λ phage repressor protein which is required to establish and maintain lysogeny of *Escherichia coli*. The story that emerges from their work is that there are two pathways leading to the synthesis of λ repressor which is specified by the *CI* gene. This gene apparently has two promoter sites *prm* (promoter for repressor maintenance) and *pre* (promoter for repressor establishment). Two proteins specified by the genes *CII* and *CIII* activate the *pre* promoter during the establishment of lysogeny by binding to a DNA site *cY*. Mutants of genes *CII* or *CIII* of site *cY* prevent the transcription of gene *CI* and hence the synthesis of repressor and the establishment of lysogeny.

Once repressor has been made with wild type λ phage using the *pre* promoter and lysogeny has been established, the smaller amounts of repressor required to be made continuously to maintain the lysogenic state are transcribed from the *prm* promoter which becomes active when repressor molecules made from the *pre* promoter bind to an operator site *Or*. The *pre* promoter activated by *CII* and *CIII* gene proteins is more efficient, in the sense that it allows greater amounts of repressor to be made, than the *prm* promoter activated by repressor itself. This dual pathway therefore allows the phage to make repressor in large quantities early in the infection and so to establish lysogeny and in smaller amounts subsequently. Echols and Green certainly do not exaggerate by saying that "the pattern of regulation for even so simple a creature as phage λ can be relatively complex".

More about Radio Emission from Air Showers

RADIO methods of studying extensive air showers are comparatively new: the first announcement that radio pulses had been detected in coincidence with the shower particles was made by Jelley *et al.* in 1965 (*Nature*, **205**, 327; 1965). Several features of the emission now seem to be firmly established. First, the principal cause of the emission is the transverse deflexion of the shower particles by the Earth's magnetic field—the so-called geomagnetic mechanism; and second, the pulses are large enough to be detectable only because of coherence—in other words, because of the superposition in phase of radiation coming from different stages in the shower development. The radiation pattern on the ground—the radio pulse lateral distribution—is essentially a diffraction pattern determined by the longitudinal development of the shower, and a promising way to study the longitudinal development is through the radio emission. A major astrophysical interest at the present time is the chemical identification of the high-energy cosmic ray primary particles that produce extensive showers in the atmosphere, because this has an important bearing on the problem of cosmic ray origins. It seems that a study of the longitudinal development through the radio emission will contribute significantly to the solution of this problem.

It is therefore of more than passing interest to have a thorough understanding of the mechanism by which the radio emission is produced. Geomagnetic deflexion is certainly the dominant process, but other possible mechanisms have been suggested, and it is important to know quantitatively their relative importance. A careful study by Prescott and his colleagues in Calgary (see the next issue of *Nature Physical Science*) gives a first answer to this question.

The starting point is the knowledge that geomagnetic deflexion is greatest when a shower moves with its axis at right angles to the Earth's magnetic field lines and is zero when the axis and the field lines are parallel. The experimental aim is to plot the radio pulse amplitude as a function of the sine of the angle θ_g, between the shower axis and the field lines, keeping other parameters constant. From the graph, the residual amplitude is determined when $\theta_g=0$. For a pure geomagnetic mechanism this amplitude would be zero.

The experiment is complicated by the fact that showers with different θ_g usually have different zenith angles as well. So although two showers have the same number of particles at sea level, their total energy can be different, and the shower maxima can be at different distances from the radio antenna. Unless a correction is made for these differences, it is inadmissible to attribute changes in observed radio pulse amplitude entirely to θ_g. Prescott and his collaborators consider this problem in detail and use other experimental information to determine the necessary corrections. They also make allowance for the background noise level, subtracting this quadratically from the measured amplitude to obtain the true amplitude of the radio pulse.

The measurements were made at a frequency of 22 MHz on showers of size $\sim 10^6$ particles, or shower energy $\sim 10^{16}$ eV. A residual pulse height was found at $\theta_g=0$ of amplitude 14 ± 6 per cent of the amplitude when $\sin\theta_g=1$. This component of the radio emission cannot be attributable to geomagnetic deflexion, and Prescott *et al.* speculate that it may possibly be caused by the quasi-Čerenkov charge excess mechanism which was one of the first mechanisms to be considered as the source of the radio emission.

NUCLEAR PHYSICS

Oxford Meeting

from a Correspondent

THE Institute of Physics conference on nuclear and particle physics (Oxford, September 22 to 24) was attended not only by a good representation of British institutions but also by a pleasing number of European nuclear physicists. It thus achieved a standing aim of any such meeting, namely to promote within it the numerous informal discussions on which the subject feeds. The proportion of contributions on nuclear reactions seemed smaller than hitherto, perhaps reflecting some shift in effort. In the nuclear structure papers there was a numerical majority of more than 2:1 in favour of light nuclei (say $A<50$) over heavy nuclei (say $A>70$).

On the first day, Professor H. J. Mang (Technical University, Munich) reviewed the problem of making the collective model respectable, physically and mathematically. For some time the collective model has enjoyed much success in "explaining" the properties of many nuclear excited states in terms of the quantized rotations or vibrations of a drop of nuclear fluid. The ultimate aim, of course, is to relate apparent collective motion to individual particle motion and inter-nucleon focus. A unified model, however, which embodies both the collective (macroscopic) and single particle (microscopic) motions thereby reaps the problem of redundant variables, that is, it has more co-ordinates than necessary to specify a nuclear state. Perhaps few nuclear physicists would abandon a relatively successful model in deference to this mathematical nicety. Professor Mang indicated, however, that a more sophisticated and satisfactory model could reproduce the results of, for example, the *ad hoc* phenomenological variable-moment-of-inertia model.

Dr A. Lane (AERE, Harwell) surveyed non-statistical effects in resonance reactions. The compound nucleus theory of nuclear reactions, where the reaction between two nuclei proceeds through states of the aggregate intermediate nucleus, has been available for a long time. Often the nuclear quantities which arise—the spacings and widths of the compound nucleus energy levels and the magnitudes and phases of reaction scattering amplitudes—are determined by so much unknown nuclear detail that they have been traditionally subject to an overall statistical analysis. As Dr Lane has emphasized, departures from the statistical theory are of special interest and beg physical explanation. For example, a doorway state (a brief engagement between nuclei before complete wedding) may explain the observed correlation between cross-sections for different nuclear reactions which proceed through the same compound nucleus. Dr Lane gave examples of other apparent correlations, too numerous to give here, and ideas which may explain them. It seems that this subject has gained impetus from more and improved data.

Professor G. Goldring (Weizmann Institute of Science, Rehovot) spoke about the hyperfine interactions of nuclei in highly ionized atoms. The angular distribution of decay gamma rays from an excited nucleus may be perturbed when the nucleus recoils in a highly ionized atom out of a target. The perturbation arises from the precession of the nucleus caused by the interaction of its excited state magnetic moment with the atomic magnetic field. This phenomenon provides an interplay between atomic and nuclear physics. Dr Goldring concentrated on results of recent work with light recoil ions. He reported an elegant experiment in which the angular distribution of gamma rays from an excited state of ^{18}O was found to be dependent on the charge state of the recoiling oxygen ions. This was done by counting γ-rays in coincidence with ^{18}O ions which were separated in their various charge states by a magnetic spectrometer. The angular distribution associated with the 6^+ ionic charge state was not attenuated whereas that associated with the 7^+ ion usually had its one electron in the 1s atomic ground state. It was inferred that this followed from the rough rule that electron exchange in the target is most likely when the orbital velocity of an ionic electron equals the velocity of the moving ion. Thus if the atomic states of the electron are well separated in energy (that is, the lower states), it may be possible to populate preferentially a particular state by proper choice of recoil velocity.

From the point of view of the high energy physicist, the meeting was a stimulating experience. The most interesting facet was perhaps the devotion of a whole day to a review, in four talks, of the present status of some of the problems in the field of astrophysics. It seems that the parochial attitudes common in nuclear and particle physics are not reflected in astrophysics, and that any physicist interested in escaping from the rat race of, say, particle physics would be a welcome credit to the ranks of those people studying the astrophysical implications of their work. High and low energy nuclear and particle physics have vital parts to play in accounting for many of the phenomena. Professor D. Sciama (University of Oxford) discussed the growing evidence that the origin of the universe lies in the "hot, big bang" rather than in the alternative form of a steady state. The evidence comes from studies of the integral number intensity distribution of radio sources, the redshifts in quasars and the galactic background of microwave radiation. All point to an evolving universe as one goes back in time. It is also evident from a knowledge of the relative abundances of hydrogen and helium that nuclear genesis requires more than thermonuclear reactions to account for these abundances; neutrino reactions at temperatures in the region 10^9–10^{10} °K can lead to the known He/H abundance ratio.

Aspects of high energy astrophysics that have emerged with the discovery of pulsars, the study of X-ray and γ-ray spectroscopy, and, of course, the

---

Cosmic X-rays not from Galaxy Clusters

THE importance of diffuse cosmic X-rays to cosmology lies in the agreement of most theories that this background must contain a significant contribution from sources at a redshift of $z=1$. According to most models the degree of isotropy in this X-ray background provides an insight into the isotropy of the universe at the epoch corresponding to $z=1$. But the scale of fluctuations observed over the sky is not consistent with any origin for the radiation in clusters or superclusters, and refinements in technique have now resulted in a lower limit of 5×10^5 sources spread uniformly across the sky, if the radiation does indeed originate in discrete sources. If the sources are assumed to have constant absolute luminosity, rather than constant apparent luminosity, this limit is raised to 4×10^6 discrete objects, according to the latest evaluation of the statistics by Schwartz *et al.* in next Monday's issue of *Nature Physical Science*.

The rocket detector used in this work operated in the range 2–32 keV, roughly comparable with earlier work on the isotropy in the 10–40 keV range (Wolfe and Burbidge, *Nature*, **228**, 1170; 1970). It seems clear that the supercluster or cluster models for the origin of the diffuse cosmic X-rays cannot stand up in the light of the work of Schwartz *et al.* and Wolfe and Burbidge, at least in the naive form of a direct proportionality between cluster density and X-ray intensity. The limits set by the latest work provide the constraints to which any alternative models must conform.

need to explain the nature of the acceleration of cosmic rays to very high energies were reviewed by Dr M. Rees (Institute of Theoretical Astronomy, University of Cambridge). According to Dr Rees, the possible identity of some pulsars with neutron stars suggests that there is no need to look further than neutron stars for an accelerating mechanism for cosmic rays; an estimate of the potential difference between the pole and equator of a neutron star is $\sim 10^{16}$ eV. (Food for thought among high energy physicists might be found in the comparison of the energies available in cosmic rays—upward of 10^{15} eV per particle in many cases—with those from the highest energy man-made accelerators.) The nature of neutron stars was considered from the point of view of a solid state physicist by Professor P. W. Anderson (University of Cambridge and Bell Telephone Laboratory), who showed how considerations from this field of physics which deals with normal matter can lead to speculations about the nature of the matter in white dwarf and neutron stars. Starting from basic ingredients, that is, a knowledge of the gas, liquid and crystalline solid states and superfluidity, Professor Anderson postulated a model for the structure of a neutron star. It is a relatively cool object ($\sim 10^8$ °K) and, at the surface, is thought to be composed of matter of white dwarf densities. Passing inwards towards a core there are liquid and solid phases and then a gas phase which has superfluid properties. The nature of central core region is not defined, but is thought to contain nucleons in their excited states (hyperons).

The role of the nuclear weak interaction has long been recognized in speculations about the source of stellar energy, and Professor L. Radicati (University of Pisa) discussed this work. It now seems clear from the work of Dr R. Davis (Brookhaven National Laboratory) that solar neutrinos are emitted at a rate less than the original theoretical estimate (by a factor of 2 to 3) and, because cross-sections can be confidently predicted from present knowledge of the weak interaction, the conclusion is that the temperature of the Sun is lower than previously predicted. Some discussion of the nature of the production processes involving neutrinos in stellar environments (at temperatures 10^8–10^{10} °K) was also presented. It is clear that details of the physics of these and other weak interaction reactions can be sought by further astrophysical observations, in particular by work on the cosmological abundance of the elements.

Professor L. Van Hove (CERN, Geneva) considered the production mechanisms of high energy particles involving the strong nuclear interaction. There is considerable information already contained in bubble chamber photographs of multi-body processes, and by analysing such events for correlations between pairs or triplets of particles, details can be extracted of the intermediate resonance states or exchanged particles in the various interactions. The theory of this field is very complex. "World data tapes" could be searched to elucidate any theoretical model having as its basis, as Professor Van Hove pleaded, the important criteria of "simplicity" and "power of prediction". The experimental analysis of data from reactions such as $pp \rightarrow \pi^- p \Delta^{++}$, $pp \rightarrow \pi^+ \pi^- pp$, and $\pi^- p \rightarrow 2\pi^- \pi^+ p$ has already shown that patience and attention to detail pay considerable rewards in this field and more such analyses will follow in future. Professor Van Hove included in this talk a brief report of the first work to come from the CERN Intersecting Storage Rings (ISR) for protons of 28 GeV energy. (The equivalent energy of a machine where the protons strike a stationary proton target in the laboratory would be more than 1,600 GeV.) The two-body elastic scattering reaction $pp \rightarrow pp$ is well known to have a differential cross-section in the forward direction given by $d\sigma/dt \sim e^{Bt}$. Work on the intersecting storage rings shows that B seems to be continuing to increase up to the highest energies ($B \sim 12$ (GeV/c)$^{-2}$ at the equivalent momentum in the static target case $\sim$ 1,600 GeV/c). This implies that the pp diffraction pattern is continuing to shrink, although greater accuracy in determining B is necessary to establish whether or not the rate of shrinkage with energy is decreasing to zero as energy tends to infinity.

The importance of detailed experimental data on differential cross-sections, polarization and asymmetries in strong interaction production processes was stressed by Dr G. L. Kane (Rutherford High Energy Laboratory, Didcot) in talking about the Regge and duality models of high energy collisions. Some experimental work, particularly in relation to pion interactions with polarized targets, has been done, but it seems that more detail is needed to satisfy the theorists and the insatiable appetites of their computers to sort out the theoretical aspects. In this connexion, a plea was made for experimental work both above and below 5 GeV for data on pion–nucleon reactions leading to elastic $d\sigma/dt$, and on polarization effects in and charge-exchange scattering, and also for a study of polarization effects in strange particle production.

Electromagnetic interactions and hadron production in photon and electron interactions were discussed by Professor R. E. Taylor (Stanford Linear Accelerator Center and CERN) and Professor G. Salvini (Frascati and University of Rome). Professor Taylor described a series of elegant scattering experiments from SLAC on elastic and inelastic scattering of electrons from all types of nuclei ranging from protons to gold, from which there are indications that nuclei exhibit no Glauber shadowing effect of the type observed for the strongly interacting particles. This may prove to contradict some earlier work on the nature of photons, in which the total photoabsorption cross-section of nuclei was measured and the results of which indicated that photons exhibited at least some partial shadowing effects in heavy nuclei. Further work is clearly required to clear up this possible anomaly from these two types of experiment. Professor Salvini was especially concerned with the experiments carried out on the electron storage rings, and in particular, those from the Frascati machine "Adone". It is impressive how such a machine works to give new and "clean" data on a variety of processes which would not be so readily interpretable or, in some cases, would not even be studied if the interaction had taken place between an electron and a proton. In the case of testing quantum electrodynamics (QED) over ever smaller distances, the deviation from the Coulomb law ($\sim 1/r$) can be written in the form $1/r[1 \pm \exp(-kr/\hbar)]$ and the lower limit on k is now 6 GeV from Frascati $e^+ e^-$ data. In boson pair production the pion form factor seems to be steadily decreasing up to the highest q^2 studied, and in the fermi pairs $p\bar{p}$, the proton form factor limit is ~ 0.02 that predicted from Dirac theory.

Professor D. H. Wilkinson (University of Oxford) gave an account of β-decay data which threaten the idea of the charge independence of nuclear forces. According to the last mentioned concept isobaric nuclei have states which, apart from the interchange of protons and neutrons, have wave functions that are otherwise identical. Thus, for example, ^{12}B and ^{12}N may be regarded as mirror nuclei and the β decays of these two to ^{12}C should be closely related. The ratio of the probabilities of the β transitions from ^{12}B and ^{12}N in fact differ slightly but significantly from that prescribed by charge independence. There are reasons to expect residual differences between the decays of members of an isobaric multiplet, but the largest of these, the so-called binding energy effect, is nowhere near large enough to explain the observed difference. Nor is the ^{12}B–^{12}N example alone. Roughly speaking, the average observed effect in p-shell nuclei is some three times larger than the average which could be theoretically accounted for.

Egg Transfer in Domestic Animals

L. E. A. ROWSON
ARC Unit of Reproductive Physiology and Biochemistry, University of Cambridge, 307 Huntingdon Road, Cambridge

This article is based on the Hammond Memorial Lecture which Mr Rowson delivered in December 1970 and reviews some of the present trends in egg transfer in domestic animals.

ALTHOUGH the first successful experiments on egg transfer were carried out by Walter Heape of Cambridge late in the nineteenth century, the technique was almost totally disregarded both as a method of livestock improvement and as a tool for reproductive research until after the Second World War. When one considers the enormous value of the technique in genetics and studies on reproductive physiology, this fact is quite astonishing. Egg transfer is, however, now used as a routine technique for research in many small mammals and during the past ten or fifteen years has been extensively used for studies in the larger domestic species, particularly the sheep, goat, pig and cow. In the polytocous species such as the small mammals and even the pig, it is not normally necessary to induce superovulation in order to provide the requisite number of eggs for experimentation but in most of the domestic species this is an important requirement.

Superovulation

Two chief sources of gonadotrophins have been used. In the small mammals and in some of the earlier work on the domestic species, gonadotrophins of pituitary origin were extensively used and were obtained either from the horse, pig or sheep. Their use in the larger animals was, however, rather expensive and to obtain optimum results it was usually necessary to give repeated injections during the follicular phase of the cycle. The alternative source, gonadotrophins obtained from the serum of mares pregnant between 50 and 80 days (PMSG), is readily obtainable more cheaply and is effective when administered as a single injection. The follicular response to such gonadotrophins was consistently satisfactory but the ovulatory response often left a great deal to be desired. Many animals developed large numbers of follicles of which either only a few or none at all ovulated and this phenomenon seemed to vary with differing batches of PMSG; some induced both follicular response and ovulation very efficiently but others induced only the first of these. This problem has not yet been completely solved, but it is beginning to look as though the ratio of the two components of PMSG (that is, the follicle stimulating hormone (FSH) and the luteinizing hormone (LH)) may be important. It is well known that a proportion of both cattle and sheep injected with PMSG do not exhibit oestrus at the expected time or, even if they do, the eggs are in some cases unfertilized. It has also been clear for several years that the injection of such gonadotrophins may cause the premature ovulation of a follicle which is present in the ovary at the time of injection and it seems likely that this effect is related to the LH content of the injected gonadotrophin. In such circumstances either the animal would not exhibit oestrus or the effect of the FSH component would cause sufficient follicular response for oestrus to occur but the progesterone secreted by the newly ovulated follicle might adversely affect fertilization. This effect could be explained in a number of ways, including failure of capacitation or failure of sperm transport to the site of fertilization. It is quite common in these conditions to find an absence of spermatozoa in the zona pellucida of the ovulated eggs.

Once a satisfactory batch of PMSG has been identified, however, it is very efficient, provided that the animal is not overstimulated. In the cow and sheep, for example, it is inadvisable to stimulate the animal to produce more than about twenty ovulations because stimulation beyond this point seems to upset completely the physiological mechanisms of the reproductive tract and the proportion of unfertilized eggs increases rapidly. It is usual, therefore, to aim at an ovulation rate of about ten eggs per animal in these species. The response also varies from species to species; for example, a dose of 1,200 IU of PMSG given to a medium to large sheep will produce a response similar to a dose of 2,000 IU given to a cow weighing six or seven times as much. In most species it is unnecessary to give exogenous LH to obtain ovulation if normal oestrus is exhibited.

Recovery of Eggs

To obtain optimum results after egg transfer it is necessary to transfer the egg to a site where it would be expected to be in normal physiological conditions; because transfer to the uterus is simpler than transfer to the oviduct, it is usual to attempt to recover the eggs at a time when they have entered or are just about to enter the uterus. The timing of this will vary with the species, but is usually 4 to 5 days from the onset of oestrus in the cow and sheep and rather earlier for the pig. A surgical approach is necessary if maximum recovery of egg is to be achieved. This involves a simple laparotomy; the introduction of a fine cannula into the ovarian end of the oviduct and the injection of the flushing fluid into the ovarian portion of the uterus. The eggs are collected in special cups which can be placed directly under the microscope without further manipulation; the state of cleavage of the recovered eggs can then be identified. In the horse and pig there is a valve-like structure at the utero-tubal junction and it is not always possible to carry out retrograde flushing; in such cases it is usual to clamp off the top portion of the uterus, flush the oviduct towards the clamp and insert the recovery tube in the tip of the uterus, so that the fluid containing the eggs can be milked back into the collecting cup. Although this technique seems rather crude, egg recovery is as efficient as recovery by the direct flushing method. About 90% of the eggs shed can usually be collected. The most effective flushing medium varies with the species—for the sheep it is homologous serum, for the pig Tyrode plus a little albumen and for the cow TCM 199.

Transfer and Synchronization

The technique of egg transfer is simple—the egg is merely picked up in the medium selected, using a Pasteur pipette attached to a 2 ml. syringe, and the tip of the pipette stabbed through the uterine wall; the egg is then expelled directly into the lumen. A minimum of flushing medium is used, seldom more than 0.1 ml.

The stage of the cycle of the donor and recipient animals must be closely synchronized to obtain optimum pregnancy rates. The degree of variability which can be tolerated is known to be ±2 days in the sheep and ±1 day in the cow; the best results are obtained, however, when oestrus in donor and recipient occurs on the same day (Fig. 1).

The results obtained in cases of exact synchronization are remarkably good and are possibly influenced by the fact that any unfertilized, abnormal or retarded eggs are rejected and not transferred after egg recovery from the donor animal.

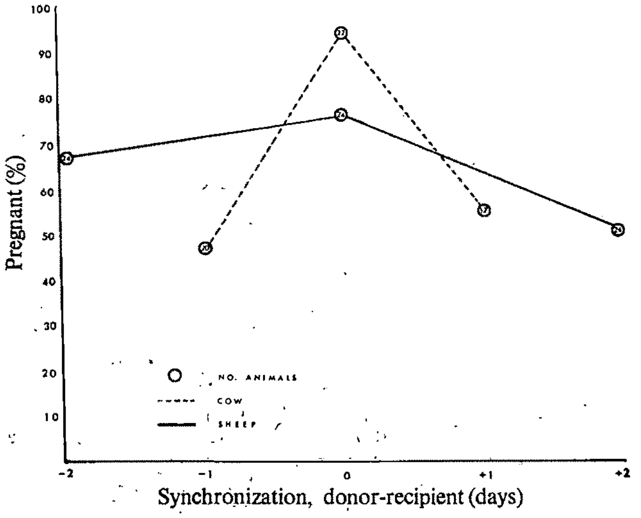


Fig. 1 The effect of varying the degree of synchronization of oestrus on fertility in cattle (———) and sheep (- - -). Numbers of animals are given in the figure.

If the eggs are to be transferred on the day of recovery it is quite satisfactory to store them in the flushing medium in an incubator at 37° C. Sheep pregnancies have been achieved successfully after up to 72 h of storage at 8° C, but the percentage of successful transfers decreases rapidly after 24 h. It has also been shown that the transfer of fertilized eggs of the pig, cow and sheep to the oviduct of the rabbit will result in their continued normal development for longer periods; they can then be recovered and successfully retransferred to suitable recipients. This shows that the requirements for culture of eggs of these species are not very specific and augurs well for *in vitro* culture of such eggs. The use of the rabbit as an incubator would permit the export of large numbers of eggs, for example, of cattle at very little cost, to various parts of the world. The transfer of such eggs after prolonged storage in the rabbit does, however, mean that development of the conceptus will only take place to a certain stage, after which it dies; the membranes are eventually expelled or resorbed. The ultimate solution to the problem of egg storage will lie in deep freezing.

Non-surgical Transfer

Most of the successful work carried out on egg transfer involves a laparotomy and the surgical recovery and transfer of the egg. Access to the uterus of the cow by way of the cervix is quite easy, but attempts to obtain pregnancies after the transfer of eggs in this way have been very disappointing. There are two reasons for this. First, the luteal phase uterus of the cow is very susceptible to infection and the introduction of any organism—very likely when the non-surgical approach is used—will cause pyometritis. Second, the insertion of a catheter, which might be used to introduce the egg, has a stimulating effect on the cervix and uterus so that uterine contractility is enhanced; eggs deposited in this way are frequently expelled and are found in the vagina within a few hours of their deposition. This effect is believed to be caused by the release of oxytocin from the pituitary as a result of the cervical and uterine stimulation, but we have so far been unable to confirm this. We are endeavouring to find an explanation of this phenomenon and to find ways in which the problem of egg expulsion can be overcome.

Reproductive Physiology

The technique of egg transfer has been used in the large domestic animals to study various fundamental and applied problems of reproductive physiology. One of the most interesting aspects has been the use of egg transfer in experiments on utero-ovarian-embryo relationships. The factors concerned with the initiation and formation of the cyclical corpus luteum are well known, but those associated with regression are far less clearly understood.

The uterus is known to be closely involved in the process because hysterectomy in the domestic animal results in persistence of the corpus luteum for prolonged periods and grafting of the removed endometrium between the flank muscles will restore cyclical behaviour and normal corpus luteum regression. The question which arises, therefore, is how the embryo overcomes the normal regression; it would seem from the repeated injection of embryo homogenates into the uterus that it acts in an anti-luteolytic manner. In an attempt to establish at what stage the presence of the embryo within the uterus is essential to prevent corpus luteum regression, eggs have been transferred at later and later stages until it is no longer possible to maintain the corpus luteum as one of pregnancy. In the sheep with a 16 to 17 day cycle the embryo must be present within the uterus by day 12½, otherwise regression cannot be prevented. Similar requirements have not been worked out in detail for other domestic animals, but there are indications that a relatively earlier presence of the embryo within the uterus is necessary in the cow and pig.

There is a remarkable unilateral utero-embryo-ovarian relationship in the cow and the sheep, which has been demonstrated by the use of egg transfer. If, for example, an egg is confined to the uterine horn adjoining the corpus luteum, the pregnancy continues normally; but if a similar egg is confined to the contralateral horn, pregnancy never occurs and the corpus luteum of the non-pregnant side regresses normally. This can be shown to be a unilateral luteolytic effect by removal of the non-pregnant horn adjoining the corpus luteum; the pregnancy will then continue normally. The situation is slightly modified in the case of the pig, for if a similar situation is created by confining embryos to one uterine horn, the lytic effect of the non-pregnant side will eventually result in regression of both sets of corpora lutea. The unilateral nature of these effects would suggest that it is the uterus itself rather than an indirect effect through some other gland which is responsible for lysis. It has been recently shown that radioactive material within the uterine vein is present in higher concentration in the ovarian artery, which runs in close apposition to it, than in other parts of the circulation; this suggests some form of direct transfer or communication between vein and artery although this has not been demonstrated anatomically and would involve a blood flow against the normal pressure differences.

Maturation and Culture

For the study of maturation and *in vitro* fertilization and for the satisfactory culture of eggs, laboratory techniques can only be a guide and it eventually becomes necessary to transfer eggs treated in this way to assess viability and the continuation of normal development. In experiments to assess the fertility of frozen semen it is often convenient to use the two oviducts separately, one as a control, to be injected with normal semen, and the other as a receiver for the experimental sample. Eggs transferred to each oviduct can then be examined and compared. The possibility of using the oviduct connected by fine tubes leading to the exterior as an *in vivo* means of fertilizing follicular eggs, both within and between species, is being investigated.

The demonstration that excellent pregnancy rates after transfer could be achieved with TCM 199 as a storage and flushing medium for cow eggs was followed by an attempt to increase production in this species by the introduction of two eggs into one uterine horn to produce twins. The first experiments were disappointing and only 12.5% produced twins. It was later found that when the two eggs were both deposited in a single uterine horn, migration of one egg did not occur and both implanted on the side; there was consequent competition for nutrients and death of one of the embryos frequently occurred. One egg was deposited in each horn during later experiments and a twinning rate of 73% was obtained. The economic implications of this finding are obvious and a vigorous attempt is being made to solve the problem of the reduced pregnancy rate obtained by non-surgical transfer of eggs so that the twinning technique can be put into practice.

Chimaerism in Cattle

Fusion of the foetal membranes occurs very early in pregnancy in many cattle twins and there is an interchange of haemopoietic and other types of cell between the embryos. The testes cells of the male in mixed sex twins contain many of the *XX* variety, but it is not yet established whether germ cells of this type continue through to actual spermatozoa. It seems likely that such a situation would occur in the case of chimaeric male calves. If so, it would mean that a proportion of the offspring of one twin may genetically be by the brother and this could seriously interfere with the accuracy of progeny tests, in which such bulls are used for artificial insemination. The percentage of twins born is not high (usually about 2 to 3%), but from our earlier experiments it was obvious that one of a pair present within a single horn frequently died by day 60 of gestation; as fusion of membranes occurs well before this stage, the fact that a cow has a single calf does not, therefore, mean that it did not start life originally as a twin. Twin ovulations have been estimated to be as high as 11% in the cow by some workers.

Our experiments on twinning open up a simple method of determining whether one twin can sire his brother's offspring. We have been able to transfer the egg of one breed of cattle to one uterine horn and that of a different breed to the other, using breeds which would colour mark their offspring. The use of such twins to inseminate a fairly large number of females should establish with certainty whether the interchanged germ cells of the chimaeras are capable of carrying through spermatogenesis. The use of similar sets of females will also be of interest because one can determine if the mixture of blood types has any effect on milk production and quality by producing twins of widely differing production characteristics such as the Jersey and Friesian. Such chimaeras are also tissue tolerant, so it should also be possible reciprocally to interchange half of the udder and to study milk production at the cellular level with the blood supply remaining constant.

Larger Litters

Transplantation has been used quite extensively to investigate the possibility of increasing the litter size, particularly of the sheep and to some extent of the pig.

Initial experiments involving the transfer of either two or five eggs to recipient sheep indicated that the uterus had a severely limiting effect on the number of offspring which would continue development and it became obvious that the problem was not solely one of availability of sufficient eggs. Lawson has recently investigated this problem in breeds of sheep of normally high, medium and low fertility, by the transfer of five eggs from a common genetic source to each group. At the time of transfer the potential fertility of each group was obtained by counting the number of corpora lutea (indicating the number of eggs shed). This work showed that the high and medium fertility breeds were producing eggs at almost their maximum potential, but that the uterus of the low fertility breed was capable of carrying about twice the maximum number assessed by corpora lutea counts. The last of these is the chief breed of sheep in New Zealand and clearly selection on the basis of high ovulation is necessary if litter sizes are to be increased. Although the litter size of the pig can be increased to some extent, the same uterine limitations apply as described previously for the cow and sheep.

Selection for an overall increase in litter size does, therefore, involve many factors, and in the cow and sheep, for example, there are great variations in cotyledon numbers; these are obviously of importance in multiple births and an examination of their inheritance would be worthwhile. This can only be done at slaughter and would, therefore, require superovulation and removal of fertilized eggs from such animals so that they could be transferred to recipients if a high cotyledon count was found.

Identical Twins

Although identical twins are rarely produced by domestic animals, they are invaluable for research and the possibility of producing them artificially has been enhanced by work on small mammals and the pig. It has been shown that the removal of all but one blastomere of even a four or eight-cell egg can result in normal offspring after the transfer of that egg to a suitable recipient. In principle, therefore, the transfer of removed blastocysts to emptied zonae could lead to the production of identical offspring, but a number of problems have arisen, the chief one being that the blastomere injected into the new zona is very liable to be exuded through the crack made at the time of injection. Attempts are being made to overcome this loss by temporary transfer of such eggs to the rabbit oviduct which coats them with a layer of mucin (Fig. 2); these experiments are still in progress. An alternative approach which seems to be successful in the small mammals is to divide the blastocyst and then to culture the two halves before transfer until they become spherical again. Attempts to use this technique have not yet been made in the domestic animals.

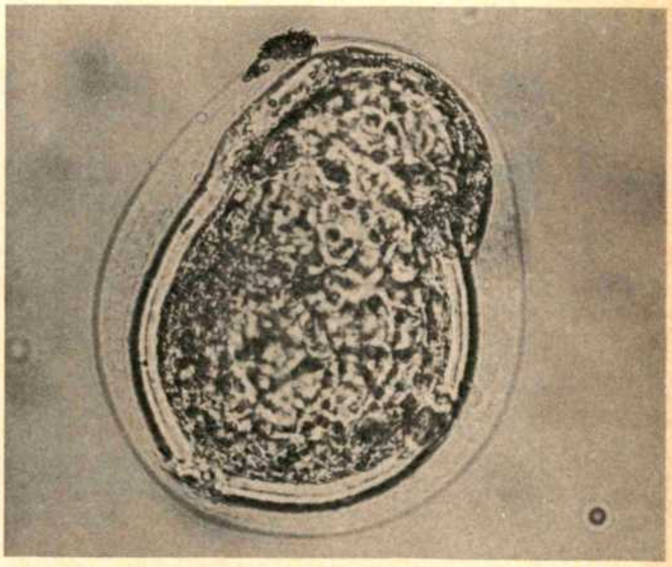


Fig. 2 A sheep blastocyst injected with cells (lower left) when at the eight cell stage and transferred to the rabbit oviduct to become sealed with a mucin coat.

It is clear that the value of egg transfer has only relatively recently been fully realized and there is little doubt that it will be extensively used as a tool for research in reproductive physiology in the future.

Global Atmospheric Research Programme

B. J. MASON
Meteorological Office, Bracknell

This worldwide programme is making a contribution to the numerical simulation and prediction of the global atmosphere.

THE Global Atmospheric Research Programme (GARP) together with the closely associated operational World Weather Watch (WWW) constitutes an ambitious, long term, international programme involving practically all the countries of the world. It aims at improving knowledge and understanding of the global circulation of the atmosphere, the physical basis of weather and climate and the production of weather forecasts of greater reliability and range. The conception of such a programme was fostered by two major developments. First, there were advances in the science of meteorology making the subject that was once largely descriptive and empirical steadily more quantitative and firmly based on the disciplines of experimental physics and fluid dynamics, largely through the development of physico-mathematical models to simulate and predict atmospheric motions. Second, there was the introduction of artificial Earth satellites with their potential for observing the global atmosphere and for providing rapid worldwide communications. The programme is being sponsored jointly by the World Meteorological Organization and the International Council of Scientific Unions, and so involves both government organizations and the academic community.

Objectives of GARP

The objectives of GARP centre chiefly on the improved understanding of the physical and dynamical processes that determine the global circulation of the atmosphere up to heights of 30 km. This includes both the statistical properties which should lead to a better understanding of the physical basis of climate, and the transient behaviour as manifest in the large scale fluctuations which control changes of weather. This implies three requirements. First, the formulation of improved physico-mathematical models of the global atmosphere. Second, the design and implementation of an optimum global observing system that will supply the input data for the theoretical models. Last, provision of adequate facilities, including the most powerful computers, to process the observational data and to test the adequacy of existing models for describing the global circulation and determining its predictability, thus providing a sound scientific basis for longer range weather forecasts and a rational basis for the design and implementation of a fully operational global observing system.

Before describing the aims and plans for GARP in more detail, it seems appropriate to review the present status of numerical modelling of the global circulation, of numerical weather prediction, and of the global observational system on which GARP programmes will have to build.

Numerical Simulation

During the past decade, the traditional, empirical and subjective methods of forecasting the evolution of weather systems for one to three days ahead have gradually given way to objective mathematical predictions based on a firm structure of physical theory that treats the atmosphere as a vast compressible fluid with energy sources and sinks. The theory is based on the physical principles of conservation of momentum, mass and energy, on the Newtonian (Navier-Stokes) equations of motion applied to a parcel of air, the first law of thermodynamics, and the equation of state of a gas.

The behaviour of a dry atmosphere containing no water substance may be described by a set of six differential equations involving six dependent variables—the three components of the wind, the pressure, density and temperature of the air, all expressed as functions of space and time. Starting from a given initial situation and specified boundary conditions, the problem is to solve a system of simultaneous non-linear partial differential equations in three spatial dimensions with time as the fourth independent variable. In fact, the equations are formulated in such a manner that they allow the time variations of the above quantities to be determined from their spatial variations. Thus, in principle, if we can observe the initial values of all six variables at a network of discrete points filling the entire or a large part of the atmosphere, we can compute the initial time rate-of-change of each variable from the governing equations and then extrapolate over a short time interval to find a predicted value at each point in the network. Repeating this process step by step, we can build up a forecast of the fields of pressure, wind, temperature and so on.

In practice, the meteorologist measures the horizontal winds (by tracking balloons with radar), the atmospheric pressure and temperature, but not the vertical component of the air motion since this is usually too small to measure directly although of the greatest importance in controlling cloud and rain forming processes. To overcome this difficulty and, at the same time, eliminate the density of the air as a variable from the equations, we use pressure rather than height as the vertical coordinate, regard this as an independent variable and introduce, as a dependent variable, the contour height, which is the height of a constant pressure (isobaric) surface. Maps of contour heights are very similar in configuration and in their relation to the winds to maps of the corresponding pressure fields. The thermal structure of the atmosphere is described in terms of the thickness of the layers between isobaric surfaces, the thickness being proportional to the average temperature of the layer. In this system the vertical velocity of the air is represented by the rate of change of atmospheric pressure, and is defined entirely by the horizontal wind field.

So far we have considered only a dry atmosphere. To include the effects of evaporation, condensation and precipitation of water we have to add equations for the continuity of moisture and modify the energy equation to include the latent heat released by condensation. Such a model is being developed in the Meteorological Office by Bushby *et al.*[1,2] to simulate the behaviour of a moist atmosphere on a finer scale than in any current operational prediction model. Observed fields of pressure, temperature, wind and humidity derived from radiosonde ascents are being used to study the dynamics of individual depressions and fronts and to make quantitative predictions of their rainfall for periods of up to 48 h ahead. Computations are made over an area of 6,500 × 5,000 km centred on the British Isles and covering most of Europe with over 12,000 grid points spaced only 50 km apart with each dependent variable being computed at alternate grid points and for ten vertical levels between 1,000 mbar and 100 mbar. The computations include the heights of these pressure surfaces, the mean temperatures of the nine layers, the horizontal and vertical components of the wind and the humidity and condensed water at the seven levels below 300 mbar above which the atmosphere is assumed to be dry. The model makes allowance for the effects of topography (for example, the Alps, Welsh, Scottish and Scandinavian mountains), for the frictional drag of the land and sea on the air, for horizontal eddy diffusion, and some adjustments are made to the vertical thermodynamic stability to cater for the influence of convective clouds that are smaller than the grid length. (The simulation of these sub-grid scale and boundary layer phenomena is described in more detail later.)

Preservation of computational stability requires the equations to be integrated in time steps of only 90 s so that a forecast for 24 h ahead involving about 10^{10} numerical operations takes 11 h on the ATLAS computer, but will only take 0.5 h on the IBM 360/195 machine which the Office will acquire later this year.

The results obtained so far from a limited number of experimental forecasts encourage us to believe that the Meteorological Office should soon be able to issue these quantitative and quite detailed forecasts of rainfall on a daily basis after the new computer becomes operational.

Meanwhile, much simpler models having a horizontal resolution of only 200–400 km and only three to six levels in the vertical, and which do not attempt quantitative prediction of rainfall, have been used for several years in several countries to make twice-daily predictions of the broad features of the pressure (contour height), temperature (thickness) and wind fields, the position and evolution of synoptic-scale systems such as depressions and anticyclones, for up to three days ahead, and for the greater part of the northern hemisphere. They have produced significant improvements in the prediction of the contour, thermal and wind fields at aircraft levels, and although improvements may not be so evident in the forecasts of surface conditions where the complex boundary layer processes are much more important, the computer products have introduced a greater degree of continuity, consistency and confidence in the weather forecast, especially in the outlooks for the second and third days, than was possible with the traditional subjective methods.

Experience gained both in the Meteorological Office and by the United States Weather Service from partly numerical and partly empirical methods of forecasting for five to seven days ahead suggests that there should be no inherent difficulty in predicting numerically the behaviour of synoptic-scale systems for at least one week given adequate observations to define the initial state. Accordingly, the Meteorological Office is now adapting the ten level model described here to an octagonal grid centred on the north pole and covering the northern hemisphere with a grid length of about 150 km, and plans to use this to make predictions for five to seven days ahead with the aid of the new computer.

In assessing the possibilities of forecasting for even longer periods, it is necessary to recognize the limitations likely to be imposed by the intrinsic indeterminism of atmospheric behaviour, as well as by those of the initial observations and the defects of the models. The promise and simultaneously the limitations of our present observations and models are demonstrated by recent long term integrations on sophisticated hemispheric models carried out by Smagorinsky *et al.*[3] in the United States and by Corby *et al.*[4,5] at the Meteorological Office. Both models incorporate the effects of radiative heating and cooling, the release of latent heat by condensation, the transfer of heat and moisture by advection, convection and turbulent diffusion from the underlying land and ocean surfaces, the effects of topography and surface friction, and the consequences of thermal contrasts between land, sea and ice surfaces.

Smagorinsky *et al.*, using a nine-level model, carried out two predictions for up to fourteen days. The movements of the planetary long waves, only five or six of which girdle the hemisphere, were generally successfully forecast, with a close correspondence between the forecast and observed positions of the main troughs and ridges, especially at the 500 mbar level where the correlation coefficients between the two sets of patterns remained above 0.5 for thirteen days from the initial time in one case, and for nine days in the other. In general the predicted contour patterns were too smooth in the middle scale so that the intensities of the depressions and anticyclones were underestimated while the strength of the tropospheric westerlies was overestimated. The forecast temperatures were too low, and the forecast rainfall too high, especially in the tropics. In spite of these defects, these first attempts at two week predictions from real but often inadequate initial data must be regarded as a major scientific achievement, particularly as the model was able to predict the birth of some second and even third-generation extra-tropical cyclones.

Furthermore, the group at the Meteorological Office has

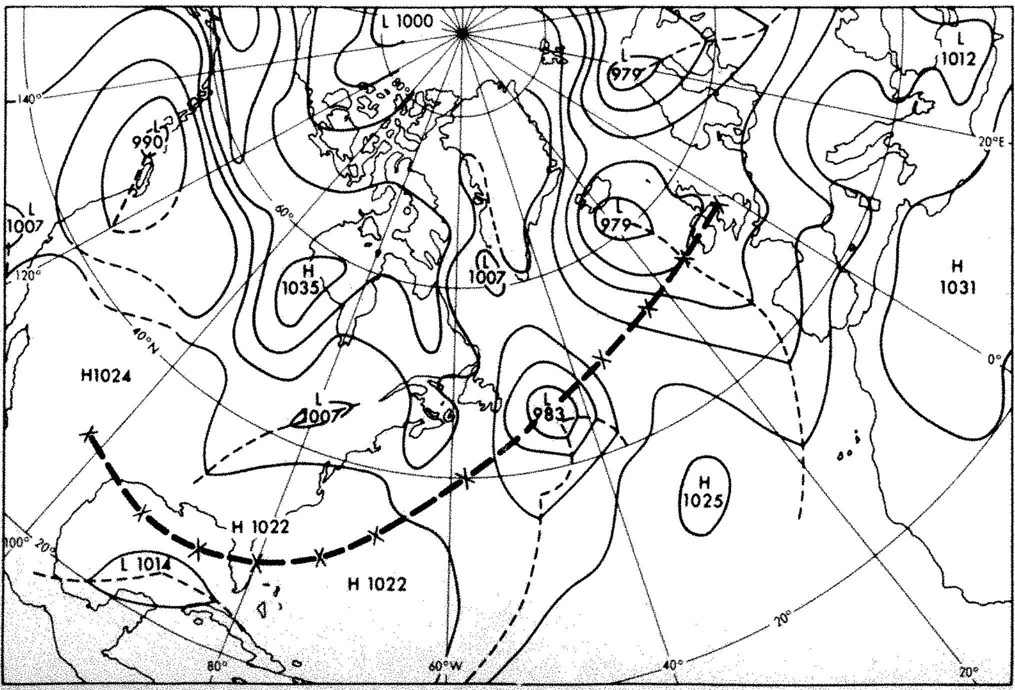


Fig. 1 The mean sea level pressure as predicted by the Meteorological Office model of the global atmosphere for the thirty-seventh day after the start of the integration. The heavy dashed line shows the track of a depression between day 30 and day 40, the crosses marking the predicted positions of its centre on successive days.

integrated a five-level model for up to sixty-five days and finds that once it has overcome the initial reluctance to generate sufficient eddy kinetic energy (a fault shared by most models, perhaps because of inadequate spatial resolution), it continues to generate new and very realistic cyclones every few days for several weeks. The detailed three-dimensional structures of cyclones produced by the model after thirty days are remarkably realistic, as illustrated in Figs. 1 and 2, with Fig. 2 showing the temperature, wind and humidity distributions within a cyclone seven days after its birth on day 30. These computations suggest that the behaviour of the atmosphere on the synoptic and larger scales may be determinate, in principle, on the time scale of at least two weeks and probably longer.

The extent to which these hopes will be realized in practice will largely depend on the solution of a number of scientific and technical problems. The first is connected with the formulation of much more realistic and detailed models of the global atmosphere and the second with a much improved global coverage of observations, especially over oceanic and tropical regions to allow adequate description of the initial mass, motion and moisture fields that provide the starting point for the numerical integrations. Last, there is a need for much more powerful computers than are currently available. These requirements largely define the objectives of GARP.

Problems in Numerical Simulation

I shall now consider some of the major deficiencies and difficulties encountered in building numerical models of the global circulation of the atmosphere. The basic problem is to formulate realistic physico-mathematical models that will adequately represent the complex physical and dynamical processes that are likely to control developments on the appropriate space and time scales. In other words, the model must properly represent the relevant or significant scales of motion and their non-linear interactions but smooth (or filter) out all the individual smaller scale motions that may introduce "noise" into the system while allowing for their overall contribution to transport and energy conversion processes by methods of statistical averaging.

In practice, the size of the smallest systems that can be individually represented in a model is limited by the spatial density of the initial observations and by the available computer power, both of which influence the design and resolution of the computational grid. Most current numerical models suffer from inadequate spatial resolution and are unable to represent adequately the meso-scale and smaller scale systems that may contribute materially to the production of eddy kinetic energy. On a deeper level, the model can accommodate explicitly only those phenomena that are adequately represented by the basic hydrodynamic and thermodynamic equations, that is, systems large enough to be strongly influenced by the Earth's rotation and to obey the hydrostatic equation. Thus in the numerical simulation of synoptic-scale and larger scale motions, many smaller scale phenomena have to be parameterized preferably in terms of observable, large scale dependent variables. These phenomena include: molecular-scale processes, such as radiative transfer as a function of the larger scale distributions of CO_2 O_3, water vapour, cloud and aerosol particles; micro-scale processes, such as turbulent exchanges of heat, momentum and water vapour in the planetary boundary layer, internal turbulent diffusion and dissipation, and convective transports; meso-scale phenomena, such as frontal circulations, orographic disturbances, and deep convective systems that are especially important in the tropics. GARP identifies three principal areas in which mechanisms operating primarily on the meso-scales

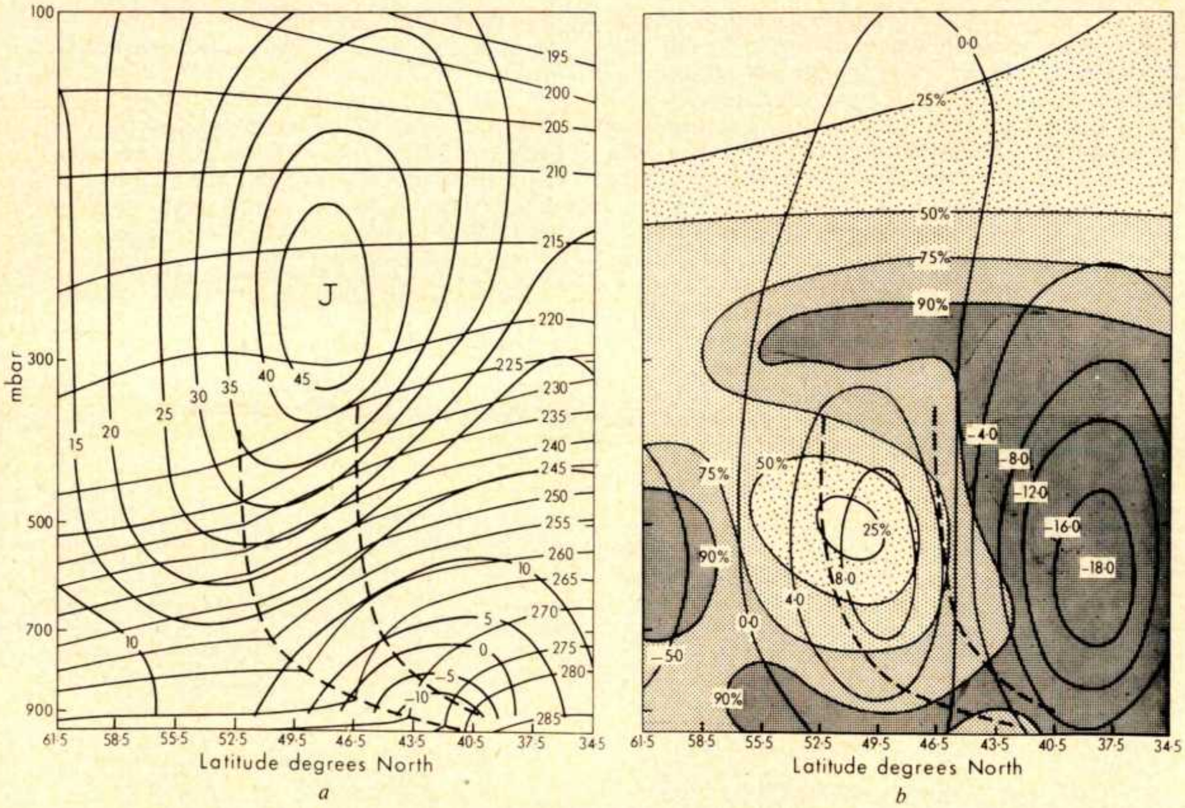


Fig. 2 *a*, Vertical cross-section of the atmosphere at 55° W for day 36 showing the predicted distributions of temperature and zonal wind near the warm front of the depression referred to in Fig. 1. A jet stream with winds exceeding 45 m s^{-1} ($\sim$90 knots) at about 250 mbar ($\sim$35,000 feet) is marked J. The wind is plotted at intervals of 5 m s^{-1} and the temperature at 5 K intervals. *b*, The same cross-section as in *a* but showing the distribution of vertical motion (negative values indicate upward motion, positive values downward motion) at intervals of 4 mbar h^{-1}, and of percentage relative humidity. There is a deep mass of rising moist air ahead of the frontal zone (shown dashed) with a region of subsiding dry air ahead of the front at middle levels but surmounted by moister air at high levels simulating the spread of high cloud ahead of the front.

and smaller scales (that is, < 500 km) are probably of vital importance in the energetics of the global atmospheric circulation. These concern: the evolution of convective cloud systems in the tropics; the turbulent exchange of heat, momentum and water vapour between the ocean surface and the overlying atmosphere; and the study of similar transfer processes in the atmospheric boundary layer over land.

Studies of Tropical Convection

All three areas mentioned above combine to be of major importance in the tropics where the large scale circulations, for example, the easterly trade-wind intertropical convergence zone (ITCZ) circulation, are maintained primarily by the release of latent heat of condensation in deep, moist, convective systems on a range of scales below 1,000 km.

Pictures from satellites reveal that over the tropical oceans cumulus and cumulonimbus clouds, individually 1–10 km across, tend to group into meso-scale convective units up to 100 km in diameter and that these, in turn, form "cloud clusters" of 100–1,000 km diameter that are generally associated with the troughs of lower-troposphere wave disturbances of characteristic wavelength 2,000–10,000 km, or with the ITCZ. It is likely that the latent heat released by cumulus convection in the ITCZ drives the trade-winds, while frictionally induced convergence in the trade-wind boundary layer feeds the cumulus convection in the ITCZ.

The task, then, is to study the genesis, growth, structure, size, organization and life cycle of the deep convective cloud systems, their interaction with the meso-scale circulation patterns in the rain areas, to determine the magnitudes of the energy-conversion processes and the associated fluxes of heat, moisture and momentum, all in relation to such factors as convergence/divergence, vertical motion, lapse rate and humidity distribution of the immediate environment, the sea-surface temperature, and the properties of the synoptic and large scale flows.

This will be the chief objective of the GARP tropical experiment planned for 1974 in the eastern Atlantic, where the structure of cloud clusters will be investigated by soundings from an array of twenty to thirty research ships in a 1,000 × 1,000 km square, by several fully instrumented research aircraft and observed by a geostationary satellite over the area, all in relation to the large scale flow as determined by wind soundings over an area stretching from the west coast of South America to Ethiopia. The whole operation, in which the United States, USSR, United Kingdom, Germany, France and Canada are expected to be the principal participants, will be planned and executed as a fully integrated international experiment under the leadership of an international team of scientists recruited specially for the task and which will work together for several years at the Meteorological Office in Bracknell.

Transport Processes in Boundary Layer

Examples of energy transfer between the ocean and the atmosphere occur when cold continental air flows over a warm ocean and during the formation of trade-wind cumuli over large areas of the tropical oceans. Energy in the form of sensible and latent heat, and moisture, are transported across the interface. These fluxes, which may be relatively uniform at the surface, become strongly concentrated higher up in cumulus towers which, in turn, may emit sufficient radiation to have appreciable influence on the large scale dynamics. Mechanical processes transport momentum into the sea surface at a rate dependent on the lapse rate of temperature and on the sea-surface roughness, but in turn affecting both of these. These processes are all much smaller in scale than the resolution of the grids of current numerical models, but it is evident that in altering the lapse rate and adding moisture and so on they influence the large scale motions in a way that cannot be determined explicitly by the prediction scheme but must nevertheless be represented in a manner that makes physical sense. The land surface exerts a drag on the atmosphere and therefore acts as an energy sink. Again the dynamics of the motions in the boundary layer is very different from that expressed by the model equations for the large scale flow.

Thus, to evaluate the role of turbulent transfer processes in the planetary boundary layer, it will be necessary to establish the size and magnitude of the fluxes of sensible and latent heat, momentum and moisture, in relation to measurable parameters of the temperature, wind and humidity fields and the characteristics of the underlying surface. Again this is difficult, not only because of the obvious problem of logistics, instrumentation and representativeness of the measurements over complex terrain but because we have yet to discover unique relationships between the fluxes, the turbulent kinetic energy balance and measurable properties of the large scale flow that apply throughout the whole depth of the boundary layer and hold in conditions ranging from the stably stratified to the strongly convective.

The difficulties of observing and understanding these meso-scale and small scale processes in sufficient detail to permit their satisfactory parameterization and incorporation in numerical models are likely to present the largest obstacle to understanding and predicting the evolution of major weather systems during the next two or three decades. The properties of the large scale flow are both easier to observe and to represent in numerical models. Accordingly, GARP should give priority to detailed studies of the meso-scale phenomena which are likely to prove intellectually and logistically more intractable than the synoptic and planetary scale systems.

Computer Requirements

If we learn to incorporate these various sub-grid scale phenomena into the numerical models without destroying their stability, we may hope to represent and predict the important synoptic and larger scale developments with a global model based on a horizontal grid length of 1° (110 km at the equator), having, say, twenty levels in the vertical and eight dependent variables (for example, winds, temperature, humidity, precipitation, radiative flux). For an integration carried out in 150 s time steps, the model would require the calculation of 4×10^9 variables or about 2×10^{12} computer operations per simulated day. Thus completion of the calculation in 0.5 h would require a computer capable of handling about 10^9 operations s^{-1}, that is, about 100 times as powerful as the IBM 360/195 or the CDC 7600. Speeds of this magnitude are likely to be achieved only by an array or matrix of central processors operating in parallel mode as envisaged in the ILLIAC IV computer, and which may become available within the next five years.

First Global Observing Year

Of crucial importance will be the acquisition of global observations adequate to define the physical state of the atmosphere at particular instants of time and so provide initial conditions for the start of the numerical integrations. At this stage it is not possible to specify precisely the minimum and optimum standards for the coverage, spatial resolution, frequency and accuracy of the observations. This will be one of the main tasks of GARP which proposes to undertake intensive observations of the global atmosphere over a period of one year—the First Global Observing Year in 1976, with a view to determining the minimum observational, data collection, and data processing facilities that will be necessary to meet the objectives listed above and to fulfil the requirements of the fully operational phases of the World Weather Watch.

To represent and predict the atmospheric processes that will determine the essential features of the weather everywhere for a week or more ahead, it seems likely that the following observations will be necessary.

In extra-tropical latitudes there is need for vertical soundings of temperature, pressure and wind up to heights of 30 km (10 mbar) once and perhaps twice daily, with horizontal spacing of 400 km and similar soundings of humidity up to 10 km with corresponding surface parameters including sea-surface temperature.

In tropical regions there is need to measure speed of winds at the 900, 700, 400, 200 and 50 mbar levels at least once a day, temperature and humidity soundings with a horizontal spacing of 500 km with corresponding surface parameters including sea-surface temperatures and air–sea temperature differences.

As to the required accuracy of the observations, air temperatures to within 1 K, atmospheric pressures to 0.3 per cent (3 mbar at the surface), winds to within ±3 m s^{-1}, vapour pressures to within ±10 per cent, and sea-surface temperatures to within 0.25° C may prove adequate. For verification of predictions, the cloud cover should be known within ±20 per cent and precipitation intensity to within ±10 per cent. All these assumptions, however, will need to be tested, first in long term numerical simulation experiments on the computer and ultimately in predictions using real initial data.

Even these apparently modest requirements cannot be met at present, except in limited areas of the populated part of the northern hemisphere. There are large deficiencies in coverage over the oceanic and tropical regions and in most of the southern hemisphere. Moreover, it is neither economically nor logistically feasible to fill the gaps by extension of existing types of facility such as radiosonde stations, and we must look hopefully to satellites to provide global measurements of meteorological parameters by measuring the intensity, polarization, angular and spectral variation of radiation emitted and reflected by the Earth and the atmosphere. In polar orbit they have the great advantage of being able to survey the whole globe every 12 h and, if placed in Sun-synchronous orbit, stay at a set local time with respect to the Earth and so greatly facilitate the ground system for receiving and using the data on a regular schedule. A geosynchronous satellite, that is, one in equatorial orbit synchronized in period with the rotation of the Earth, remains stationary over a particular point on the equator and therefore allows a particular area to be viewed continuously. Current US satellites of this type carry cameras which scan laterally while the satellite spins about its axis and so cover about one-quarter of the Earth's surface every 20 min. They therefore provide a time lapse film showing the evolution and movement of cloud systems from which valuable information on the winds at cloud level may be deduced. Operational meteorological geostationary satellites planned by the United States for 1975 will have much improved cameras capable of producing pictures with a horizontal resolution of 1 km in the visible and 7 km in the infrared from a height of 36,000 km.

Current American and Russian polar orbiting satellites provide television pictures of cloud, snow and ice cover with a horizontal resolution of about 1 km. Scanning radiometers working in the 11 μm window take pictures by day and night with a resolution of 5–10 km from a height of about 1,000 km and determine the equivalent black-body temperatures of the surfaces to within an accuracy of perhaps 2 K. In the future, television cameras in US satellites will be replaced by high resolution scanning radiometers to give improved resolution in both the visible and infrared parts of the spectrum.

Detailed analysis and interpretation of satellite pictures have provided valuable information on the location, structure and evolution of large cloud systems and on such meso-scale features as hurricanes, thunderstorms, squall lines, jet streams and mountain waves, often from oceanic and other remote areas where the observing networks are too sparse to make their detection likely by conventional methods. A complete global daily coverage of cloud pictures will be required for GARP and might be achieved by four geosynchronous satellites equally spaced round the equator and two polar orbiting satellites both in Sun-synchronous orbits but phased 6 h apart.

Although the cloud pictures and radiation data from relatively simple radiometers currently provide a great deal of valuable information, it is mainly only qualitative in character and difficult to incorporate directly into numerical dynamical models which require data on atmosphere temperature, pressure, composition and winds. We shall now examine the possibilities of obtaining such data on a global basis for GARP by remote sensing of the atmosphere from satellites.

Remote Sensing

In contrast to the simple radiometers required for the determination of total fluxes and surface temperatures, a number of very sophisticated and ingenious spectrometers and radiometers are being developed to obtain the vertical distribution of atmospheric temperature from measurements of the spectral distribution of radiation received from constant constituents such as carbon dioxide and molecular oxygen.

Various techniques are being developed for selecting the narrow frequency intervals and measuring the radiation received in them. The NIMBUS III satellite launched by the United States in April 1969 carries an Ebert infrared spectrometer (SIRS) which uses a fixed grating to select seven narrow spectral intervals of width about 5 cm^{-1} in the 15 μm carbon dioxide band, and one interval in the 11 μm window. The satellite also carries an infrared interferometer spectrometer (IRIS), in essence a Michelson interferometer, to accomplish the same task by scanning the entire spectrum from 6 to 20 μm with a resolution of about 5 cm^{-1}.

A simpler device developed at Oxford and Reading universities and described in detail by Houghton *et al.*[6] is operating on the NIMBUS IV satellite launched in April 1970. It uses narrow-band (3.5 cm^{-1}) interference filters to select six channels in the 15 μm band and absorption cells containing carbon dioxide to filter and further select the required radiation. The equipment weighs only 10 kg and consumes only 5 W of power.

The SIRS instrument on NIMBUS III produced some excellent vertical temperature profiles, especially over the northern hemisphere in the absence of clouds. Fig. 3 shows one of these and, for comparison, the radiosonde sounding taken from Kingston, Jamaica, about 400 km to the north-west and 4 h earlier in time.

Close agreement between the two is evident, the chief differences being in the middle troposphere, where the SIRS sounding is warmer by 2 or 3 K. Most such soundings, how-

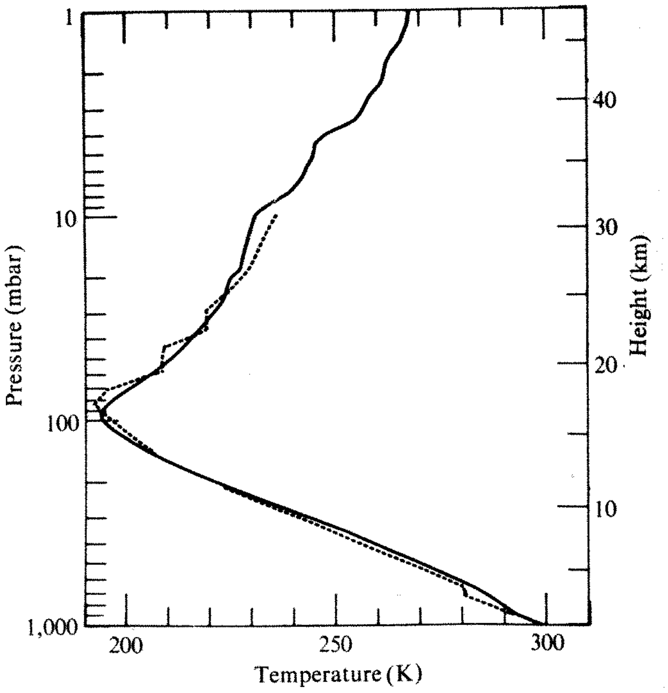


Fig. 3 Comparison of SIRS sounding with radiosonde ascent at Kingston, Jamaica, April 14, 1969. ....., Kingston, 1200 GMT ; ——, SIRS, 16° N, 73° W, 1612 GMT in clear sky conditions.

ever, require adjustment because of the presence of clouds and this is the most important hurdle to be overcome. Future sounding instruments will have a much smaller field of view, about 30 km square, and this will greatly increase the number of soundings obtained in clear air regions between cloud masses. Meanwhile the experiments on NIMBUS III and IV have demonstrated convincingly that atmospheric soundings from satellites are feasible. With further improvements in instrumentation and analysis techniques, it should be possible to produce for the first Global Observing Experiment, a global coverage of temperature data at least as good as that now being provided over only limited areas by conventional methods. In the stratosphere the satellite data are probably already more reliable than the radiosonde measurements which are subject to large instrumental errors at these levels. The cloud problem could, in principle, be solved by using microwave instead of infrared sounders but no such instruments are likely to be flown before 1976.

In principle, the methods developed for obtaining the temperature profile from measurements of radiation emitted by CO_2 (the concentration of which is known) can also be used to obtain the (unknown) vertical distribution of water vapour if the temperature profile is known and corrections are made for the radiation from underlying cloud or land surfaces. With these provisos, it should be possible to obtain the weighted average of the relative humidity of the upper troposphere from measurements of emitted radiation in the 6.3 μm band and in a window between 8 and 12 μm; and by making additional measurements in the broad band between 19.5 and 24 μm it should be possible to deduce the mean humidity in the lower and middle troposphere as well. The SIRS instrument on the NIMBUS IV satellite includes six additional channels for this purpose.

Measurement of Pressure and Winds

Accurate determination of the vertical temperature distribution would allow the pressure profile to be calculated from the hydrostatic equation,

$$\frac{1}{p}\frac{dp}{dZ} = \frac{-g}{RT}$$

provided that the surface pressure p_0 is known. Accurate measurement of the surface pressure on land is readily achieved by modern automatically recording aneroid barometers. The problem is much more difficult over the oceans where it would be possible, but very expensive, to mount aneroid barometers on floating buoys that could be located and interrogated by satellites. This may, however, be rendered unnecessary by the recent development in the United States of a simple, lightweight radar altimeter which, when mounted on a constant level balloon (see next section) floating at, say, 100 mbar (~16 km), can measure the height z of the balloon above the sea surface to within an accuracy of ±10 m. Hence, if the balloon carries instruments to measure the ambient pressure and temperature, the parameters p, T and z in the hydrostatic equation are known, and p_0 may be calculated. Given now the surface pressure, and T as a function of z from satellite infrared spectrometer data, the pressure may be determined as a function of height z from the hydrostatic equation. It seems that about 300 balloons would be required to establish the pressure reference level (at, say, 100 mbar) over the southern hemisphere, but many fewer may be required in the northern hemisphere where there are many more ships that can measure surface pressure directly.

Determination of the pressure and temperature fields as just described would allow the winds to be calculated in middle and high latitudes where the winds are closely related to the pressure field through the controlling action of the Coriolis force due to the Earth's rotation. This would not be the case in the tropics where the horizontal gradients of pressure and temperature and the Coriolis force are all weak, so that determination of winds in the tropics is one of the most important problems of GARP. American meteorologists are devoting considerable effort to the extraction of winds from the movements of clouds on the pictures transmitted on successive frames from the spin-scan cameras carried on geostationary satellites but this tech-

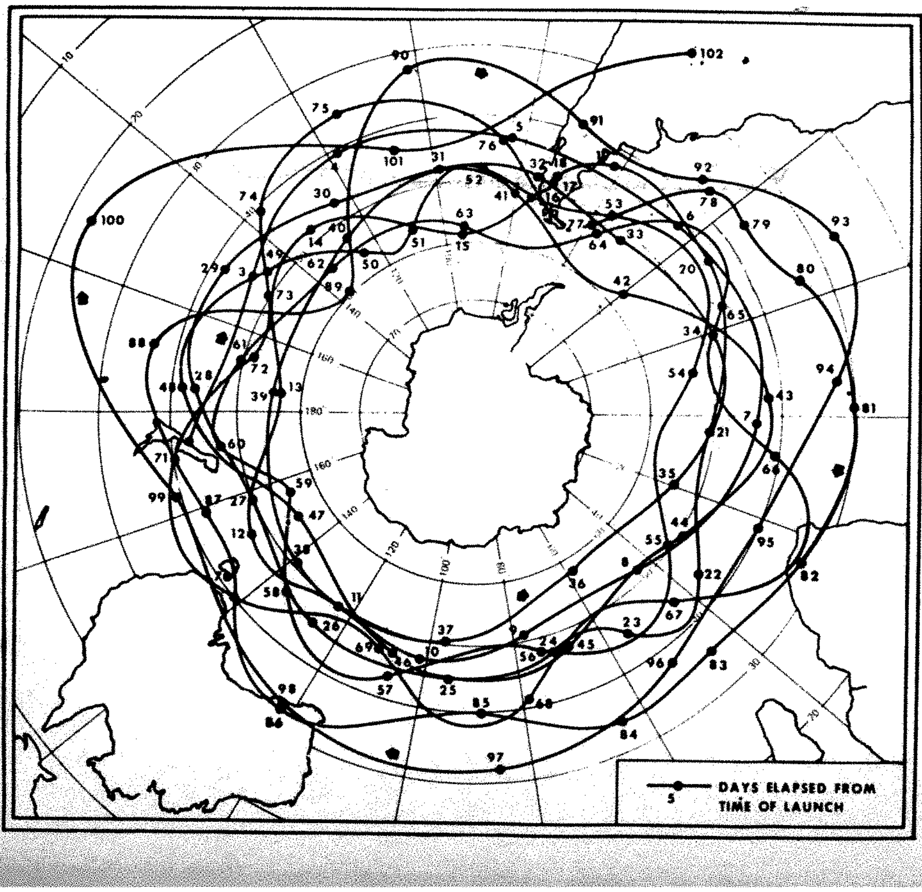


Fig. 4 Trajectories of a balloon released from Christchurch, New Zealand, making 8 circuits at 200 mbar (~40,000 feet) during 102 days.

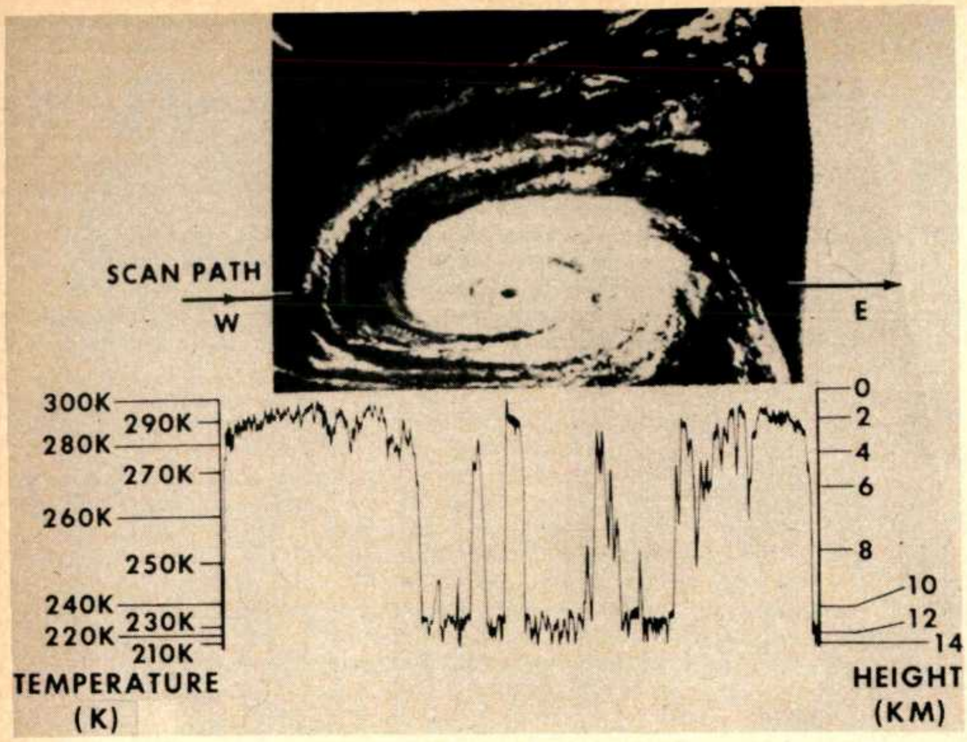


Fig. 5 An infrared photograph of a hurricane taken from a NIMBUS satellite. The temperatures of the ocean or cloud surfaces are determined from measurements of emitted radiation as shown by the record below the photograph.

nique can provide only a limited horizontal coverage of winds and information at only about two levels.

Considerable attention has therefore been paid to the measurement of winds by tracking inextensible constant volume, super-pressurized balloons that drift with the winds along surfaces of constant atmospheric density. In the United States GHOST (Global Horizontal Sounding Technique) Project, more than 200 such balloons have been released from Christchurch, New Zealand, and tracked by the signals from a simple telemetering photoresistor device that measures the elevation of the Sun. Balloons flying at 100 mbar higher are able to remain aloft for many months and make many circuits of the globe. One has flown for more than 450 days circling the southern hemisphere more than 25 times at the 100 mbar level. Balloons flying at the 200 mbar level (40,000 feet) have an average life of about 100 days, Fig. 4 showing the trajectories followed by one such balloon in making eight circuits of the southern hemisphere over 102 days. Unfortunately, balloons drifting at lower levels suffer with varying severity from the accumulation of frost, ice and snow, which limits their duration to weeks or even a few days, and so balloons can be flown economically only above 200 mbar, and below 850 mbar in the tropics, the most vital part of the troposphere being barred. Thus, unless the icing problem is solved, it seems that the constant level balloon will play a much less prominent role in GARP than once seemed likely, and this leaves the difficult problem of obtaining adequate wind data in the tropics unsolved. It has been suggested, however, that a large constant level balloon flying at, say, 100 mbar might release very lightweight dropsondes carrying transponders and fitted with parachutes that would drift with the wind, and that these could be located and tracked by a navigational system such as LORAN or OMEGA. Such a system may be tested within the next year or so.

There is little doubt that the developments described in this article will, during the next decade or two, lead to major changes in the methods and techniques of meteorological observation and measurement and that the direct measurement of some of the basic atmospheric parameters will be gradually supplemented and perhaps ultimately replaced by indirect methods involving the remote sensing of electromagnetic radiation from atmospheric constituents by satellites. For the next ten years or so, the global observing system will probably consist of a mixture of satellites, conventional radiosondes, dropsondes, balloons released from ground stations and ships and tracked by navigational aids such as LORAN and OMEGA, constant level balloons carrying accurate radar altimeters, and so on. Satellite methods, however, are likely to become more and more dominant and, although it may be necessary to retain some ground based sounding stations for the calibration and checking of the satellite system, it will probably be possible to dispense with many of the radiosonde stations in the northern hemisphere and the weather ships well before the end of the century.

Satellites will also play an important role in locating and interrogating automatic instruments on remote land stations, ocean buoys and drifting balloons and in rapidly transmitting the great quantities of data to a few major centres for processing and analysis by giant computers. An adequate global system would consist of four geostationary satellites equally spaced around the equator and two polar orbiting vehicles. On present expectations, it is likely that the system in 1976 will be deficient by one, and probably two, geostationary satellites; this and the lack of adequate wind data in the tropics are likely to be the most serious gaps in the requirements of the first global experiment planned for that year.

Progress in understanding and predicting the behaviour of the global atmosphere, however, is likely to be limited not so much by deficiencies of observational data or computer power, but by the difficulty of adequately simulating in numerical models, however complex, the wide variety of strongly interacting processes and motions encountered in the real atmosphere in such a way that the models remain realistic and stable within the limits set by random disturbances.

[1] Bushby, F., and Timpson, M., *Quart. J. Roy. Meteorol. Soc.*, **93**, 1 (1966).
[2] Benwell, G., and Timpson, M., *Quart. J. Roy. Meteorol. Soc.*, **94**, 12 (1968).
[3] Smagorinsky, J., Miyakoda, K., Strickler, R., and Henbree, G., *Monog. Weath. Rev.*, **97**, 1 (1969).
[4] Corby, G., Gilchrist, A., and Newson, R. L. (in the press).
[5] Gilchrist, A., *Quart. J. Roy. Meteorol. Soc.*, **97**, 340 (1971).
[6] Houghton, J., and Smith, S. D., *Prac. Roy. Soc.*, A., **320**, 23 (1970).

Succession of Cainozoic Vertebrate Assemblages from the Northern Kenya Rift Valley

W. W. BISHOP, G. R. CHAPMAN & A. HILL
Department of Geology, Bedford College, University of London, Regents Park, London NW1
J. A. MILLER
Department of Geodesy and Geophysics, University of Cambridge, Cambridge CB3 OE2

A succession of twelve different fossiliferous units in the Baringo area ranges in time from about 14 m.y. to the Holocene. The early part of the sequence provides for the first time faunal and stratigraphic evidence for the period between 5 m.y. and the Fort Ternan sediments dated about 14 m.y.

In Africa south of the Sahara very little is known of the late Miocene and Pliocene vertebrate fossil assemblages. Until recently a gap in the fossil record extended for more than 12 m.y. from the well documented earlier Miocene assemblages older than 17 to 18 m.y.[1,2] to those faunas, sometimes including australopithecines, which range in time from about 5 m.y. to 1 m.y. Only the Fort Ternan fossiliferous sediments, dated about 14 m.y.[3–5] and first described in 1962, were known with certainty to fall within this period. The investigations described here have been carried out since 1965 in the vicinity of Lake Baringo and the Tugen Hills (or Kamasia Range) in the northern Kenya Rift Valley (Fig. 1). The stratigraphic succession includes twelve different sedimentary units which contain vertebrate fossils. The early part of the sequence spans the former large faunal gap.

Between northern Tanzania and the Ethiopian border, the floor of the rift is generally covered by either Pleistocene volcanic rocks or epiclastic sediments which infill fault-bounded basins. Together these obscure the record of earlier rift history. Very little of the Kenya Rift has at its surface rocks older than 3.0 m.y., and thus the Tertiary succession in the Baringo area is particularly interesting. West of Lake Baringo, between latitudes 0° 15′ N and 1° 00′ N, a major tilted block, which forms the Tugen Hills, occurs within the rift graben; exposed in its eastern fault scarp, and in foot hills to the east, is a succession of lavas and sediments totalling 3,000 m in thickness that represents a sequence from mid-Miocene to Holocene. Basement gneisses outcrop beneath the Tertiary succession in the lowest 300 m of the main escarpment[6–8].

The first geological observations in this area were made by Gregory[9,10] who identified the principal lithological units and inferred the former existence of a "Lake Kamasia" to account for some of the sedimentary formations. The area was later accepted as the type locality for a "Kamasian Pluvial"[11,12], in spite of opposition from Shackleton[13]. The term Kamasian has no stratigraphic or climatic significance, and to avoid confusion the topographic feature is called here the Tugen Hills. Fuchs first recorded[14] vertebrate fossils in the area; these and other vertebrates found recently in the same formation[15] have been shown to be of later Pleistocene age. Officers of the Kenya Geological Survey[16] began systematic mapping in the south of the area in the early 1960s. The research reported here was initiated after discoveries made during mapping by members of the East African Geological Research Unit (EAGRU) based at Bedford College, University of London, and under the direction of Professor B. C. King. Stratigraphic and palaeontological investigations have been undertaken in more detail and some preliminary results have

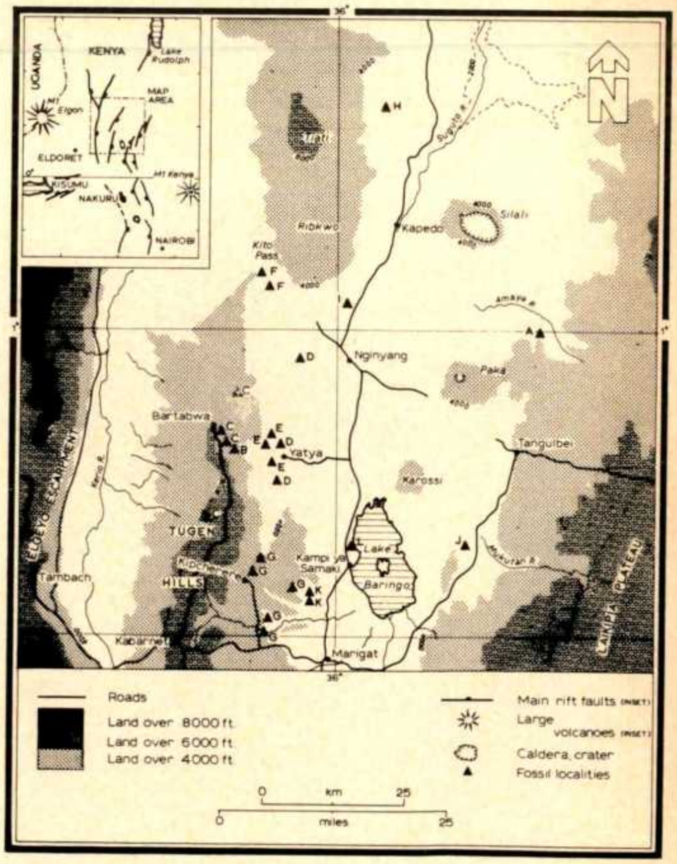


Fig. 1 Distribution of the fossiliferous localities. *A*, Alengerr beds; *B*, Muruyur beds; *C*, Ngorora Formation; *D*, Mpesida beds; *E*, Lukeino member; *F*, Kaperyon formation; *G*, Chemeron formation; *H*, Aterir beds; *I*, Karmosit beds; *J*, Chemoigut beds; *K*, Kapthurin formation; *L*, Kokwob beds. (Inset: location of the map area in the Kenya Rift.)

been published[17-19]. On completion of the study the whole palaeontological collection will be lodged in the Kenya National Museum, Nairobi.

Stratigraphy

Provisional lithostratigraphic names have been assigned to all the principal stratigraphic units; most of these are unpublished, although recorded in PhD theses[20]. Names in Fig. 2 are of rock units (formation, member) and not of localities or sites.

The succession in the northern Kenya Rift has been divided into five groups of formations[8]. Group I, the earliest, basaltic, group is absent from the Tugen Hills succession (Fig. 2) where the lowest group present (II) is dominated by phonolite flood-lavas and rests directly on the basement. The oldest phonolite formation is almost certainly older than the Uasin Gishu and other so called plateau phonolites in Kenya. Undersaturated basaltic lavas in the middle of group II may be equivalent to the Elgeyo Basalts of Walsh[21], and from the evidence of stratigraphic correlation and isotopic dates the group II phonolites above the basaltic unit seem to be the equivalents of the Uasin Gishu phonolites[22], and range in age from 12.0 to 13.6 m.y.[1]. Rocks equivalent in age to the Ngorora Formation[18] and the highest group II phonolite unit have not been located elsewhere in the rift area.

A distinct unconformity, representing a major rift faulting episode, separates the rocks of group II from those of the younger groups. The lavas of groups III, IV and V are only rarely of undersaturated types[8]. In the Tugen Hills the lowest lavas of group III are the Kabarnet Trachytes[21], a very extensive formation of holocrystalline flood-trachytes from which several isotopic dates close to 7.0 m.y. have been obtained. The dominant lavas of group III, however, are alkali basalts. Group IV, which is composed of large complex trachyte volcanoes, is absent in the Tugen Hills-Lake Baringo area but is strongly developed farther north (Figs. 3 and 4). The petrologically varied lavas of group V above the Chemeron formations are, at least in part, the same age as the Dispei-Lake Hannington Phonolites (largely phonolitic trachytes) of McCall[23]. The stratigraphy of the Chemeron and Kapthurin formations was established by McCall *et al.*[16] and elaborated by Martyn[17,20].

Figs. 3 and 4 show the upper part of the succession on the Baringo-Turkana border where the dominant features are the large trachyte volcanoes of group IV. Rock units above these in the sequence are essentially local developments and cannot be directly traced by mapping into the Tugen Hills succession farther south.

Although the stratigraphy shown in Figs. 2-4 was established entirely by mapping, a large number of isotopic (^{40}K/^{40}Ar) dates has been obtained from the lavas and from feldspar crystals in tuffs. Some of these have already been mentioned and will be reported in detail elsewhere. The dates available at present, although sometimes showing disparate results in single rock units, enable an approximate time curve for accumulation to be related to the succession illustrated in Figs. 2-4. This effects an approximate dating of the fossil assemblages and assists in correlation with other areas.

The fossiliferous sediments to the east of Lake Baringo can be related approximately to the succession shown in Fig. 2: the Alengerr beds outcrop in the eastern rift escarpment in the area of the Amaya river within a succession, chiefly of phonolite lavas, which is the equivalent of group II in the Tugen Hills. Potassium-argon dates on the lavas indicate that the age of the fossiliferous Alengerr beds is between 12.0 and 14.0 m.y.

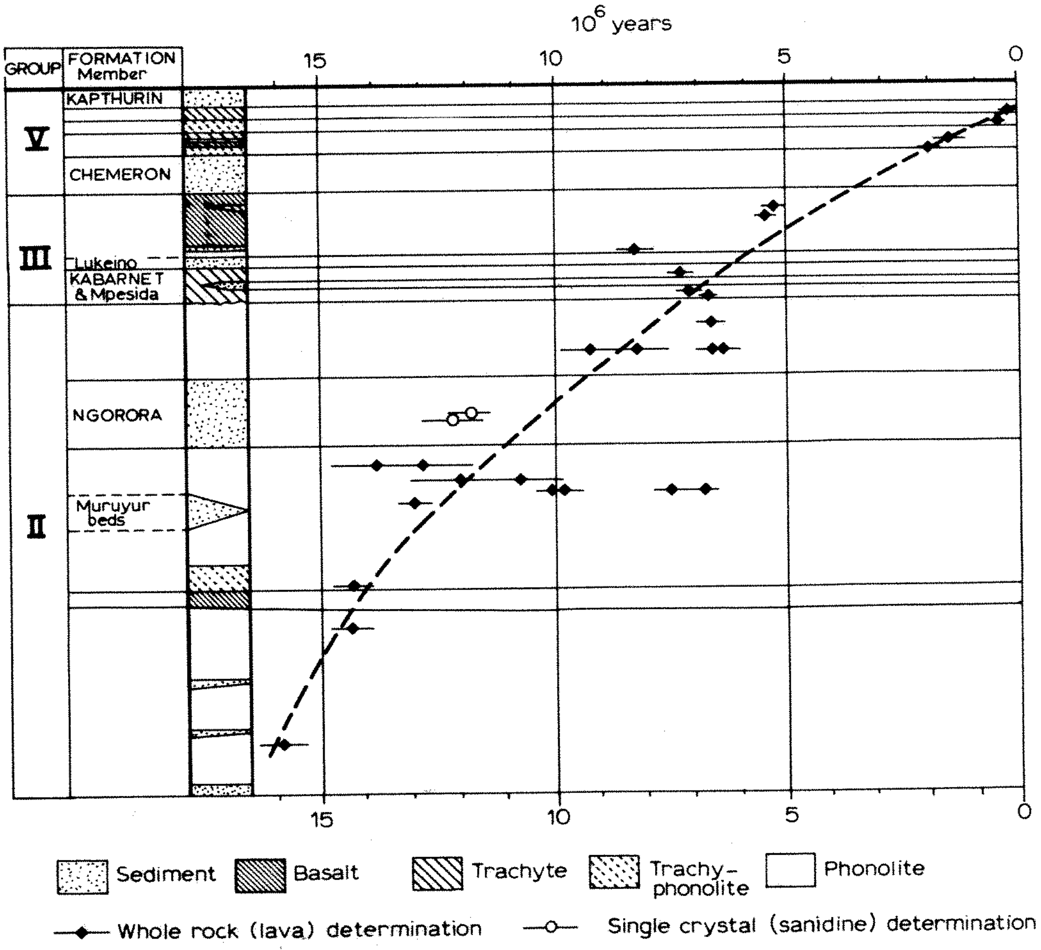


Fig. 2 Stratigraphy and isotopic (^{40}K/^{40}Ar) dates in the Tugen Hills (Kamasia Range). Dotted line: approximate accumulation curve for the sequence.

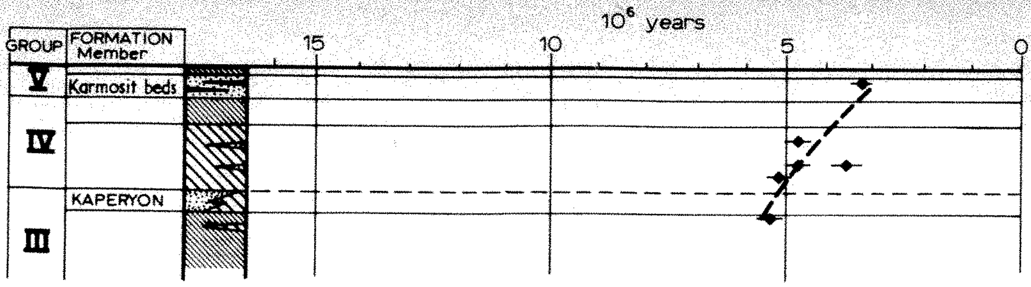


Fig. 3 Stratigraphy and isotopic ($^{40}K/^{40}Ar$) dates in the Ribkwo area.

The Chesowanja sediments[19] were deposited in part of the Chemoigut sedimentary basin immediately to the east of Lake Baringo. Potassium–argon determinations on trachyte lavas which possibly ponded the former Chemoigut lake gave results of 1.1 and 1.2 m.y.

Faunal Assemblages

(*a*) Alengerr beds

MAMMALIA	
Proboscidea	
Deinotheriidae	*Deinotherium* c.f. *hobleyi* Andrews
Gomphotheriidae	
Perissodactyla	
Rhinocerotidae	*Dicerorhinus* c.f. *leakeyi* Hooijer

The Alengerr beds are exposed at two small fossiliferous areas along one horizon of coarse fluviatile sediment, dated between 12 and 14 m.y.

(*b*) Muruyur beds

PISCES

REPTILIA
Crocodilia

There is one fossiliferous locality in the Muruyur beds, probably between 13 and 14 m.y. old.

(*c*) Ngorora Formation

PISCES	*Clarias* sp. *Tilapia* sp.
REPTILIA	
Chelonia	
Pelomedusidae	*Pelusios* c.f. *sinuatus*
Trionychidae	
Crocodilia	
AVES	
Ciconiidae	*Leptoptilos* sp.
MAMMALIA	
Primates	
Cercopithecoidea	
Hominoidea	Hominid?
Carnivora	
Proboscidea	
Deinotheriidae	*Deinotherium hobleyi* Andrews
Gomphotheriidae	
Hyracoidea	sp. nov.?
Perissodactyla	
Rhinocerotidae	*Chilotherium* sp. *Aceratherium* sp. (? *D. leakeyi* Hooijer) *Brachypotherium* sp.
Artiodactyla	
Suidae	
Hippopotamidae	
Giraffoidea sp.	
Giraffidae sp.	
Bovidae	
Boselaphini	*Protragocerus* sp.
Tragelaphini/Boselaphini	
Cephalophini/Boselaphini	
Caprinae/Alcelaphini	

The geological situation, sediments and fauna of the Ngorora Formation, which consists of deposits older than 9 m.y. and younger than 12 m.y., have been described by two of us (W. W. B. and G. R. C.)[18]. In a note in this article[18] L. S. B. Leakey described the hominoid tooth as hominid, having some affinities with "*Kenyapithecus*" and some with both *Homo* and *Australopithecus*. From the teeth, the *Deinotherium hobleyi* material seems more advanced than that from Gebel Zelten[24] and the known East African Miocene localities. An age of 12.5 m.y. has been suggested for the arrival of *Hipparion* in Europe[25]. Consequently its apparent absence from the Ngorora Formation is interesting, because there seem to be neither palaeoecological nor taphonomical features of the formation that would preclude *Hipparion* being represented in the assemblage had it been in sub-Saharan Africa at that time. If *Hipparion* is indeed absent it emphasizes that care should be taken when attempting to date the faunas of one continent by the date of appearance of *Hipparion* on another[26]. The *Brachypotherium* represents the new species recently described from Lothagam I[27]. The *Chilotherium* is also a new species. The hippo remains, which include primitive premolars, are the oldest dental evidence of the family. The small giraffoid may be comparable to one at Fort Ternan; and the *Protragocerus* sp. is similar dentally to a Fort Ternan form, *P. labidotus*, but the horn cores resemble more closely a *Protragocerus* from Langebaanweg, South Africa[28].

(*d*) Mpesida beds

PISCES	
Siluriformes	
REPTILIA	
Chelonia	
Trionychidae	
Crocodilia	
MAMMALIA	
Proboscidea	
Elephantidae	*Stegotetrabelodon orbus* Maglio
Perissodactyla	
Equidae	aff. *Hipparion*
Rhinocerotidae	
Artiodactyla	
Hippopotamidae	*Hippopotamus* sp.
Bovidae	

There are three fossiliferous localities, in three separate outcrops of the Mpesida beds, probably with the same age of about 7 m.y. List (*d*) given here is that for the chief locality, but no additional taxa have been recorded from either of the two subsidiary localities. Maglio has mentioned[29] the unit and its possible correlatives, in connexion with the Elephantidae. *Stegotetrabelodon orbus* is found elsewhere only from Lothagam I[29–31] and from the Kaperyon formation. The "*Hipparion*" is the earliest recorded equid in Africa south of the Sahara. The hippopotamus is similar to a new species from Lothagam I.

(*e*) Lukeino member

REPTILIA	
Chelonia	
Trionychidae	
Crocodilia	
MAMMALIA	
Proboscidea	
Gomphotheriidae	
Perissodactyla	
Equidae	aff. *Hipparion*
Artiodactyla	
Suidae	*Nyanzachoerus* c.f. sp. "C"[32]
Hippopotamidae	*Hippopotamus* sp.
Bovidae	Reduncini sp. Antilopini sp.

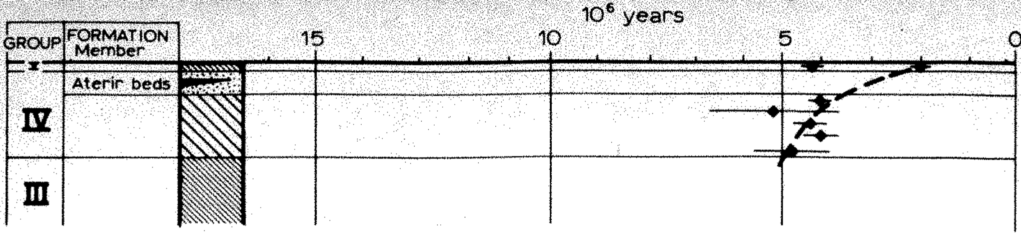


Fig. 4 Stratigraphy and isotopic ($^{40}K/^{40}Ar$) dates in the area north of Kapedo.

There are three fossiliferous localities in the Lukeino member at the same horizon along a continuous outcrop. The fauna is younger than 6.7 m.y., but possibly older than 6 m.y. The *Nyanzachoerus* sp. seems to be more primitive than sp. nov. "C" from Lothagam I, but also has some resemblances to sp. nov. "B" from Kanapoi[32]. The hippopotamus resembles a new species from Lothagam I and from the Mpesida beds. There is a similarity between the Reduncini sp. and a species from the Dhok Pathan and Tatrot levels of the Siwalik sequence.

(*f*) Kaperyon formation

PISCES	
REPTILIA	
Chelonia	
Trionychidae	
Crocodilia	
MAMMALIA	
Proboscidea	
Elephantidae	*Stegotetrabelodon orbus* Maglio
	Primelephas gomphotheroides Maglio
Perissodactyla	
Equidae	aff. *Hipparion*
Artiodactyla	
Suidae	
Hippopotamidae	*Hippopotamus* sp.
Bovidae	
Rodentia	
Hystricidae	

The Kaperyon formation contains two fossiliferous localities, the fauna from which is about 5 m.y. old. The formation is referred to by Maglio[29]. The specimen of *Stegotetrabelodon orbus* seems to be closer to that from Lothagam I than does the Mpesida beds form. This is also the case with the *Hippopotamus*, which is like a Lothagam I species, and is more advanced than that from the Mpesida beds.

(*g*) Chemeron formation

PISCES	† *Clarias* sp.
	† *Tilapia* sp.
REPTILIA	
Chelonia	
Trionychidae	
Crocodilia	c.f. † *Crocodylus niloticus* Laurenti
MAMMALIA	
Primates	* *Papio baringensis* Leakey
	* *Paracolobus chemeroni* Leakey
	‡ Hominidae gen. et sp. indet.
Proboscidea	* *Deinotherium bozasi* Arambourg
	Anancus sp.
	Mammuthus subplanifrons Osborn
	Loxondonta adaurora Maglio
	* *Elephas recki* Dietrich
Perissodactyla	
Equidae	aff. *Hipparion*
Rhinocerotidae	† * *Ceratotherium simum* (Burchell)
Artiodactyla	
Suidae	*Nyanzachoerus* sp. "A"
	Nyanzachoerus c.f. sp. "B" or *Mesochoerus heseloni* (Leakey)
	c.f. *Mesochoerus* sp.
	c.f. *Sus* sp.
	c.f. *Potamochoerus* sp.
Hippopotamidae	† *Hippopotamus* c.f. *imagunculus* Hopwood
	Hippopotamus sp. nov.
Giraffidae	
Sivatheriinae	
Bovidae	
Tragelaphini	
Alcelaphini	
Rodentia	
Hystricidae	c.f. *Xenohystrix crassidens*

Table 1 Distribution of Vertebrate Fossils through the Succession of Sedimentary Units in the Baringo Area

			(*a*) Alengerr	(*b*) Muruyur	(*c*) Ngorora	(*d*) Mpesida	(*e*) Lukeino	(*f*) Kaperyon	(*g*) Chemeron	(*h*) Aterir	(*i*) Karmosit	(*j*) Chemoigut	(*k*) Kapthurin	(*l*) Kokwob
Pisces				×	×	×		×	×			×		×
Reptilia	Chelonia	Pelomedusidae			×					×		×		
		Trionychidae			×	×	×	×	×			×		
	Crocodilia			×	×	×	×	×	×	×		×		
Aves		Ciconiidae			×									
Mammalia	Primates	Cercopithecidae			×				×			×	×	
		Hominidae			?				×			×	×	
	Carnivora				×								×	
	Proboscidea	Deinotheriidae	×		×				×			×		
		Gomphotheriidae	×		×		×		×	×				
		Elephantidae				×		×	×	×	×	×		
	Hyracoidea				×								×	
	Perissodactyla	Equidae				×	×	×	×	×		×		
		Rhinocerotidae	×		×	×			×	×	×	×		
	Artiodactyla	Suidae			×		×	×	×	×	×	×	×	
		Hippopotamidae			×	×	×	×	×	×	×	×	×	
		Giraffidae			×				×		×	×		
		Bovidae			×	×	×	×	×	×	×	×	×	
	Rodentia	Hystricidae						×	×					
		Others											×	
	Tubilidentata												×	

The sediments in the Chemeron formation have been described by Martyn[17,20] and by McCall, Baker and Walsh[16]. There are several fossil localities in two principal areas of outcrop. These areas were interpreted by Martyn as representing two separate basins of deposition, the Chemeron basin and the Kipcherere basin, in which the successions were broadly equated stratigraphically and lithologically. In list (*g*) taxa marked * are from JM91(90), a locality in the basal member (member 1) in the Chemeron basin. Because of the anomalous presence in the JM91 assemblage of teeth assigned to *Elephas recki* stage 2 it has been suggested that the fauna from this locality is younger than the basal faunas elsewhere in both depositional basins[32]. The geological evidence available at present does not support this view.

Fossils also come from localities higher in the succession. Taxa marked † in the faunal list are found in the lower fish beds (member 2) as well as in the basal beds, and the hominid (marked ‡) comes from a low level in the upper fish beds (member 4). An isotopic age of 2.0 m.y. has been obtained for a lava conformably overlying the formation in the Chemeron basin. L. S. B. Leakey has provided a preliminary note on the fauna[17]; the hominid fragment has been described and discussed by Tobias[33]; R. E. F. Leakey has described the cercopithecoids[34]; Maglio[29-31] has mentioned the Elephantidae; and Hooijer has described[35] the rhinocerotidae—the *Ceratotherium simum* is a primitive form comparable to the *C.s. germanoafricanum* from Laetolil. Upper third molars from Olduvai Bed II are intermediate between those from JM91 and those from Olduvai Bed IV and the recent forms. The new species of *Hippopotamus* resembles the one to be described from Kanapoi.

(*h*) Aterir beds

REPTILIA	
Chelonia	
Pelomedusidae	
Crocodilia	
MAMMALIA	
Proboscidea	c.f. *Anancus* sp.
	Mammuthus subplanifrons Osborn
Perissodactyla	
Equidae	aff. *Hipparion*
Rhinocerotidae	*Ceratotherium* sp.
Artiodactyla	
Suidae	c.f. *Sus* sp.
	Nyanzachoerus c.f. sp. "B"
Hippopotamidae	*Hippopotamus* sp.
Bovidae	

Several fossiliferous areas occur at the same horizon in the Aterir beds in a sedimentary basin about 4.5 × 1.5 km in extent. The unit is a little younger than 4 m.y. in age. Maglio has referred[29] to the locality as "Atirir" (*sic*) in connexion with the Elephantidae. The *Anancus* sp. is not *A. osiris*, but is comparable to an undescribed species from Kanapoi[27] and to one from Langebaanweg. The *Ceratotherium* is probably a new species to be described from Lothagam.

(*i*) Karmosit beds

MAMMALIA	
Proboscidea	
Perissodactyla	
Rhinocerotidae	
Artiodactyla	
Suidae	*Notochoerus euilus* (Hopwood)
Hippopotamidae	*Hippopotamus* sp.
Giraffidae	
Bovidae	
Tragelaphini	
Reduncini	
Antilopini	c.f. *Aepyceros* sp.

The Karmosit beds include one fossiliferous locality at an isolated outcrop. The fauna is probably a little older than 3.4 m.y. The locality was discovered and the initial collection made by Dr G. J. H. McCall. *Notochoerus euilis* is not found later than the level of Tuff C of the Shungura formation in the Omo Valley (about 3 m.y.).

(*j*) Chemoigut beds (Chesowanja locality)

MOLLUSCA	
Gastropoda	*Bellamya* sp.
Bivalvia	
PISCES	
Siluriformes	
REPTILIA	
Chelonia	
Trionychidae	
Pelomedusidae	
Crocodilia	*Crocodylus niloticus* Laurenti
MAMMALIA	
Primates	
Cercopithecidae	*Simopithecus* sp.
	sp. indet.
Hominidae	*Australopithecus* sp.
Proboscidea	
Elephantidea	*Elephas* c.f. *recki* Dietrich
Deinotheriidae	*Deinotherium bozasi* Arambourg
Perissodactyla	
Equidae	*Equus* sp.
Rhinocerotidae	*Ceratotherium* sp.
Artiodactyla	
Suidae	*Pronotochoerus jacksoni* Leakey
	"*Metridiochoerus*" sp.
Hippopotamidae	*Hippopotamus* c.f. *amphibius* Linn.
	Hippopotamus sp.
Giraffidae	
Bovidae	
Tragelaphini	*Tragelaphus* sp.
? Tragelaphini	
Bovini	
Reduncini	*Kobus* sp.
Alcelaphini	? *Megalotragus kattwinkeli* (Schwarz)
	? *Connochaetes* sp.
	small sp.
	smaller sp.
Antilopini	? *Antidorcas* sp.

Descriptions of the geology of the Chemoigut beds and of a hominid partial cranium, together with a faunal list, have already been published[19]. The specimen of *Deinotherium bozasi* is the latest occurrence of the genus so far known. The *Kobus* sp. is as large as the largest *K. sigmoidalis* from the Omo and Olduvai deposits, and the extant *K. ellipsiprymnus*. The middle size group of the Alcelaphini is about the size of *Damaliscus niro* or *Parmularius angusticornis*.

(*k*) Kapthurin formation

MAMMALIA	
Primates	
Cercopithecidae	*Colobus* sp.
Hominidae	*Homo* sp.
Carnivora	indet.
Hyracoidea	
Procaviidae	*Heterohyrax* sp.
Artiodactyla	
Suidae	*Potamochoerus* sp.
	Tapinochoerus sp.
	Phacochoerus sp.
Hippopotamidae	*Hippopotamus amphibius* (Linn.)
Bovidae	
Tragelaphini	*Tragelaphus* c.f. *scriptus* (Pallas)
Reduncini	*Redunca* sp.
Alcelaphini	indet.
Cephalophini	*Cephalophus* sp.
	Sylvicapra sp.
Tubulidentata	
Orycteropodidae	*Orycteropus* sp.
Rodentia	
Muridae	*Oenomys* sp.
	Rattus c.f. *rattus* (Linn.)
Thryonomyidae	*Thryonomys* sp.

The Kapthurin formation includes two fossiliferous localities and it is younger than a single unconfirmed potassium-argon age of 230,000 yr. The sediments have been described by Fuchs[14] and later by Martyn[15,17,20] and by McCall, Baker and Walsh[16]. Margaret Leakey has discussed the archaeology[15] and the same reference has contributions from Martyn on the sediments, Tobias on the hominid which he believes is probably *Homo erectus*, and R. E. F. Leakey on the rest of the fauna. The identifications and list above were kindly provided by Dr J. Harris.

(*l*) Kokwob beds

The fauna of the Kokwob beds consists of abundant mollusca and fragments of fish and mammalia in beach and shallow water deposits at various levels up to 20 m above the present level of Lake Baringo. The age of the deposits is probably Holocene.

Fossiliferous horizons recently investigated at Omo, East Rudolf, Kanapoi and Lothagam have extended knowledge of the East African Pliocene/Pleistocene mammalian faunas back from about 1.8 m.y., the age of the base of the sequence at Olduvai Gorge, to about 5 m.y. As well as representing this time span, the Baringo succession provides the first faunal and stratigraphic evidence between about 5 m.y. and the Fort Ternan faunal assemblage at 14 m.y. Further detailed work on the Baringo faunal assemblages together with more isotopic dates will facilitate more confident comparisons with other African localities and should produce valuable information on the still little known earlier Pliocene and late Miocene record in Africa.

This work was financed by the Kenya Government, the Natural Environment Research Council, the Royal Society, the Wenner-Gren Foundation for Anthropological Research and the Boise Fund of the University of Oxford. We thank the EAGRU members, J. N. Carney, Dr M. P. McClenaghan, Dr J. E. Martyn, J. S. C. Sceal and Dr P. K. Webb, whose work has led to the elucidation of the succession and who discovered many of the fossil localities; and Professor B. C. King, Dr L. S. B. Leakey, R. E. F. Leakey, Dr G. J. H. McCall, Dr A. C. Walker, Dr J. Walsh and Dr L. A. J. Williams for their help. Dr N. J. Snelling of the Institute of Geological Sciences allowed us to use the results of several isotopic age determinations on the lavas. For identification and preliminary comments on the fauna we thank Mrs S. C. Savage (Hippopotamidae), Professor H. B. S. Cooke (Suidae), Dr A. Gentry (Bovidae), Dr J. Harris (Deinotheriidae), Dr D. A. Hooijer (Rhinocerotidae), Dr V. J. Maglio (Elephantidae) and Dr R. Wood (Chelonia).

Received July 23, 1971.

[1] Bishop, W. W., Miller, J. A., and Fitch, F. J., *Amer. J. Sci.*, **267**, 669 (1969).
[2] Van Couvering, J. A., and Miller, J. A., *Nature*, **221**, 5181 (1969).
[3] Leakey, L. S. B., *Ann. Mag. Nat. Hist.*, **4**, 47 (1961).
[4] Bishop, W. W., and Whyte, F., *Nature*, **196**, 1283 (1962).
[5] Evernden, J. F., and Curtis, G. H., *Curr. Anthrop.*, **6**, 4 (1965).
[6] King, B. C., *Proc. Geol. Soc. Lond.*, **122**, 1629 (1966).
[7] King, B. C., in *African Magmatism and Tectonics* (edit. by Clifford, T. N., and Gass, I. G.), 263 (Oliver and Boyd, Edinburgh, 1970).
[8] King, B. C., and Chapman, G. R., *Proc. Roy. Soc.*, A (in the press).
[9] Gregory, J. W., *The Great Rift Valley* (Murray, London, 1896).
[10] Gregory, J. W., *The Rift Valleys and Geology of East Africa* (Seeley, Service, London, 1921).
[11] Leakey, L. S. B. (ed.), *Proc. First Pan-African Congr. Prehist.* (Nairobi, 1947) (Blackwell, Oxford, 1952).
[12] Clark, J. D. (ed.), *Proc. Third Pan-African Congr. Prehist.* (Chatto and Windus, London, 1957).
[13] Sandford, K. S., and Blondel, F. (eds.), *Proc. Eighteenth Intern. Geol. Congr. Lond., 1948*, 14 (Assoc. Services Geol. Africains Proc., 1951).
[14] Fuchs, V. E., *Geol. Mag.*, **87**, 148 (1950).
[15] Leakey, M., *Proc. Prehist. Soc.*, **35**, 3 (1969).
[16] McCall, G. J. H., Baker, B. H., and Walsh, J., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.), 191 (University of Chicago Press, 1967).
[17] Martyn, J. E., *Nature*, **215**, 476 (1967).
[18] Bishop, W. W., and Chapman, G. R., *Nature*, **226**, 914 (1970).
[19] Carney, J., Hill, A., Miller, J. A., and Walker, A., *Nature*, **230**, 509 (1971).
[20] Martyn, J. E., thesis (Univ. London, 1969).
[21] Walsh, J., *Rep. Geol. Surv. Kenya*, 84 (1969).
[22] Shackleton, R. M., *Quart. J. Geol. Soc. Lond.*, **106**, 345 (1951).
[23] McCall, G. J. H., *Rep. Geol. Surv. Kenya*, 78 (1967).
[24] Savage, R. J. G., *Proc. Geol. Soc. Lond.*, No. 1657, 167 (1968).
[25] Van Couvering, J. A., and Miller, J. A., *Nature*, **230**, 559 (1971).
[26] Simons, E. L., Pilbeam, D., and Boyer, S. J., *Nature*, **229**, 408 (1971).
[27] Hooijer, D. A., and Patterson, B., *Bull. Mus. Comp. Zoo. Harv.* (in the press).
[28] Hendey, Q. B., *Ann. S. Afr. Mus.*, **56**, 75 (1970).
[29] Maglio, V. J., *Nature*, **225**, 328 (1970).
[30] Maglio, V. J., *Breviora*, **336**, 1 (1969).
[31] Maglio, V. J., *Breviora*, **341**, 1 (1970).
[32] Cooke, H. B. S., and Maglio, V. J., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.) (Scottish Academic Press, in the press).
[33] Tobias, P. V., *Nature*, **215**, 479 (1967).
[34] Leakey, R. E. F., in *Fossil Vertebrates of Africa*, **1**, 53 (Academic Press, London and New York, 1969).
[35] Hooijer, D. A., in *Fossil Vertebrates of Africa*, **1**, 71 (Academic Press, London and New York, 1969).

Antigens of Developing *Drosophila melanogaster*

DAVID B. ROBERTS
Genetics Laboratory, Department of Biochemistry, South Parks Road, Oxford OX1 3QU

The changes in the antigenic complements of extracts of soluble proteins of *Drosophila* have been followed through development.

THE genetic control of development in eukaryotes can be investigated in terms of the control of synthesis of one or a few proteins. Thus many people[1-4] have studied the relationship of enzymes to development in *Drosophila*. I have studied simultaneously many proteins detected immunologically in *Drosophila* at all stages from the unfertilized egg to the eclosed adult, and a study is in progress (Gouveia and myself, unpublished results) of the spatial distribution during development of these proteins. Some of the proteins have been identified as known enzymes, but the function of most of them is unknown. The apparent concentrations of the antigens, however, suggest that the proteins are enzyme or structural proteins rather than being concerned exclusively with control. Development has already been studied in terms of changes in the antigens in extracts of soluble proteins of amphibia[5-10], echinoderms[11,12] and insects[13,14], but usually for a limited period of development or for one tissue. The presence or absence of an antigen is taken to reflect the state, active or inactive, of the gene or genes responsible for the synthesis of that antigen. But this concept overlooks genes with reduced activity, which may be below the resolution of the techniques used. It also overlooks control at the level of translation, although this has been investigated with one protein. Because of the complexity of the problem, however, such a simple approach is necessary. Although the simultaneous appearance of many antigens seems to correlate with phases of sensitivity to the action of lethal mutations, no attempt has yet been made to associate the appearance or disappearance of these proteins with specific morphological events.

To obtain different developmental stages, about 10,000 flies, Oregon-R, were placed in a 'Perspex' population box with Petri dishes containing medium for egg laying (4% agar, 10% glucose, 10% live yeast dissolved in 100 ml. water, and autoclaved at 15 pounds/inch^2 for 15 min, and with the addition of nipagin just before the agar set). The Petri dishes were changed at hourly intervals, the samples when harvested were $X \pm 0.5$ h from the mid-laying time. All times are given from this mid-laying time. Because eggs may be retained by the female after fertilization, no eggs collected in the first hour of any working day were used, so that each stage contained few individuals significantly older than the stated age. The mean hatching time was 22 h, the mean time of puparium formation 128 h and the mean time of eclosion 215 h.

Rabbits were immunized with extracts of eggs (laid over 4 h), embryos (eggs laid over 22 h), first instar larvae (eggs laid over 4 h and collected at 40 h), second instar larvae (eggs laid over 4 h and collected at 60 h); third instar larvae (eggs laid over 4 h and collected at 96 h); adult flies. All immunized rabbits produced antibodies which reacted with the extracts used for immunization. Differences in the antigenic complement of different developmental stages were obvious on double diffusion plates (Fig. 1). Because of the many precipitin bands formed the results were more readily interpreted on immunoelectrophoresis plates (Fig. 2). Using the antibody preparations described above, thirty-three different protein antigens were identified which appeared at some time during the development of *Drosophila* (Fig. 3). Ten of these proteins are detected in newly fertilized eggs and, of these ten, eight are found in unfertilized eggs (Fig. 4) and probably represent the products of genes active in the egg during oogenesis or the products of genes active in adjacent nurse cells.

The other two proteins may represent the products of mRNA transcribed from the maternal genome and masked in the unfertilized egg but released by fertilization. This suggestion is supported by the observations of Lockshin[15] that no RNA synthesis could be detected in the eggs of two species of Coleoptera until the nuclei appeared in the cortex of the egg, which is at about 1.5 h in *Drosophila*[16]. The suggestion is also supported by the observations of Gross[17] and others on masked mRNA and early protein synthesis in sea urchins. Five new proteins appear in 3 h old extracts, probably translated from mRNA transcribed from the zygote genome after the nuclei have migrated to the cortex and which therefore represent the earliest paternal proteins in this organism. New proteins are observed in extracts of late embryos and of young larvae. At these times, studies using electrophoretic variants of enzymes have shown that many enzymes, which must be translated from mRNA transcribed from the zygote genome, are synthesized[3]. This is also the first sensitive phase for lethal point mutations[18]. New proteins are again detected in extracts from the other developmental periods, from mid second instar to mid third instar and late in pupal life. The first of these coincides with the two lethal phases, early and late third instar detected by Hadorn and Chen[19], with the death of the larvae occurring some time after the normal appearance of the essential protein. The second of these periods may reflect the pupal lethal period of Hadorn and Chen.

No new proteins were detected at the time of puparium formation although extracts were made of larvae/puparia-pupae at 3 h intervals from 115 h to 145 h. The absence of new proteins at this period was surprising in view of the consider-

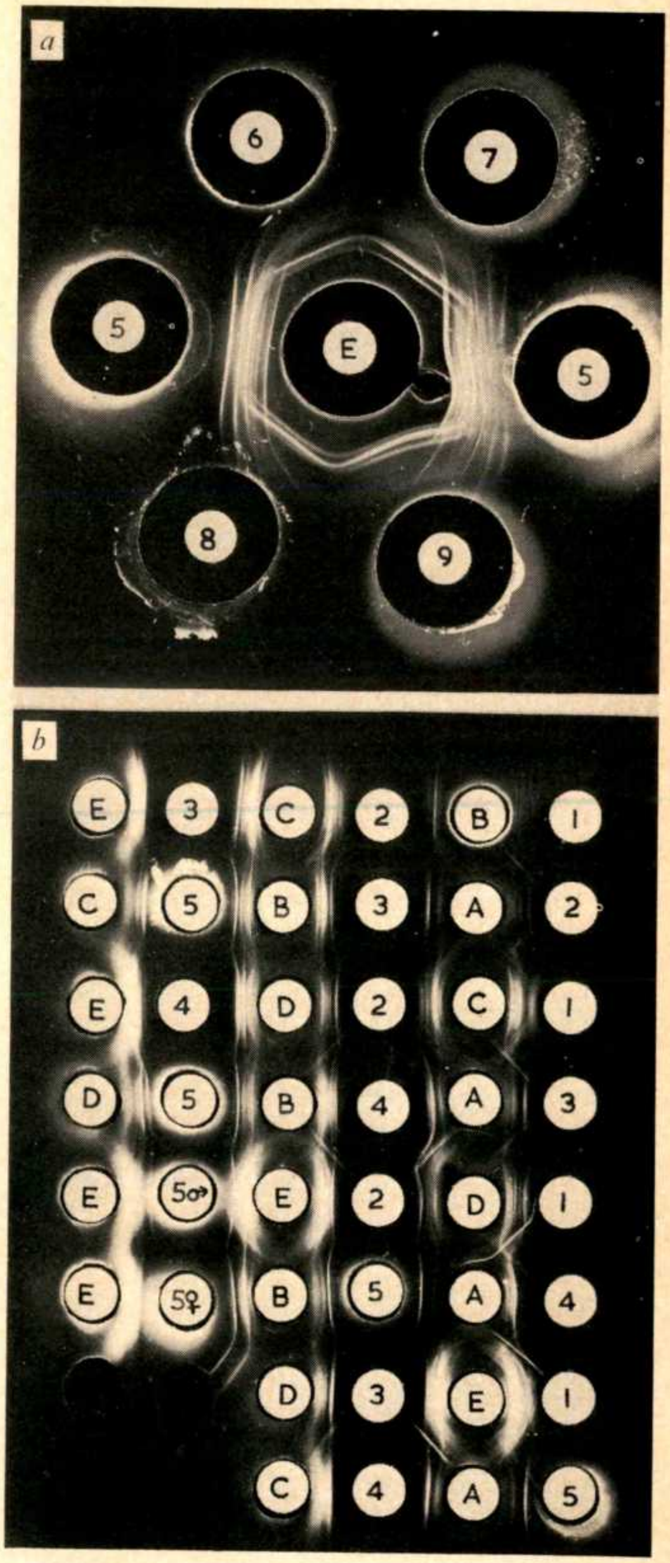


Fig. 1 Double diffusion plates of extracts from different developmental stages. *a*, The double diffusion plates were set up as indicated in the text. *b*, Horizontal precipitin band represents a heterologous interaction, that is, an antigen reacting with antibodies prepared against another antigen; a diagonal precipitin band represents a homologous interaction. 1, Egg; 2, first instar larvae; 3, second instar larvae; 4, third instar larvae; 5, whole fly; 6, 3 h old eggs; 7, 6 h old eggs; 8, 9 h old eggs; 9, 12 h old eggs; A, anti-egg; B, anti-first instar larvae; C, anti-second instar larvae; D, anti-third instar larvae; E, anti-fly.

able changes in puffing pattern that occur at this time[20]. One protein (No. 28) precipitated by antibodies against extracts of third instar larvae was subjected to a more detailed study than the others. Its molecular weight was determined on a sucrose density gradient[21] with reference to catalase as 420,000 daltons. The protein was detected on the gradient by analysing alternate drops on double diffusion plates with antibodies. This antigen is found at a high concentration in haemolymph and at a very low concentration in salivary gland/fat body extracts. The protein is first detected at about 60 h and cannot be detected in extracts of mature adults.

Molecular weight studies, time course studies and location studies suggest that this protein is similar to protein No. 1 found by Laufer[22] in *Hylophora crecopia* which has a molecular weight of about 450,000 daltons and is located in the haemo-

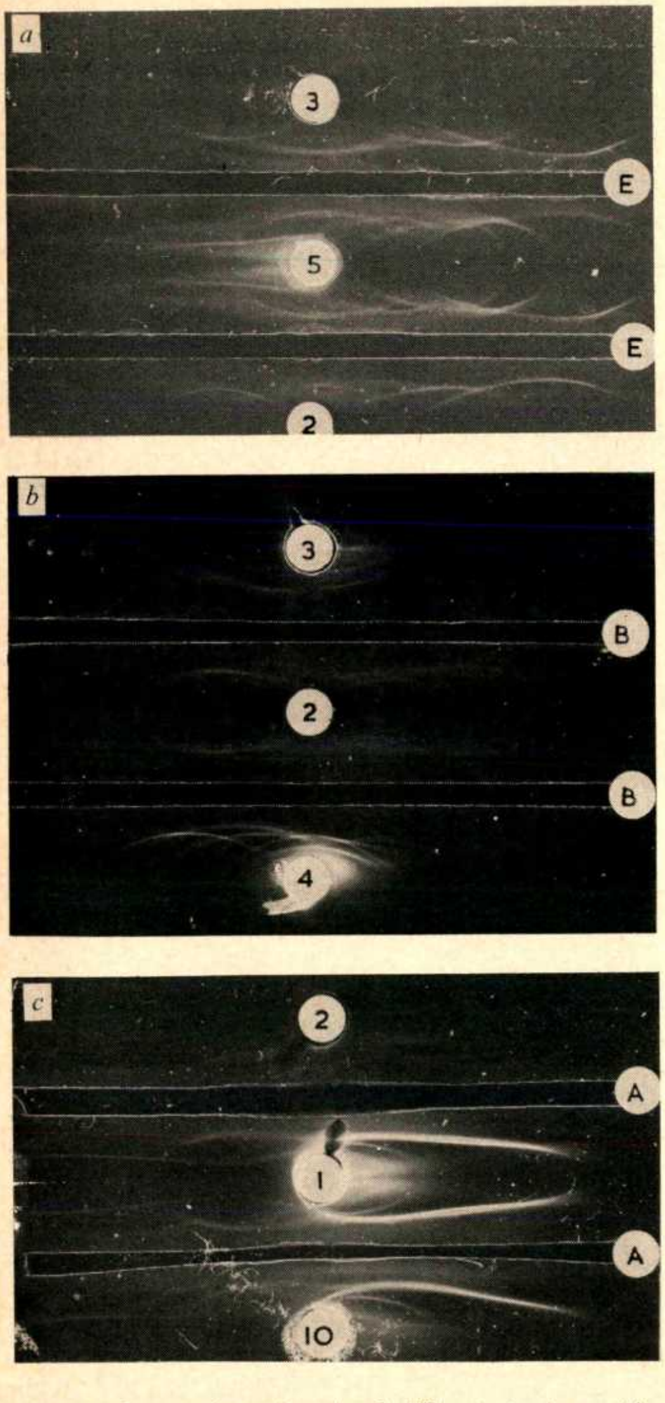


Fig. 2 Immunoelectrophoresis of different developmental stages. The plates were set up as indicated in the text. Ten embryos were used.

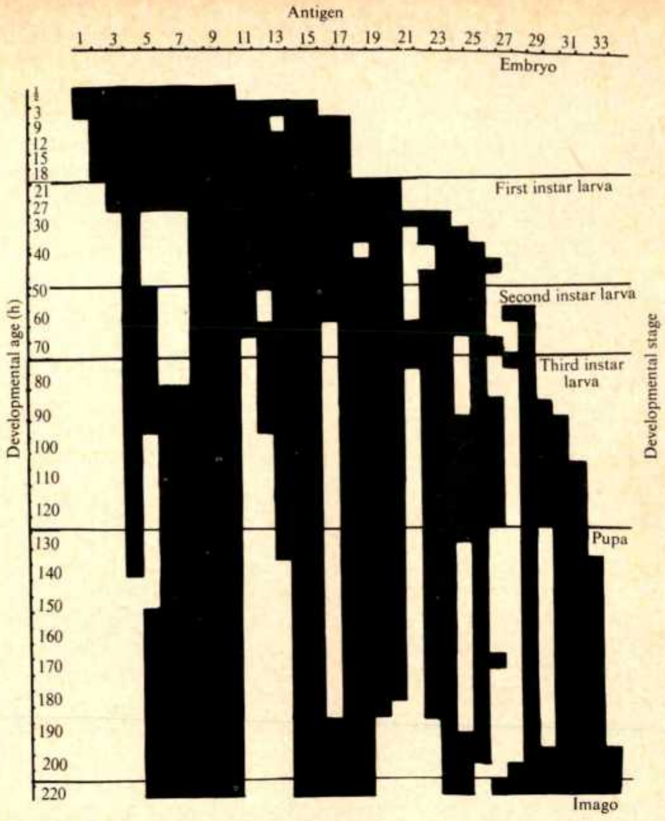


Fig. 3 Antigens appearing during the development of *Drosophila melanogaster*. Flies laid eggs for 1 h periods, the eggs, larvae, pupae or flies were harvested at the times indicated and extracts of soluble proteins were prepared from these by sonicating for 30 s in 0.05 M phosphate buffer (*p*H 7.2) containing 0.8% NaCl, using an MSE sonicator. Third instar larvae, pupae and flies were homogenized with a tissue grinder before being sonicated. Extracts were kept cold at all times. The extracts were centrifuged at 30,000*g* for 20 min and the protein concentration of the supernatant was estimated by the micro-Biuret method[24]. All extracts were adjusted to the same protein concentration and were analysed by immunoelectrophoresis using the antibody solutions described above. The table shows the presence of the antigens at different developmental ages tested.

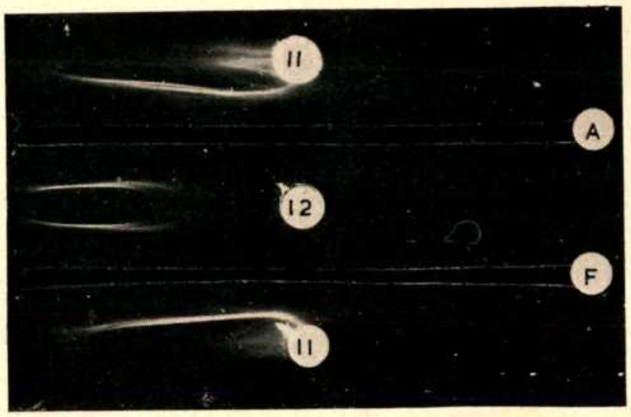


Fig. 4 Immunoelectrophoresis plate of fertilized and unfertilized eggs. The unfertilized eggs were collected from virgin female flies which laid eggs for 1 h periods. The eggs from many collections were stored at −20° C until a sufficient quantity for making an extract had been collected. The fertilized eggs were collected after flies had laid for 0.5 h periods and again stored at −20° C until sufficient eggs had been collected. Both extracts were adjusted to the same protein concentration. 11, Newly fertilized eggs; 12, unfertilized; F, anti-embryo.

lymph at all stages of development excepting egg, embryo and mature adult and to calliphorin described by Munn and Greville[23] in *Calliphora erythrocephala* and other insects. Calliphorin shows a similar distribution but has a higher molecular weight.

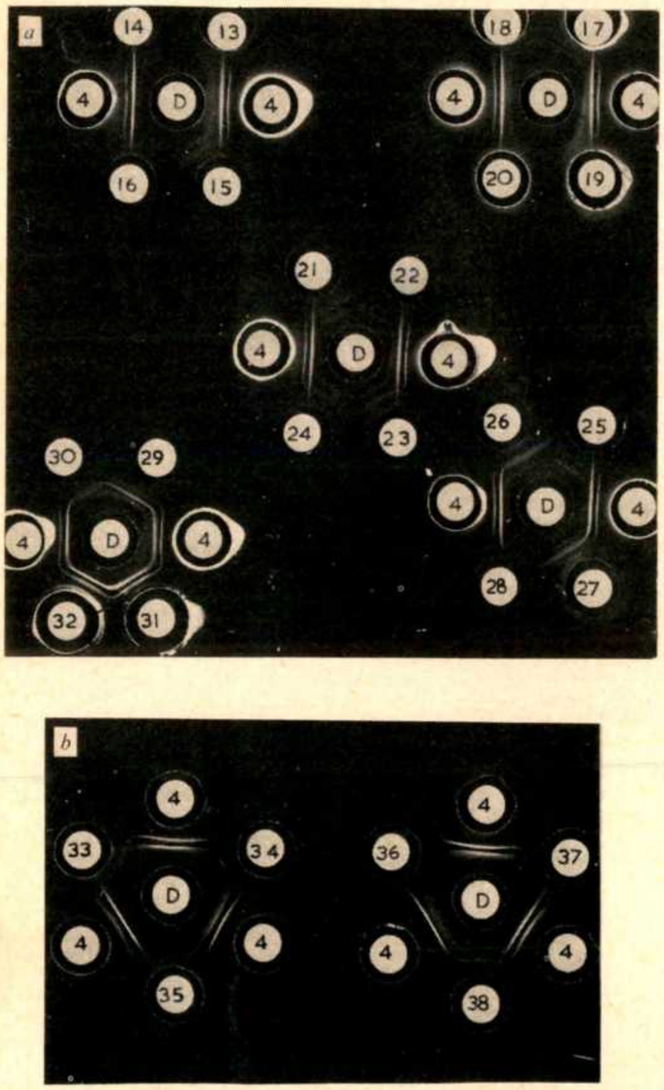


Fig. 5 Analysis of the control of the synthesis of protein No. 28. *a*, Double diffusion plate showing the time of appearance of two antigens during development. No. 28 at 60 h and No. 29 at 75 h. *b*, Double diffusion plate of larvae grown on normal medium and on medium-containing actinomycin D. The larvae were either harvested at the time shown (ii) or transferred to actinomycin D at the time shown and harvested at 85 h (i). 13, 1 h old egg; 14, 4 h old egg; 15, 8 h old egg; 16, 12 h old egg; 17, 16 h old egg; 18, 20 h old egg; 19, 25 h old larvae; 20, 30 h old larvae; 21, 35 h old larvae; 22, 40 h old larvae; 23, 45 h old larvae; 24, 50 h old larvae; 25, 55 h old larvae; 26, 60 h old larvae; 27, 65 h old larvae; 28, 70 h old larvae; 29, 75 h old larvae; 30, 80 h old larvae; 31, 85 h old larvae; 32, 90 h old larvae; 33, 54 h old transfer; 34, 57 h old transfer; 35, 60 h old transfer; 36, 54 h old larvae; 37, 57 h old larvae; 38, 60 h old larvae. All extracts adjusted to the same protein concentration.

The control of the appearance of antigens could be mediated at the level of transcription or at the level of translation. To test this a detailed study was carried out on protein No. 28. This protein was detected in extracts of 60 h old larvae grown on normal medium but not in extracts of 57 h old larvae. Larvae transferred to medium containing actinomycin D had not synthesized this protein 30 h later if transferred at 54 h but had synthesized the protein at this time if transferred at 57 h (Fig. 5). This suggests that the structural gene for this protein is transcribed between 54 h and 57 h and the protein first appears between 57 h and 60 h. Thus there is little delay in the translation of the mRNA so that the control of the synthesis of this protein is at the level of transcription. Actinomycin D had no effect on embryogenesis nor on proteins appearing during that time, probably because of permeability factors; in contrast actinomycin D seriously disturbed larval development and proteins appearing during larval life.

Antibodies against extracts of egg and of whole flies showed a significant difference in the antigenic complement of male and female flies. Two extra antigens were present in the female. Fractionation of the adults into head, thorax and abdomen showed that this difference was confined to the abdomen. These two antigens were also present in extracts of eggs and seem to be proteins either synthesized in the egg during oogenesis or in the nurse cells and transported to the eggs. No other differences could be detected between adult male and female flies.

These results show which proteins should be studied further and what questions such studies can hope to answer. The preparation of antibodies against different organs and the use of fluorescent antibodies will decide which proteins characterize an organ and which are found generally. A study of wild type strains for electrophoretic variants of the antigens will reveal the formal genetics of these proteins, and indicate the earliest time of mRNA synthesis from the paternal genome in the zygote. The role played by these proteins in development could be elucidated by the study of the disturbed antigenic complement of developmental lethal mutations.

I thank Mr J. Hewitt and Miss Susan Moffitt for technical assistance. This work was assisted by a grant from the SRC. Actinomycin D was a gift from Merck Sharp and Dohme Ltd.

Received July 9; revised September 14, 1971.

[1] Ursprung, H., Dickinson, W. J., Murison, G., and Sofer, W. H., in *Problems in Biology, RNA in Development* (edit. by Hanly, E. W.), 55 (University of Utah Press, 1969).

[2] Doane, W. W., in *Problems in Biology, RNA in Development* (edit. by Hanly, E. W.), 72 (University of Utah Press, 1969).

[3] Wright, D. A., and Shaw, C. R., *Biochem. Genet.*, **4**, 385 (1970).

[4] Fox, D. H., Abacherli, E., and Ursprung, H., *Experientia*, **27**, 218 (1971).

[5] Cooper, R. A., *J. Exp. Zool.*, **101**, 143 (1946).

[6] Cooper, R. A., *J. Exp. Zool.*, **107**, 397 (1948).

[7] Cooper, R. A., *J. Exp. Zool.*, **114**, 403 (1950).

[8] Spar, I. L., *J. Exp. Zool.*, **123**, 467 (1953).

[9] Clayton, R. M., *J. Embryol. Exp. Morphol.*, **1**, 25 (1953).

[10] Romanovsky, A., *Folia Biol.*, **10**, 1 (1964).

[11] Perlmann, P., and Gustafson, T., *Experientia*, **4**, 481 (1948).

[12] Perlmann, P., *Exp. Cell. Res.*, **5**, 394 (1953).

[13] Telfer, W. H., and Williams, C. M., *J. Gen. Physiol.*, **36**, 389 (1953).

[14] Zaman, V., and Chellapah, W. T., *Exp. Parasitol.*, **13**, 108 (1963).

[15] Lockshin, R. A., *Science*, **154**, 775 (1966).

[16] Sonnenblick, B. P., in *Biology of Drosophila* (edit. by Demerec, M.), 62 (Wiley, New York, 1950).

[17] Gross, P. R., Malkin, L. I., and Moyer, W. A., *Proc. US Nat. Acad. Sci.*, **51**, 407 (1964).

[18] Hadorn, E., *Developmental Genetics and Lethal Factors* (Methuen, London, 1961).

[19] Hadorn, E., and Chen, P. S., *Arch. Jul. Klaus-Stiftg.*, **27**, 147 (1952).

[20] Ashburner, M., *Chromosoma*, **21**, 398 (1967).

[21] Martin, R. G., and Ames, B. N., *J. Biol. Chem.*, **236**, 1372 (1961).

[22] Laufer, H., *Ann. NY Acad. Sci.*, **89**, 490 (1960).

[23] Munn, E. A., and Greville, G. D., *J. Insect Physiol.*, **15**, 1935 (1969).

[24] Zamenhof, S., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **3**, 70 (Academic Press, New York, 1957).

Bacterial Virus Gene Expression in Human Cells

CARL R. MERRIL & MARK R. GEIER
Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20014

JOHN C. PETRICCIANI
Laboratory of Pathology, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014

Human fibroblasts, from a patient with congenital lack of α-D-galactose-1-phosphate uridyl transferase activity, have been infected with transducing bacteriophage that harbours either wild type or defective transferase gene. Infection only by the former phage initiates transferase synthesis.

IF the genetic code is truly universal it might be possible to make genetic changes in mammalian cells by introducing a specific gene by bacteriophage transduction[1,2]. Human fibroblasts from a patient with galactosemia served as a model system to test for such a possibility. Galactosemia is the result of an autosomal recessive inborn error of metabolism in which there is a congenital absence of the enzyme α-D-galactose-1-phosphate uridyl (GPU) transferase activity[3,4].

The viruses which were tested in this system were lambda ($\lambda_{\mathrm{CI}}857$, $\lambda_{\mathrm{CI}}857S_7$)[5-7] and two transducing viruses derived from λ: the first, $\lambda pgal_8C_I857(K^+T^+E^+)$, contains the complete galactose operon (ref. 8, and personal communication from D. Court and S. Adhya), and the second, $\lambda pgal_8C_I857(K^+T^-E^+)$, contains the galactose operon with a mutation that renders the transferase gene inactive (made for us by M. E. Gottesman).

To obtain a functional enzyme from a bacterial virus, a mammalian cell would have to transcribe at least part of the phage genome into mRNA and then translate this into protein. Transcription was demonstrated in this study by the discovery of λ-specific RNA in cells exposed to either λ virus or λ DNA. GPU-transferase activity was also detected after exposure of the cells to λpgal T^+ virus or its DNA.

Transcription was studied by using galactosemic human fibroblasts (CCL-72 obtained in the sixteenth passage from the American Type Culture Collection). In each experiment, flasks infected with λ virus and control flasks were labelled with radioactive uridine 24 h before the cells were collected. When RNA was extracted and hybridized with denatured λ DNA on cellulose nitrate filters, the production of λ-specific RNA reached a maximum of 0.2% of labelled total RNA within 4.5 days after infection (Fig. 1). Cultures exposed to λ have remained at this level through two 3 : 1 subcultures for longer than 40 days. A similar result was obtained when the cells were exposed to λ DNA. Less than 0.005% of the labelled RNA extracted from the control cells was bound to the λ DNA filters. As a control for any contaminant which might be homologous with *E. coli*, the labelled RNA from both the λ infected and non-infected cells was hybridized with *E. coli* DNA filters. The binding was less than 0.005%.

Transferase activity in fibroblasts was assayed by a modification of the method of Russell[12-14]. In this assay, galactose-1-phosphate (Gal-1-P) is converted to UDP-galactose (UDP-Gal) by the transferase enzyme and by using ^{14}C labelled Gal-1-P it is possible to follow this reaction (Fig. 2). This activity was linear with time in the conditions used and proportional to the amount of cell lysate used (Fig. 3). All activity was lost by boiling the cell lysate. Uninfected galactosemic cells had an activity ≤0.2 nmol of UDP-Gal/60 min/mg of protein (Table 1). No difference in enzyme activity could be detected between uninfected galactosemic fibroblasts and fibroblasts infected with λ virus or λpgal $T(^-)$, with a mutation in transferase structural gene. The λpgal T^+ DNA-infected cells had enzyme levels which were generally greater than the λpgal T^+ whole virus-infected cells. Infected cell cultures have

Table 1 Assays for Transferase Activity

Cell strain	Exposure	Multiplicity of infection	Time after exposure	Transferase* activity
Normal fibroblasts				
Rat embryo fibroblasts	None	—	—	46.0
VMK	None	—	—	23.2
WI–38	None	—	—	6.3
Galactosemic fibroblasts (CCL-72)				
(*a*) Uninfected	None	—	—	<0.2
(*b*) Infections with transferase negative virus or DNA				
	λ virus	4×10^5	96 h	<0.2
	λpgal $(K^+T^-E^+)$ virus	4×10^5	96 h	<0.2
	λpgal $(K^+T^-E^+)$ DNA	6×10^4 phage p.f.u. equivalents†	96 h	<0.2
(*c*) Infections with transferase positive virus or DNA				
	λpgal $(K^+T^+E^+)$ virus	4×10^5	96 h	2.0
	λpgal $(K^+T^+E^+)$ virus	4×10^5	52 days	10.1
	λpgal $(K^+T^+E^+)$ DNA	6×10^4 phage p.f.u. equivalents†	96 h	15.6

* Galactose-1-phosphate uridyl transferase activity is expressed in terms of nmol of UDP-Gal formed in 60 min/mg of protein at 37° C.

† 1 μg of λDNA = 2×10^{10} p.f.u. equivalents.

Rat embryo fibroblasts were prepared as a primary culture from Holtzman rat embryos (donated by V. Huebner), VMK is a primary cell strain from African green monkey kidney cells (prepared by the cell biology section of the Division of Biologics Standards) and WI-38 is a normal human diploid cell line (CCL-75, passage 16, from the American Type Culture Collection). The cells were grown, infected and assayed for transferase activity as described in Figs. 2 and 3. $\lambda_{\mathrm{CI}}857$[5] virus was grown vegetatively on *E. coli* N205 (a spontaneous gal$^+$ revertant of strain W3102 gal K^2 and carries recA allele from strain 152 gal$^+$ of Meselson)[17]. λpgal$(K^+T^-E^+)$ was also grown on N205. λpgal$(K^+T^+E^+)$ was grown vegetatively on N205 and also on N580 (a transferase negative strain, W3104)[18]. These viruses were purified and banded in CsCl prior to use[9]. Virus DNA was prepared as described in Fig. 3.

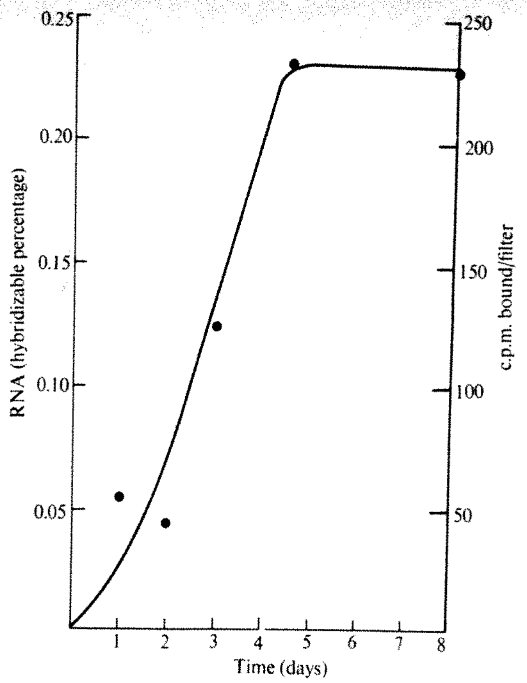


Fig. 1 Appearance of λ-specific RNA after infection of CCL-72 human galactosemic fibroblasts with $\lambda_{CI}857S_7$ virus at a multiplicity of infection (m.o.i.) of 1×10^5 λ plaque forming units (p.f.u.)/cell. The data presented are in c.p.m. above background as well as hybridizable percentage which is defined as c.p.m. bound to filter-scintillation system background (20 c.p.m.)/total c.p.m. input. Hybridization with RNA extracted from uninfected cells gave ≤ 5 c.p.m. above background. Cells were cultured in Eagle's minimal essential media (EMEM) containing sodium bicarbonate (1 g/l.), foetal calf serum (10%), penicillin (100 U/ml.), streptomycin (50 μg/ml.), and glutamine (0.17%) in 10 cm Petri dishes. This mixture was used for all tissue culture unless otherwise indicated. $\lambda_{CI}857S_7$ was heat-induced from *E. coli* M5107 (given by E. Signer)[6,7], precipitated with polyethylene glycol, and banded in a gradient of CsCl[9]. The virus was dialysed in 0.015 M Tris-HCl, *p*H 7.6 buffer containing 0.01 M $MgSO_4$, and before use it was diluted into 1 ml. of Earle's balanced salt solution without bicarbonate (Earle's BSS) and passed through 'Millipore' filters (0.45 μm pore size). The filters were rinsed with an additional 1 ml. of Earle's BSS. The cells were infected by the addition of 2 ml. of λ virus (5×10^{10} p.f.u.) in Earle's BSS. The virus was allowed to adsorb for 1 h with gentle rocking at 37° C, followed by the addition of 10 ml. of tissue culture media. Incubation was conducted at 37° C in an atmosphere of 5% CO_2 in air. The cellular RNA was labelled by removing the media and adding 10 ml. of fresh media plus 100 μCi of ^3H-5-uridine (Lot No. 1745–40 from ICN, 21.7 mCi/mmol). The cells were incubated a further 24 h, rinsed twice with phosphate buffer saline (calcium and magnesium-free), and trypsinized. The RNA was extracted using SDS to break the cells followed by phenol, chloroform–isoamyl alcohol extractions and DNAase digestions using the method of Biswal and Benyesh-Melnick[10]. The extracted RNA was precipitated with ethyl alcohol and resuspended in a solution containing 0.003 M EDTA (adjusted to *p*H 7.4 with HCl). Hybridization was carried out in 6 × 50 mm test tubes in a total volume of 0.480 ml. containing a final concentration of 2 × SSC and 50% formamide. Cellulose nitrate filters (Schleicher and Schuell, B-6) containing 50 μg of denatured DNA each (the DNA is in excess at this concentration) were placed in the tubes and the ^3H RNA was added. The tubes were sealed and incubated at 37° C for 24 h (hybridization was complete within 24 h). These filters were then extensively washed with 2 × SSC, treated with 20 μg of RNAase A (Worthington) and 10 units of RNAase T_1 (Sankyo) at 37° C for 10 min. They were again washed with 2 × SSC and incubated in 2 ml. of 1 × SSC containing 70% formamide for 10 min at 37° C. The filters were again washed with 2 × SSC, dried, and counted in a Nuclear Chicago refrigerated scintillation counter (model 8914). The scintillation system contained 12 g of BBOT (Packard)/gallon of toluene. This hybridization technique was developed as a modification of the Gillespie and Spiegelman[11] method by M. Das and S. Spiegelman (personal communication). All spent medium was tested for bacterial contamination by inoculating 0.5 ml. of medium on to tryptone plates and into 10 ml. of fresh tissue culture media without antibiotics. These were been assayed through repeated subcultures and enzyme activity has remained relatively constant at the level achieved 4 days after infection. All eight λpgal virus preparations give similar results. Cells were grown in the presence of strepto- cultured for 5 days at 37° C and at room temperature. Cells were also periodically monitored for mycoplasma contamination. All such tests were negative.

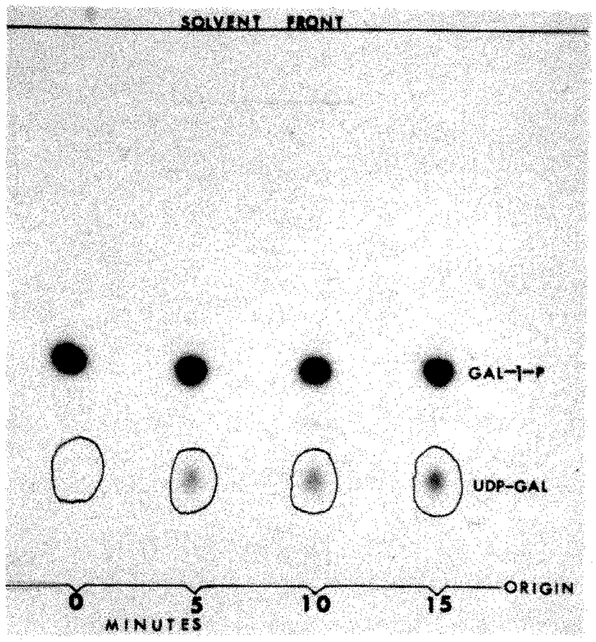


Fig. 2 Chromatography of 5 μl. aliquots at 5 min intervals from an assay for α-D-galactose-1-phosphate uridyl transferase activity. The ^{14}C-Gal-1-P reactant is clearly visible in the autoradiogram, while the product UDP-Gal becomes more visible as its concentration increases. The circles represent a UDP-Gal marker (non-radioactive). Cells (5×10^5, normal rat fibroblasts) were grown as described in Fig. 1, and scraped off the wall, centrifuged (1,400*g* for 5 min), rinsed in PBS (calcium and magnesium-free) and resuspended in 20 μl. of glycine buffer (0.08 M, *p*H 8.7). Sodium dilauryl sulphate (2 μl. of 0.2% solution) was added and the cells were freeze–thawed three times and centrifuged at 12,000*g* for 2 min. The supernatant was then assayed for transferase activity and protein concentration was determined by the Lowry method[15]. The reaction mix contained 5 μl. of D-galactose-^{14}C-1-phosphate (4.5×10^{-4} M, New England Nuclear Corp., 227 mCi/mmol)*, 5 μl. H_2O, 5 μl. of glycine buffer (1 M, *p*H 8.7), 10 μl. of UDP-glucose (4×10^{-4} M) and 10 μl. of crude cell lysate. The reaction was performed at 37° C and 5 μl. aliquots were removed and applied to the thin-layer chromatography plates PEI (Brinkman). Unlabelled UDP-Gal (2 μl. 10 μmol/ml.) was spotted at the origin as a marker. The chromatogram was run for 4 cm in 1 N formic acid and 17 cm in 2 N sodium formate, *p*H 3.6. The position of the UDP-Gal could be determined by the ultraviolet absorption of the unlabelled marker. Similar results were obtained by using DEAE paper ('Whatman DE81') with 0.015 M $Na_2HPO_4 \cdot 7H_2O$ and 0.15 M citric acid, *p*H 3.8; however, this system gave a higher background. Autoradiography of the chromatogram was performed with X-ray film ('Kodak NS-2T') and the UDP-Gal spots were cut out and placed in a toluene-TLA (Beckman) scintillation system for quantitative counting. A Beckman LS250 scintillation counter was used.

* Commercial Gal-1-P gives a relatively high background in this system (1,000 c.p.m.); however, it is possible to purify this material by utilizing the PEI thin layer chromatography system described above. The Gal-1-P is located by autoradiography and the spots cut out. These are washed twice with 1 litre of distilled H_2O and then placed in 100 ml. of 0.5 M LiCl for 20 min. The LiCl solution is then decanted off and passed through a 'Dowex 50' column, 5 mm × 20 mm (which was previously washed with 0.5 M LiCl). The column effluent was diluted fifty times and pumped through a 'Dowex 1' column (which was previously washed with 2 M ammonium formate and then with distilled H_2O). The column was eluted with 1 M formic acid and the Gal-1-P collected. The sample was freeze dried and brought to the desired concentration with distilled water. This purified Gal-1-P gives a background of about 100 c.p.m.

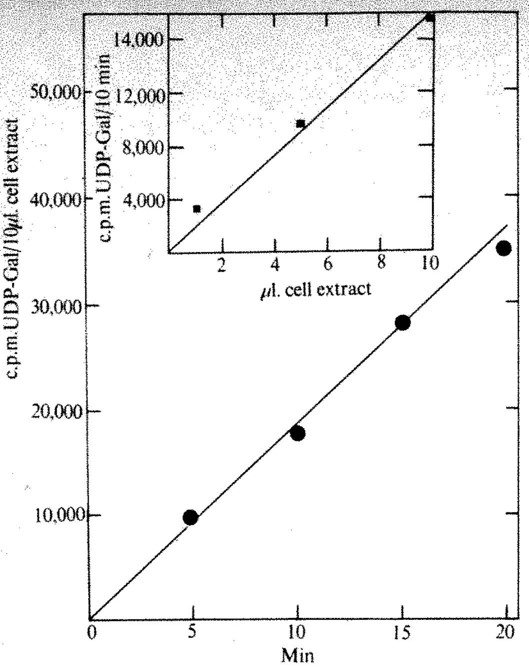


Fig. 3 Linearity of the α-D-galactose-1-phosphate uridyl transferase assay as a function of time. Inset demonstrates the linearity of the assay as a function of protein concentration. The cells used in this assay were galactosemic human fibroblasts infected with a multiplicity of infection of 6×10^4 phage plaque forming unit equivalents of λpgal T$^+$ DNA*. The DNA was added to three flasks, each containing 5×10^5 cells, in 2 ml. of Earle's BSS with 75 µg/ml. of DEAE-D (MW$=10^6$, Pharmacia)[16]. This DNA was allowed to adsorb for 1 h with gentle rocking at 37° C, followed by the addition of 10 ml. of tissue culture medium and incubated for an additional 95 h at 37° C in an atmosphere of 5% CO_2 in air. The cells were harvested and the assay performed as described in Fig. 2.

* 1 µg of λDNA $=2\times10^{10}$ p.f.u. equivalents, based on a molecular weight of $\lambda=3\times10^7$. Phage DNA was obtained by phenol extraction of 3 ml. of phage with an equal volume of freshly distilled phenol equilibrated with 2×SSC followed by an extraction with isoamyl alcohol and chloroform (1 : 100), and a second phenol extraction. The DNA was treated with 1 mg of pronase in 1 ml. of Tris-HCl (0.1 M, *p*H 7.5) buffer containing EDTA (0.003 M) for 5 min followed by three more phenol extractions and two ethyl ether extractions. It was then exhaustively dialysed against 2×SSC at 4° C.

mycin (50 µg/ml.) and penicillin (100 U/ml.). Mycostatin (50 U/ml.) was tested in cultures with no effect on enzyme production or λ-specific RNA levels. Cycloheximide (1 mg/ml.) which blocks protein synthesis in mammalian cells[19] but not in bacteria[20] prevented enzyme production in cultures infected with λpgal T$^+$. These cultures were monitored for bacterial and mycoplasma contamination in the same manner as in the hybridization study.

We determined whether λpgal T$^+$ enhances intracellular galactose metabolism. Morphological evidence of metabolic stress (granular cytoplasm, rounding up and detachment from the surface of the flask) could be detected in galactosemic cells after 72 h incubation with galactose as sole hexose source. Cells infected with λpgal, however, have survived from 1 to 3 weeks longer than control cells when both were grown in media containing 1 mg/ml. galactose and dialysed foetal calf serum. Normal human fibroblasts have survived in this medium for at least 1 week longer than any galactosemic cells infected with λpgal T$^+$ virus. Furthermore, galactosemic fibroblasts exposed to λpgal T$^+$ produce more $^{14}CO_2$ from galactose-1-^{14}C than uninfected fibroblasts (our unpublished observations).

The possibility of contaminating organisms giving rise to the data is remote as subcultures of the spent medium in thioglycollate and trypticase soy broths showed no growth, nor did cultures on tryptone plates and tissue culture media without antibiotics, and the cells were periodically shown to be free of mycoplasma. The presence of organisms in numbers sufficient to yield the transferase data (5×10^5 *E. coli*, N205) should have been detected easily and, furthermore, no *E. coli* specific RNA was detected in the hybridization experiments. As the cells were cultured in penicillin and streptomycin, the contaminant would be insensitive to these antibiotics as well as mycostatin, but inhibited by cycloheximide. As a further control 10^6 λ lysogens (*E. coli* M5107), were added to a culture of galactosemic fibroblasts and labelled for 24 h with ^{3}H-uridine. These cells were then washed in the normal manner, leaving only the fibroblasts bound to the flask surface. No λ-specific RNA could be found by hybridization, indicating that the λ-specific RNA is not produced by an *E. coli*-like organism. The possibility that the λpgal T$^+$ virus is merely transporting enzyme into the cells can be discounted as the enzyme is produced by cells infected with pronase-treated λpgal T$^+$ DNA.

The detection of λ-specific RNA supports the occurrence of transcription, while the lack of enzyme production in the presence of cycloheximide indicates that translation is necessary for transferase activity.

The experimental conditions used by us do not permit a differentiation between the various models of phage gene preservation within mammalian cells. The detection of both λ-specific RNA and transferase enzyme activity 41 days after infection with no decrease in levels indicates that the genome is not being segregated out in these conditions of growth. The phage DNA may be preserved by any of the following mechanisms: integration into the host genome, plasmid-like existence in the cytoplasm, interaction with the mitochondria (known to have some bacteria-like properties), or perhaps in some totally unknown manner.

Our results suggest that it might be possible to introduce a selected bacterial gene into human cells *in vivo*, using phage as a vehicle, and significantly to alter a specific metabolic pathway. Our results also agree with recent evidence[21,22] that there are much greater biochemical similarities between far ranging living species than had been appreciated previously[23].

We thank Dr M. Gottesman, S. Spiegelman, R. L. Kirschstein, G. L. Cantoni and R. S. Krooth for their support and encouragement.

Received October 13, 1970; revised August 5, 1971.

[1] Morse, M. L., Lederberg, E. M., and Lederberg, J., *Genetics*, **41**, 142 (1956).
[2] Morse, M. L., Lederberg, E. M., and Lederberg, J., *Genetics*, **41**, 758 (1956).
[3] Kalckar, H. M., Anderson, E. P., and Isselbacher, K. J., *Biochim. Biophys. Acta*, **20**, 262 (1956).
[4] Tedesco, T. A., and Mellman, W. J., *Science*, **172**, 727 (1971).
[5] Sussman, R., and Jacob, F., *CR Acad. Sci.*, **254**, 1517 (1962).
[6] Goldberg, A. R., and Howe, M., *Virology*, **38**, 200 (1969).
[7] Goldberg, A. R., and Howe, M., *Virology*, **39**, 152 (1969).
[8] Signer, E. R., *Nature*, **223**, 158 (1969).
[9] Nash, H. A., and Merril, C. R., *J. Mol. Biol.*, **43**, 357 (1969).
[10] Biswal, N., and Benyesh-Melnick, M., *Proc. US Nat. Acad. Sci.*, **64**, 1372 (1969).
[11] Gillespie, D., and Spiegelman, S., *J. Mol. Biol.*, **12**, 829 (1965).
[12] Russell, J. D., and De Mars, R., *Biochem. Genetics*, **1**, 11 (1967).
[13] Russell, J. D., in *Galactosemia* (edit. by Hsia, D. T.), 204 (Thomas, Springfield, Illinois, 1969).
[14] Russell, J. D., *Biochem. Genetics*, **1**, 301 (1968).
[15] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
[16] Merril, C. R., and Geier, M., *Virology*, **42**, 780 (1970).
[17] Gottesman, M. E., and Yarmolinsky, M. B., *J. Mol. Biol.*, **31**, 487 (1968).
[18] Lederberg, E. M., *Tenth Symp. Soc. Gen. Microbiol.*, **10**, 115 (1960).
[19] Wettstein, F. O., Noll, H., and Penman, S., *Biochim. Biophys. Acta*, **87**, 525 (1964).
[20] Sisler, H. D., and Siegel, M. R., in *Antibiotics*, **1**, 283 (edit. by Gottleib and Shaw) (Springer, 1967).
[21] Bryan, R. N., Gelfund, D. H., and Hayashi, M., *Nature*, **224**, 1019 (1969).
[22] Wigle, D. T., and Dixon, G. H., *Nature*, **227**, 676 (1970).
[23] Gruneberg, H., *Nature*, **225**, 39 (1970).

LETTERS TO NATURE

PHYSICAL SCIENCES

Further Data on the Optical Variable PKS 1514-24

Biraud[1] and Bond[2] have drawn attention to the close agreement in position between the variable star AP Lib and the radio source PKS 1514–24. The optical object, which was originally classified by Ashbrook[3] as an irregular variable, exhibits chaotic variations[1] in brightness over time scales of hours and days. Although the optical position given by Ashbrook[3] is not precise, there seems to be little doubt that this object is associated with the radio source. However, it is important for further investigations that the radio-optical association be more firmly established by the measurement of accurate radio and optical positions.

Radio observations of PKS 1514–24 were carried out in 1971, July 31 and August 1–2, using the pencil beam response of the cross-type telescope at the Molonglo Radio Observatory. The operating frequency is 408 MHz and the antenna beamwidth is 2′.6 EW × 2′.9 NS at this declination. Six nearby sources with accurately known positions and flux densities were also observed on each day to calibrate the telescope pointing and gain.

The optical position of AP Lib was measured on the glass copies of the Palomar Sky Survey. The techniques used for these measurements have been described in detail before[4]. The position was determined with respect to nine reference stars from the Smithsonian Astrophysical Observatory star catalogue. Measurements were made on both the E ("red") and O ("blue") plates; the two estimates agreed to within 0″.2 arc.

Table 1 Radio and Optical Data for PKS 1514-24 and AP Lib

	R.A. (1950.0)	Declination (1950.0)
AP Lib	15 h 14 m 45.28 s ±0.03 s	−24° 11′ 22″.9±0″.4
PKS 1514–24	15 h 14 m 45.64 s ±0.09 s	−24° 11′ 20″.6±1″.6
Peak flux density at 408 MHz	1.75±0.05 flux units *	

* 1 flux unit = 10^{-26} W m^{-2} Hz^{-1}.

The radio and optical positions with their standard errors are shown in Table 1. The errors in the 408 MHz radio position and peak flux density were obtained by combining the calibration uncertainty and the internal scatter among the three scans. The flux density is based on the absolute scale of Wyllie[5]. Following the procedure outlined earlier[4], the errors in the optical position represent the mean of the r.m.s. residuals for the two plate solutions. A comparison of the radio and optical positions shows that they do not in fact coincide within the errors. The difference of 4″.9 arc in right ascension corresponds to 3.8 combined standard errors and certainly appears significant, although the declination difference of 2″.3 (1.4 σ) may not be real. The radio position in Table 1 is supported by the 1,425 MHz position of Fomalont and Moffet[6] as well as by an earlier but less precise R.A. measurement by Hoskins and Murdoch[7]. There is evidence in the pencil beam scans for a weak confusing source ~0.2 flux units lying 2–3′ E and ~1′ N of the main source. A possible bias in the fitting process caused by the presence of this additional source may partly explain the observed radio-optical discrepancy. However, the narrower Molonglo fan beam is not affected by this weak source and a more accurate determination based on further scans in 1966 (D. G. Hoskins, private communication) gives the fan beam R.A. as 15 h 14 m 45.61 s ± 0.18 s (1950), in excellent agreement with the present value. It will therefore be important to carry out observations at higher resolution to search for possible small scale structure.

Table 2 Flux Densities (Flux Units) of PKS 1514-24 Measured by Different Observers and Arranged in Chronological Order

Observatory	Approx. epoch	Ref.	Frequency (MHz) 408	1,410	2,650	5,000
Parkes	1963	8	5.3±0.9	2.7±0.2	2.1±0.2	
Parkes	1964	9			1.8±0.15	
Owens Valley	1965	6		2.32±0.10 *		
Parkes	1966	10	2.6±0.4 †			
Molonglo	1966.32	7	1.9±0.3			
Parkes	1968	11				1.94±0.05
Molonglo	1971.58	Here	1.75±0.05			

Errors have been obtained directly from the references cited.
* Flux density at 1,425 MHz.
† Flux density measured at 468 MHz with the Parkes interferometer.

The published spectral data for PKS 1514–24 are given in Table 2 and the more recent measurements indicate an extremely flat spectrum. The apparent discrepancy between the two low frequency flux densities measured at Parkes[8,10] is only partly resolved by the detection[10] of a 1.4 flux units confusing source in the Parkes beam. Additional scans with the Molonglo pencil beams confirm the existence of an extended source lying 12′ E and 17′ N of PKS 1514–24. This source has an integrated 408 MHz flux density of 1.6±0.1 flux units and no further sources >0.3 flux units were found within ±30′ in R.A. or ±20′ in declination. Therefore, although there is no convincing evidence for variability, it seems possible that a secular decrease in 408 MHz flux density has occurred. Some support for this conclusion is given by the marginally significant decrease at 21 cm. A more definite indication of radio variability, however, must await further observations at all wavelengths.

When proposing the identification of PKS 1514–24, Bolton, Clarke and Ekers[12] classified the optical object as a 16 m elliptical galaxy. The object is reclassified by Westerlund and Wall[13] as an N-type galaxy, which is more consistent with its appearance and UBV colours. AP Lib lies very close to the centre of the Sky Survey plates (epoch 1955.3; seeing 3) and the image on the E plate is clearly non-stellar with an irregular nebulous envelope of small extent. The total diameter is $\sim 10''$ arc and there appears to be a small prominence or "jet" on the eastern limb; this feature lies very close to the 408 MHz radio position. There is further evidence for faint extensions to the north.

On the Palomar O plate the image is most unusual. The halo is of smaller extent and there seems to be a number of narrow radial filaments distributed around the disk and extending about $5''$–$10''$ arc from the nucleus. A close comparison of the E and O plates shows that the position angles for several of these filaments are correlated on the two plates. While recognizing that there are problems involved in correctly identifying weak features which are of the same order as the grain size of the emulsion, a careful examination shows no similar peculiarities in any of the nearby stellar or non-stellar images. Large scale photographs taken in good seeing will obviously be necessary to confirm the existence of these nebulous filaments.

It is worth considering further the possible similarity[1] between AP Lib and the peculiar variable object BL Lac (=VRO 42.22.01)[14,15]. Macleod and Andrew[14] have reported a faint envelope around BL Lac, although an examination of the Sky Survey prints shows it to be much less obvious than the nebulosity surrounding AP Lib. Photometric observations of the two objects[13,16,17] place them close together on the two colour diagram and more towards the domain of the N galaxies than the region occupied by the quasi-stellar objects. Neither object shows the presence of emission lines in the optical spectrum[16,18,19]. If the conventional interpretation of radio spectra applies, then the inverted spectrum of BL Lac suggests it is a much younger source than AP Lib. A final point of interest concerns the recent discovery that two further radio sources ON 231 and B2 1215+30 are also associated with bright variable objects[1,20,21] of neutral colour and unusual appearance. It is therefore tempting to speculate that these four objects may belong to a new class of highly active discrete sources.

I thank Dr A. J. Turtle for assistance with the radio observations and for helpful discussions. This work was supported by grants from the Australian Research Grants Committee, the Sydney University Research Grants Committee, and the Science Foundation for Physics within the University of Sydney.

R. W. HUNSTEAD

Cornell-Sydney University Astronomy Centre,
School of Physics,
University of Sydney

Received September 20, 1971.

[1] Biraud, F., *Nature*, **232**, 178 (1971).
[2] Bond, H. E., *Astrophys. J. Lett.*, **167**, L79 (1971).
[3] Ashbrook, M. D., *Harvard Annals*, **109**, No. 7 (1942).
[4] Hunstead, R. W., *Mon. Not. Roy. Astron. Soc.*, **152**, 277 (1971).
[5] Wyllie, D. V., *Mon. Not. Roy. Astron. Soc.*, **142**, 299 (1969).
[6] Fomalont, E. B., and Moffet, A. T., *Astron. J.*, **76**, 5 (1971).
[7] Hoskins, D. G., and Murdoch, H. S., *Austral. J. Phys., Astrophys. Suppl.*, 15 (1970).
[8] Bolton, J. G., Gardner, F. F., and Mackey, M. B., *Austral. J. Phys.*, **17**, 340 (1964).
[9] Shimmins, A. J., Clarke, M. E., and Ekers, R. D., *Austral. J. Phys.*, **19**, 649 (1966).
[10] Ekers, R. D., *Austral. J. Phys., Astrophys. Suppl.*, 6 (1969).
[11] Shimmins, A. J., Manchester, R. N., and Harris, B. J., *Austral. J. Phys., Astrophys. Suppl.*, 7 (1969).
[12] Bolton, J. G., Clarke, M. E., and Ekers, R. D., *Austral. J. Phys.*, **18**, 627 (1965).
[13] Westerlund, B. E., and Wall, J. V., *Astron. J.*, **74**, 335 (1969).
[14] Macleod, J. M., and Andrew, B. H., *Astrophys. Lett.*, **1**, 243 (1968).
[15] Schmitt, J. L., *Nature*, **218**, 663 (1968).
[16] Dupuy, D., Schmitt, J., McClure, R., van den Bergh, S., and Racine, R., *Astrophys. J. Lett.*, **156**, L135 (1969).
[17] Bertaud, C., Dumortier, P., Véron, P., Wlérick, G., Adam, G., Bigay, J., Garnier, G., and Duruy, M., *Astron. Astrophys.*, **3**, 436 (1969).
[18] Oke, J. B., Neugebauer, G., and Becklin, E. E., *Astrophys. J. Lett.*, **156**, L41 (1969).
[19] Searle, L., and Bolton, J. G., *Astrophys. J. Lett.*, **154**, L101 (1968).
[20] Browne, I. W. A., *Nature*, **231**, 515 (1971).
[21] Lucchetti, S. C., and Usher, P. D., *Nature*, **232**, 622 (1971).

Cosmological Evolution in Radio Galaxies

ROWAN-ROBINSON has presented evidence that radio galaxies exhibit remarkably strong evolutionary effects with cosmological epoch[1]. Examination of his evidence, however, indicates that, although an evolutionary effect may indeed be present, no reliable estimate of the time scale involved can yet be made in the way he proposes. This is because (i) the mean value of $V/V_{\rm max}$ (defined by Rowan-Robinson) is very sensitive to changes in the limiting magnitude assumed for the sample, and (ii) the redshift corrections (*K*-corrections) which have to be applied to the apparent magnitudes of the fainter radio galaxies (for which the evolutionary effects appear to be strongest) are not sufficiently well known. These effects will now be considered in turn.

(i) In this section I shall follow the procedure of Rowan-Robinson but make use of a slightly smaller yet more complete body of data (199 out of the 220 sources used by Rowan-Robinson). These sources are all those in a statistically complete sample of 199 extragalactic radio sources from the revised 3C survey which have been mapped with high resolution at declinations $\delta > +10^\circ$ and more than 10° from the galactic plane. It is only within this area of sky that all the sources in the revised 3C catalogue have been mapped with high resolution and for which identifications have been established with a fair degree of reliability[2-4]. Following Rowan-Robinson, I have estimated $x = V/V_{\rm max}$ in an Einstein–de Sitter cosmological model for each source in the complete sample known to be a radio galaxy. In making these calculations I have used the radio spectral data of Kellermann, Pauliny-Toth and Williams[5], and the optical spectral data (*K*-corrections) of Oke and Sandage[6]. The latter are only available for redshifts $z < 0.28$ (at which redshift a galaxy would have an apparent visual magnitude $m_v = 19.0$ if its absolute magnitude $M_v = -21.60$, the mean for all the galaxies in the sample with a measured redshift) and it has therefore been necessary to extrapolate the data of Oke and Sandage to give $K_v{}^6 = 1.05$ and 1.25 at redshifts of 0.32 and 0.37 respectively. Although it will be shown later that the *K*-corrections of Oke and Sandage may not be appropriate for distant radio galaxies, they probably give a good description of the spectral properties of nearer, less luminous radio galaxies and have been used here, in the absence of any alternative data. The procedure was to fix the radio flux density limit 9×10^{-26} W Hz^{-1} sr^{-1} at 178 MHz (the limiting flux density of the 3C(Rev) catalogue), to vary the limiting apparent visual magnitude m_v in 0.1 magnitude steps between 17.75 mag and 20.35 mag and estimate the mean value $\bar{x}$ for each limiting magnitude. The results are shown in Fig. 1; the error bars represent the standard deviation in the mean expected for n values of x uniformly distributed between 0 and 1, equal to $(12\,n)^{-0.5}$. The number of sources which have contributed to $\bar{x}$ is shown above each point. As the limiting magnitude is increased, progressively more objects are included in the sample which determines $\bar{x}$. In most cases the apparent magnitudes used have been estimated from the plates and prints of the Palomar Sky Survey since very few photoelectric V magnitudes have been measured for faint radio galaxies. Because of the consequent uncertainty most are quoted[7] only to the nearest half magnitude and the number of sources which contribute to $\bar{x}$ correspondingly increases in jumps every half magnitude. If the apparent magnitude limit is chosen just to include galaxies with m_v equal to one of these discrete values, then each will contribute a value of $x \sim 1$ to $\bar{x}$ and produce the

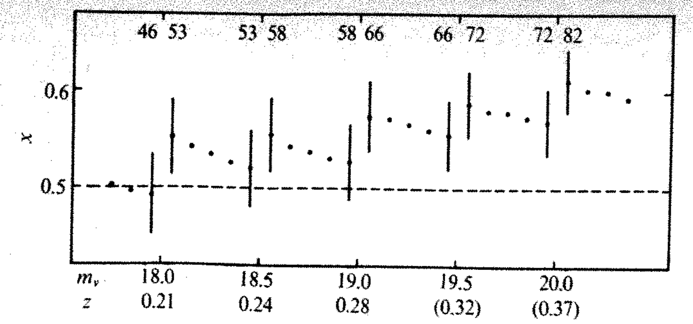


Fig. 1 The value of $\bar{x}$ (defined by Rowan-Robinson[1] and in the text) determined for samples of radio galaxies brighter than magnitude m_v. The number of sources which contribute to each value of $\bar{x}$ is shown above the point, and the expected standard errors in the mean (see text) are shown by the vertical bars. The mean absolute visual magnitude for all the radio galaxies in the present sample (excluding 3C231) with a measured redshift is $M_v = -21.60$. The redshifts at which a galaxy of this absolute magnitude would have the apparent magnitudes in the diagram are shown below each number. The values for $m_v > 19.0$ are based on an arbitrary extrapolation from the available data and are likely to be particularly unreliable.

jumps found in Fig. 1. As the magnitude limit is again increased, no further sources contribute to $\bar{x}$, so that the contribution of the faintest galaxies decreases from $x \sim 1$ and $\bar{x}$ slowly decreases. Eventually the next discrete value of m_v is reached when another jump in $\bar{x}$ occurs. The values quoted by Rowan-Robinson are found to agree well with the peak values in each saw-toothed cycle of $\bar{x}$ but it is obvious that they give an exaggerated measure of the variation of $\bar{x}$ with m_v. Rowan-Robinson has attempted to reduce this effect when estimating the rate of evolution of sources, but the rest of his discussion concentrates on the values of $\bar{x}$ and not the weighted $\bar{x}^*$. Both his results and the present results, taken at face value, still suggest that $\bar{x}$ becomes increasingly different from 0.5 as fainter limiting magnitudes are approached and that this difference is greater than the expected error in the mean for every point fainter than 19.0 mag. Unfortunately they cannot be taken at their face value because of the uncertainty of the *K*-corrections.

(ii) It is found that the sources with $v/v_{\max}$ determined by the optical limit of the sample contribute systematically larger values of x to the mean $\bar{x}$ than do those for which $v/v_{\max}$ is set by the radio limit. For example, the twelve sources which are optically limited contribute an average value of 0.679 to the point with the faintest limiting magnitude, while the seventy which are radio flux density limited contribute an average of 0.574 to $\bar{x}$. This difference becomes progressively less marked as brighter limiting magnitudes are approached and is due partly to the effect of the *K*-corrections. If appropriate *K*-corrections had been used this difference would in no way invalidate the volume-luminosity test or the evolutionary conclusions that may be drawn from it. But there is reason to believe that the *K*-correction given by Oke and Sandage may be substantially different from those appropriate when correcting the apparent magnitudes of distant radio galaxies with strong emission line spectra.

The values given by Oke and Sandage[6] are based on observations of the spectra of the nuclei of several nearby giant *E* and *SO* galaxies and *M*31. The composite spectrum from which the *K*-corrections were determined decreases very sharply for wavelengths shorter than 5000 Å, so that galaxies of this type which are at redshifts large enough for this part of their spectrum to be displaced into the visual band (centred on λ 5500 Å) will appear relatively faint. For redshifts $z > 0.2$, the *K*-corrections are therefore large and strongly redshift-dependent. A small increase in the redshift of such a galaxy will make it relatively much fainter, so that a source with $v/v_{\max}$ determined optically will tend to have $x > 0.5$. In addition *K*-corrections have not been determined[6] for redshifts $z > 0.28$ (corresponding to $m_v = 19.0$ for a typical radio galaxy) where the greatest differences between $\bar{x}$ and 0.5 are found, and it has been necessary to extrapolate from the available data at lower redshifts. The composite spectrum used by Oke and Sandage was obtained from galaxies which are almost entirely free from emission lines, whereas all but 4 of the 24 radio galaxies with $z > 0.1$ listed recently by Burbidge[8] have strong emission lines, which must obviously affect the *K*-correction. The spectrum of 3C405 (Cygnus A) is entirely dominated by the strong emission lines[8], nearly all of which have wavelengths shorter than 5000 Å. The optical spectra of distant radio galaxies are not well established but in many respects they appear to be intermediate between those of elliptical galaxies and quasars[8,9].

Sandage has estimated *K*-corrections for quasars[10] and, for the red-shift range $z = 0.1$ to 0.4, although they are much less reliably determined they are probably of similar magnitude but of opposite sign to those for elliptical galaxies. A lower limit to $\bar{x}$ of 0.53 ± 0.035 at a limiting visual magnitude of 19.5 is set if all the sources were limited by their radio flux density. Any *K*-corrections can only increase this value, but plausible values for the *K*-corrections could give values of $\bar{x}$ different from 0.5 by more than one standard deviation only for the faintest radio galaxies. The uncertainties in the magnitudes of these galaxies of between 0.5 mag and 1 mag[2-4,7] are such as to make any interpretation of this difference in terms of an evolutionary effect rather unreliable. The present results suggest, however, that Rowan-Robinson has substantially over-estimated the rate of evolution in radio galaxies. It is clear that, until accurate spectrophotometric data are available for a sample of distant radio galaxies, it will not be possible to determine the cosmological evolutionary properties of these objects with any confidence by the method used by Rowan-Robinson.

I thank Drs J. R. Shakeshaft and M. S. Longair for discussions on this subject, and the Science Research Council for a research fellowship.

CRAIG D. MACKAY

Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
Free School Lane,
Cambridge

Received May 10, 1971.

[1] Rowan-Robinson, M., *Nature*, **229**, 388 (1971).
[2] Macdonald, G. H., Kenderdine, S., and Neville, A. C., *Mon. Not. Roy. Astron. Soc.*, **138**, 259 (1968).
[3] Mackay, C. D., *Mon. Not. Roy. Astron. Soc.*, **145**, 31 (1969).
[4] Elsmore, B., and Mackay, C. D., *Mon. Not. Roy. Astron. Soc.*, **146**, 361 (1969).
[5] Kellermann, K. I., Pauliny-Toth, I. I. K., and Williams, P. J. S., *Astrophys. J.*, **157**, 1 (1969).
[6] Oke, J. B., and Sandage, A. R., *Astrophys. J.*, **154**, 21 (1968).
[7] Wyndham, J. D., *Astrophys. J.*, **144**, 459 (1966).
[8] Burbidge, G. R., *Ann. Rev. Astron. Astrophys.*, **8**, 369 (1970).
[9] Arp, H., *Astrophys. J.*, **153**, L33 (1968).
[10] Sandage, A. R., *Astrophys. J.*, **146**, 13 (1966).

Reply to Mackay

FIRSTLY, Mackay raises the issue of the completeness of the sample I used. The restriction to sources with declination greater than 10° and further than 10° from the galactic plane which he suggests does not materially affect the results I obtained (6 of the sources I used would have to be omitted).

The discontinuous dependence of the mean value of $x = v/v_{\max}$, $\bar{x}$, on the chosen limiting optical magnitude is dealt with by using the quantity $\bar{x}^*$, explained in my article[1]. The quantity $\bar{x}^*$ is by definition a continuous function of limiting optical magnitude and in fact, by taking the values given on line 6 of my Table 1, Mackay would have noted that it runs smoothly through the middle of the points plotted in his Fig. 1.

On the question of *K*-corrections for galaxies, I should explain that Oke and Sandage's[2] *K*-corrections for the "standard giant elliptical" played no part at all in my argument about 3C radio galaxies, so that Mackay's polemic seems rather misdirected. What I actually did was to determine a mean relation between estimated visual magnitude and redshift empirically, and then used this to estimate redshift in the cases where it is not yet known. Now it is true that below 19th magnitude this relation is essentially an extrapolation, since only one radio galaxy fainter than this has had its redshift measured. But on the one hand the result is still significant if attention is confined to galaxies with $V \leqslant 19$: and on the other the result is very insensitive to what extrapolation is used.

Over the redshift range $0 < z \leqslant 0.4$, Oke and Sandage's *K*-correction for the standard giant elliptical can be approximated by $K_\nu \sim 2.2\, z$, whereas Sandage's[3] composite spectrum for quasars gives, over the same redshift range, roughly $K_\nu \sim -2.0\, z$. *N*-galaxies, which have colours intermediate between ellipticals and quasars[4], may then be presumed to have *K*-corrections in the range $K_\nu \sim (0 \pm 2)\, z$. For $V \geq 17$, *N*-galaxies already constitute half the data from which my mean empirical V-*z* relation was determined. Thus our ignorance of the appropriate *K*-correction for radio galaxies can be expressed by saying that the quantity *B* in my equation (1) is uncertain by ±2 from this cause (there may well be other systematic errors).

In fact I find that $\bar{x}^*$ is hardly changed if *B* is changed by ±5, and the corresponding values of the evolutionary parameters given in my Table 2 remain within the uncertainties quoted there. From the fact that a luminosity evolution of strength $(1+z)^6$ is needed to reduce $\bar{x}^*$, corrected for this evolution, to 0.5, I would infer that the optical spectra of galaxies would have to behave like $P(\nu) \propto \nu^5$ to be the sole cause of the effect reported. Such objects would hardly have been overlooked by those making the identifications.

It is, incidentally, absurd to say, as Mackay does, that the difference between $\bar{x}$ for sources with $\nu_{\max}$ determined by the optical and radio limits respectively "is due to the effect of the *K*-corrections". A difference is entirely to be expected, since a galaxy near the optical limit must be one of the most distant in the sample, whereas one near the radio limit may be either near or far, due to the vast range of radio luminosities found in galaxies. Thus the effects of evolution will be greater for the optically limited sources. A detailed calculation, including the effects of the dispersions in optical and radio luminosity, shows that the evolutionary rates quoted in my Table 2 give practically the same ratio of $(\bar{x}-0.5)$ for optically limited sources to $(\bar{x}-0.5)$ for radio limited sources as that given by Mackay, whether quasar or elliptical galaxy colours are assumed.

Two effects, neither of them discussed by Mackay, could explain part, or possibly all, of the results I obtained.

(i) The radio galaxies that have had their redshifts measured are totally unrepresentative of the remainder. In other words, an entirely new class of objects is going to be discovered when spectra are taken of the remaining 3C radio galaxies (for example nearby dwarf radio galaxies). Fortunately a number of observers are now taking spectra of these galaxies, so that this hypothesis will shortly be tested.

(ii) A large proportion of the identifications of radio sources with faint galaxies could be mistaken. Although I gave a simple argument in my paper which suggested that the number of chance identifications should be small, it is impossible to be totally confident about this. It has to be borne in mind that the number of faint galaxies per square degree is not well known. If it were substantially more than is generally assumed (implying, incidentally, a strong evolutionary effect in the distribution of galaxies!), many of the associations of radio sources with 19–20th magnitude galaxies could be mistaken.

I thank Dr Mackay for showing me his paper in advance of publication, and Professor F. Hoyle for the hospitality of the Institute of Theoretical Astronomy, Cambridge where the additional calculations in this note were done.

M. ROWAN-ROBINSON

Mathematics Department,
Queen Mary College,
*London E*1

Received August 19, 1971.

[1] Rowan-Robinson, M., *Nature*, **229**, 388 (1971).
[2] Oke, J. B., and Sandage, A. R., *Astrophys. J.*, **154**, 21 (1968).
[3] Sandage, A. R., *Astrophys. J.*, **146**, 13 (1966).
[4] Sandage, A. R., *Astrophys. J.*, **150**, L9 (1967).

Interaction of Gravitational and Electromagnetic Fields or Another Effect?

THERE have been many articles recently on the interpretation of certain anomalous effects observed in frequency and interplanetary time delay measurements in terms of the interaction of gravitational and electromagnetic fields[1-6]. Usually the proposed explanations are inconsistent with the general theory of relativity[2,5,7,8], and often the predicted effects do not match the measurements. We show here that in the interpretation of the anomalous phenomena there is no need to contradict general relativity and the different measurements can be reconciled[4,9,10].

The anomalous phenomena can be quite satisfactorily explained by attributing them to the interaction of the electromagnetic field and the moving, inhomogeneous media traversed by the signal. Model computations show that in the time delay and light deflexion measurements the magnitude of this effect is about the same as or less than the measurement errors, but in the frequency measurements the effect exceeds the noise level[11].

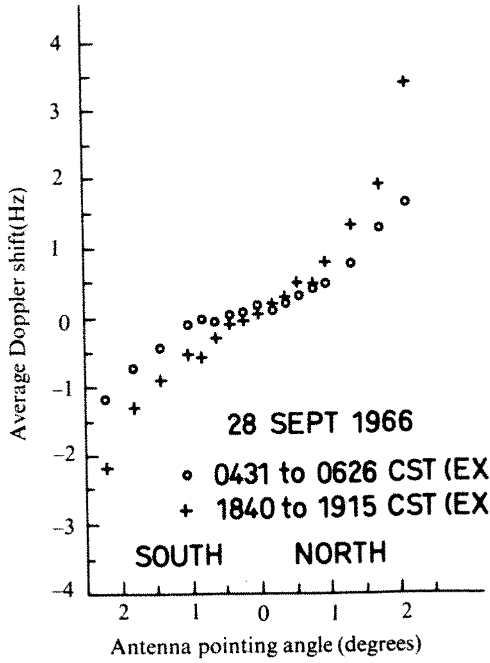


Fig. 1 Average Doppler shifts against azimuthal antenna pointing angle measured at 960 and 810 MHz using a 230 km closed loop circuit. The crosspath component of wind speed was from the north-west at all altitudes above approximately 400 m and its magnitude averaged at several m s^{-1} (after Birkemeier *et al.*[15]).

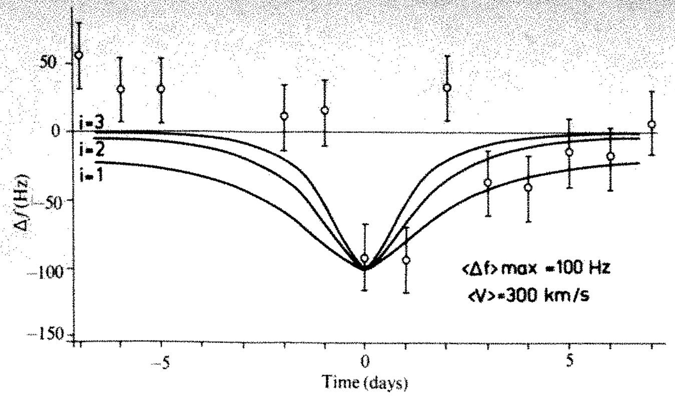


Fig. 2 Frequency decrease of the 21 cm radiation of Taurus A when it passed near to the Sun. Comparison of theoretical curves[11] resulting from the secondary effect of high velocity streams in the solar corona and combined result of 1967–68 measurements[2]. Zero time corresponds to the closest approach of the Sun.

The crux of the phenomenon is the simple physical fact that an electromagnetic wave propagating in matter inevitably interacts with the particles of the matter. This effect is usually described by the "material parameters"—the refractive index, permeability, permittivity and so on. In the case of moving media, however, one must not forget that the velocity of the signal source (and hence the location of emission, the "particle" which took part in the preceding interaction and so on) and that of the "particle" just interacting with the signal are generally not equal. Thus the "particle" observes and reradiates an electromagnetic wave, the characteristics (field intensity, frequency and so on) of which are changed according to the theory of relativity. Because the existence of an absolute system of coordinates is precluded by the theory of relativity, the phenomenon can be investigated only by tracing stepwise the path of the signal[12–14]. This is the method of relativistic ray-tracing—a search for a solution in a closed form is being made. The interaction, of course, depends on the frequency of the signal.

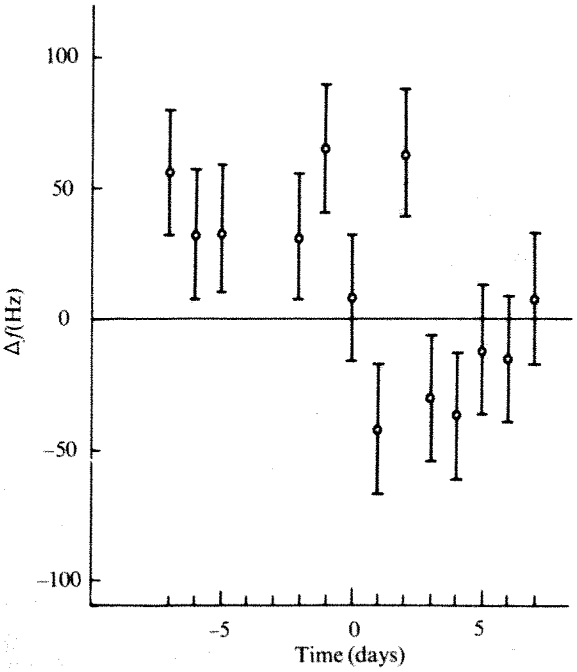


Fig. 3 Residuals of the frequency decrease of Taurus A 21 cm radiation after rejecting the theoretical value of the secondary effect. The resulting pattern can be attributed to the axial rotation of the Sun[11].

For computation purposes the moving medium is decomposed into constant velocity layers, which are also generally inhomogeneous: at the individual layer boundaries the coupling of the solutions is obtained from rigorous computations of the Doppler effect, aberration and so on. In this way the changes in the signal parameters can be studied adequately. In this communication only the frequency changes, which can be measured more reliably, will be discussed.

The frequency change due to moving inhomogeneous media was first observed by Birkemeier *et al.*[15] in terrestrial troposcatter measurements. The results were originally interpreted in terms of a mechanical model involving a "moving mirror"[15] (an optical method), but a more exact method was subsequently developed[12]. The influence of wind on the frequency of the wave propagating between two fixed stations can be seen in Fig. 1.

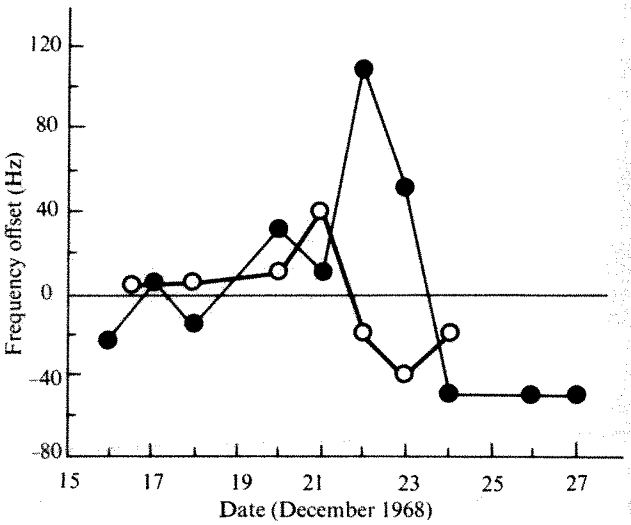


Fig. 4 Apparent frequency shift of the 1,612 MHz OH emission feature from W28S. The closest approach of the Sun occurred on December 21, 1968. ●, Agassiz data; ○, Haystack data (after Ball *et al.*[10]).

An active discussion on the frequency decrease of the 21 cm radiation when the source passed near to the Sun was initiated by the experiments of Sadeh *et al.*[1–3]. The measurements were not very reliable and their interpretation met serious difficulties[11]. According to our model computations, the redshift of the order of 10^{-7} observed near the occultation of Taurus A by the Sun is produced by high velocity streams in the solar corona. The magnitude of this effect may amount to several parts in 10^{10} to 10^{7} (Fig. 2). The residuals can be attributed to the average motion of the solar corona due to the axial rotation of the Sun (Fig. 3), which is in good agreement with the measurements carried out later by Ball *et al.*[10] (Fig. 4). Because of the strongly varying solar streaming pattern, the overall effect measured may exhibit significant variations but the effect of axial rotation is always present.

The problem of the so-called solar limb effect has remained unsolved for nearly half a century. This phenomenon is evident at the extreme of the solar limb (at radial distances greater than about 95% of the radius of the disk) where the Fraunhofer lines exhibit a redshift substantially exceeding the gravitational shift—0.636 km s^{-1} (refs. 16–23). In the inner parts of the Sun (at distances greater than about 0.5′ to 1′ from the limb) the variation of wavelengths has been successfully described in terms of source (transmitter) motions or motions of the location of absorption (receiver) due to the solar currents[24]. The interaction we proposed at that time was still not considered. Based on the streaming patterns adopted for the Sun we carried out accurate model computations

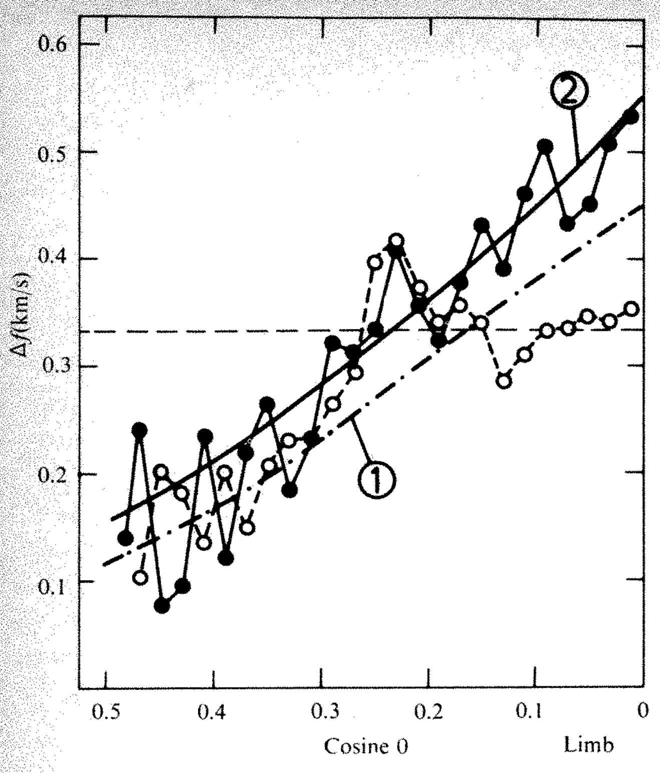


Fig. 5 Comparison of the limb effect determined by Adam[21] and theoretical curves[25]. Δf is the frequency difference from the centre value, θ is the angle between the line of sight and the radius connecting the observed point with the centre of the Sun. ●, East limb observations; ○, west limb observations; - - -, gravity shift; 1, corresponds to Schröter's radial current model[24] ($v=0$, 3 km s^{-1}); 2, a similar fit to the east limb data ($v=0.57$ km s^{-1}).

which resulted in a good interpretation of the anomalous redshift measured at the limb[25] (Fig. 5) because of the radial expanding character of the currents.

It follows that the development of new theories to explain the measured "anomalous" phenomena do not seem to be justified. Such theories will only be required if reliable measurements show anomalous effects differing from the effects of moving media. As far as we know such effects have not yet been observed. The high redshift values observed for quasars will probably be partly explained in terms of high velocity material motions (expansion and so on) associated with them. Finally it must be pointed out that the evaluation of interplanetary occultational experiments (Mariner, for example) requires great care, because motions in the medium of propagation may introduce additional terms. Similarly, in the very accurate measurements of satellite geodesy the ionospheric motions must be taken into account.

CS. FERENCZ

Committee on Space Research of Hungary,
Akadémia u.17,
Budapest 5

GY. TARCSAI

Geophysical Institute,
Eötvös University,
Kun B.tér 2,
Budapest 8

Received June 14, 1971.

[1] Sadeh, D., Knowles, S. H., and Yaplee, B. S., *Science*, **159**, 307 (1968).
[2] Sadeh, D., Knowles, S. H., and Au, B., *Science*, **161**, 567 (1968).
[3] Sadeh, D., Hollinger, I. P., Knowles, S. H., and Youmans, A. B., *Science*, **162**, 897 (1968).
[4] Markowitiz, W., *Science*, **162**, 1387 (1968).
[5] Szekeres, C., *Nature*, **220**, 1116 (1968).
[6] Shamir, I., *Nature*, **222**, 362 (1969).
[7] Woodward, J. F., and Yourgrau, W., *Nature*, **226**, 619 (1970).
[8] Reinhardt, M., *Nature*, **229**, 36 (1971).
[9] Shapiro, I. I., Pettengil, C. H., Ash, M. E., Stone, M. L., Smith, W. B., Ingalls, R. P., and Brockelman, R. A., *Phys. Rev. Lett.*, **20**, 1265 (1968).
[10] Ball, J. A., Dickinson, D. F., Lilley, A. E., Penfield, H., and Shapiro, I. I., *Science*, **167**, 1755 (1970).
[11] Ferencz, Cs., and Tarcsai, Gy., *Planet. Space Sci.*, **18**, 1213 (1970).
[12] Ferencz, Cs., *Acta Tech. Acad. Sci. Hung.*, **68**, 1–2, 233 (1970).
[13] Ferencz, Cs., *Acta Tech. Acad. Sci. Hung.* (in the press).
[14] Drahos, D., Ferencz, Cs., Ferencz, I., Horváth, F., and Tarcsai, Gy., in *Space Research X* (North-Holland, Amsterdam, 1970).
[15] Birkemeier, W. P., Merril, H. S., Sargeant, D. H., Thomson, D. W., Beamer, C. W., and Bergemann, G. T., *Radio Sci.*, **3**, 309 (1968).
[16] St John, C. E., *Astrophys. J.*, **57**, 195 (1928).
[17] Evershed, J., *Mon. Not. Roy. Astron. Soc.*, **91**, 260 (1931).
[18] Freundlich, E. F., Brunn, A. v., and Brück, A., *Z. Astrophys.*, **1**, 43 (1930).
[19] Adam, M. G., *Mon. Not. Roy. Astron. Soc.*, **108**, 446 (1948).
[20] Adam, M. G., *Mon. Not. Roy. Astron. Soc.*, **118**, 106 (1958).
[21] Adam, M. G., *Mon. Not. Roy. Astron. Soc.*, **119**, 460 (1959).
[22] Adam, M. G., *Proc. Roy. Soc.*, **A270**, 297 (1962).
[23] Higgs, L. A., *Mon. Not. Roy. Astron. Soc.*, **121**, 421 (1960).
[24] Schröter, E. H., *Z. Astrophys.*, **41**, 141 (1957).
[25] Ferencz, Cs., and Tarcsai, Gy., *Planet. Space Sci.* **19**, 659 (1971).

Age Determinations of Recent Hawaiian Lavas using Measurements of Weathering Changes

HAWAII has many recent lava flows in the age range of 200 to 1,000 years BP but carbon-14 dating of these flows is usually not possible because adequate carbonaceous material is difficult to find. Other isotopic methods, such as potassium–argon and thorium–lead ratios, and the technique of fission-track dating[1], are not applicable to this time range. Thermoluminescence dating of geologically recent lavas seems possible[2,3] although not yet widely tested. We investigated the possibility of dating late prehistoric flows by measuring weathering changes of surface rocks.

The study area was the eastern slopes of Mauna Loa and Kilauea volcanoes from 40 to 4,000 feet (10–1,200 metres) altitude with a range in mean annual temperature of 22.8°–15.5° C, and mean annual rainfall of 90 to 150 inches (2,300–3,700 mm). The area includes both aa and pahoehoe lava flows which are olivine basalt, basalt, or oceanite in composition[4]. Five dated aa flows, ranging in age from 13 to 218 years, and three undated aa flows were sampled. Sampling sites were selected by taking areas at which rainfall isohyets, suitable for comparative purposes, intersected flows of convenient age.

Small surface rocks (1–2.5 cm in diameter) were taken as representative of the weathered condition while small cubes of rock within large surface boulders (>30 cm diameter) were used as unweathered samples. Using a randomized sampling procedure, either five or ten samples of both weathered and unweathered rocks were collected at each site. The samples were ground to 100 mesh size before analysis and measurements of weathering changes made from the differences found between weathered and unweathered samples.

The principal weathering changes investigated were pH, hydration, oxidation, mineralogical and elemental changes. Many parameters were found to be unsuitable as age indices either because the amount of change in the historic rocks (≤ 218 yr) was too small or because there was no consistent relationship between age and the magnitude of the change measured. The most useful parameters were found to be: ΔpH ($p\mathrm{H}_{\mathrm{KCl}} - p\mathrm{H}_{\mathrm{H_2O}}$ of weathered rocks only), pH difference between unweathered and weathered rocks, percentage losses of total sodium and calcium, percentage gain of titanium, and a 110°–350° C weight-loss measurement of the amount of hydration and adsorbed water.

Table 1 Representative Measurements of Weathered Rock Parameters from Five Hawaiian Lava Flows

Parameter	Mauna Loa 1942 flow	Mauna Loa 1852 flow	Kilauea 1750 flow	Late prehistoric flows of Mauna Loa Lower Stainback	Upper Stainback
No. of samples	10	10	10	5	4
ΔpH* (pH_{KCl}-pH_{H_2O})	−0.22	−0.37	−0.61	−0.67	−0.84
*p*H difference (between unweathered and weathered rocks)	1.28	2.07	2.92	3.52	3.41
% loss of Na	7.2	15.0	13.0	23.5	48.3
% loss of Ca	Not significant	4.8	4.7	9.6	14.3
% gain of Ti	Not significant	2.2	2.3	25.8	30.5
110°–350° C weight loss*	0.78	0.94	2.03	2.87	3.28

* Measurements of weathered rocks only.

Table 1 shows representative results from five flows: historic flows of ages 13, 116 and 218 yr, and two late prehistoric flows whose ages were subsequently estimated at about 360 yr. The results are given in full elsewhere. In Table 1, losses and gains of the elements listed are percentages of the amounts originally present in the unweathered rocks.

The two parameters, *p*H difference and 110°–350° C weight loss, had the highest correlation coefficients with time (0.58 and 0.68 respectively). These parameters were regressed on measurements made of site factors on five sampling sites from the five dated flows, a total of fifty groups of measurements. The site factors were age, mean annual rainfall, mean annual temperature, a plant factor (based on the *p*H of the undecomposed plant litter); and four parameters of the unweathered lava: rock porosity and texture (both rated semi-quantitatively), and titanium and combined calcium and sodium content. The equations obtained were examined for confidence limits, multiple R^2 value and statistically significant regression coefficients, and checked to ensure that the residuals did not show a trend when plotted against time. Three equations were selected which met the above criteria most adequately:

$$(1)\quad (p\text{H difference})^2 = -0.54 - 0.08\ (\text{Na loss}) + 0.27\ (\text{Ca loss}) + 0.03\ (\text{age}) - 0.29\ (\text{porosity}) + 1.21\ (\text{Ti})$$

$$R^2 = 80\% \qquad \text{confidence limits: } \pm 87 \text{ yr}$$

$$(2)\quad 110°\text{–}350° \text{ C weight loss} = 0.66 + 0.008\ (\text{age}) + 0.00005\ (\text{temperature} \times \text{rainfall})$$

$$R^2 = 55\% \qquad \text{confidence limits: } \pm\ 108 \text{ yr}$$

$$(3)\quad p\text{H difference} = -0.55 + 0.008\ (\text{age}) + 0.004\ (\text{rainfall}) + 0.461\ (\text{Ti content})$$

$$R^2 = 65\% \qquad \text{confidence limits: } \pm 108 \text{ yr}$$

Using measurements of weathering changes and site factors from two of the undated flows sampled, the regression equations were solved for age with the following results:

Upper Stainback flow:	359 ± 87 yr	(1)
	383 ± 108 yr	(2)
	310 ± 108 yr	(3)
Lower Stainback flow:	362 ± 87 yr	(1)
	341 ± 108 yr	(2)
	343 ± 108 yr	(3)

Both groups of equations show some measure of internal agreement and, in particular, equations (1) and (2) which utilize different rock parameters and site factors. It is assumed that errors arising from curvilinear change in weathering parameters and from climatic change are small for the comparatively limited time span studied.

Results using these methods need to be tested against alternative dating methods so that their value in measuring absolute ages can be assessed. This approach, however, may have value as a comparatively inexpensive method of assigning relative ages to lavas. Information on relative ages can provide a time axis for many studies where such questions as rates of rock weathering and clay formation, or rates of vegetation succession, are being examined. With further refinement it seems likely that weathering changes could be used to assign relative ages to both aa and pahoehoe lavas of greater age than and differing climate from the flows included in the present study.

This work was supported by the New Zealand Government during tenure by I. A. E. A. of a National Research fellowship. Other funds came from the Hawaii Agricultural Experiment Station. We thank Drs G. D. Sherman, G. Uehara, Y. N. Tamimi, C. H. Lamoureax, R. H. Jones and C. L. Schroth for their advice.

I. A. E. ATKINSON

Botany Division Substation,
c/o Soil Bureau,
Department of Scientific and Industrial Research,
Private Bag, Lower Hutt

L. D. SWINDALE

Department of Soil Science and Agronomy,
School of Tropical Agriculture,
University of Hawaii,
Honolulu, Hawaii 96822

Received August 9, 1971.

[1] Price, P. E., and Walker, R. M., *J. Geophys. Res.*, **68**, 4847 (1963).
[2] Sabels, B. E., *Radioactive Dating* (International Atomic Energy Agency, Vienna, 1963).
[3] Hwang, F. S. W., *Nature*, **227**, 940 (1970).
[4] Macdonald, G. A., and Katsura, T., *J. Petrol.*, **5**, 82 (1964).

Experimental Study of Channel Patterns

RIVER channel patterns are described as being braided, meandering, or straight[1] with braided rivers having steeper gradients than meandering rivers at the same discharge[2]. Recent experimental studies also suggest that straight channels have gentler gradients than meandering channels for the same discharge[3]. The difference in slope has at times been assumed to be the cause of the difference between braided and meandering channels, although braided channels have also been attributed to rapid variations in discharge, high sediment load, or erodible banks[4]. All of these explanations are plausible, and the last two can be related to the higher velocity and greater tractive force associated with steep gradients.

As part of a programme of research on river morphology, experiments were undertaken to investigate further the effect of slope on channel pattern and especially to determine if at certain ranges of slope marked changes of channel pattern occur. The experiments were performed in a 30.5 m long, 7.3 m wide, and 1 m deep flume at the Engineering Research Center, Colorado State University (Fig. 1). The flume was filled with poorly sorted sand (median grain size 0.7 mm),

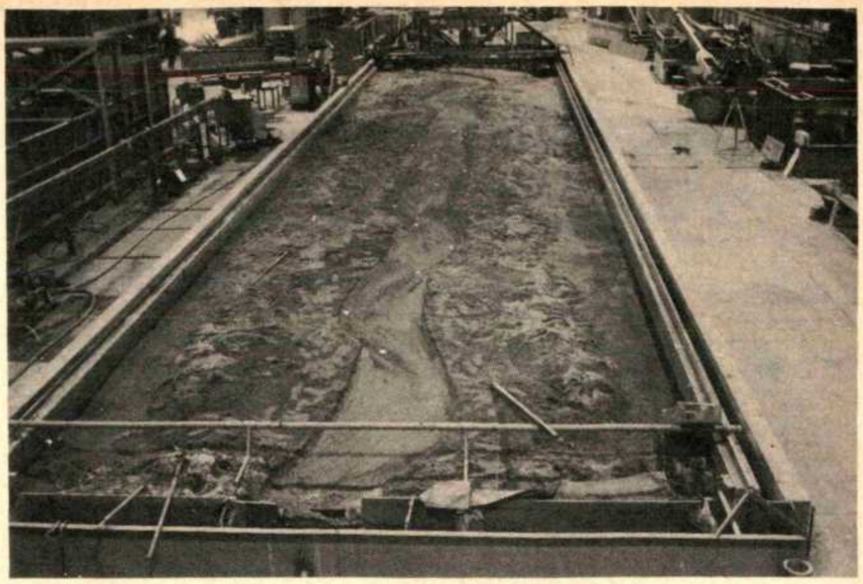


Fig. 1 Experimental facility used to study development of diverse thalweg patterns. Model channel has meandering thalweg, as identified by white spots in channel. Note that although thalweg sinuosity is about 1.2 and the banks are irregular, the channel itself is straight.

which contained particles representative of the complete range of sand sizes. The slope of the sand surface was graded to the desired inclination, and a channel 0.3 m wide and 7.6 cm deep was then cut into its surface. At the beginning of each experiment, water was introduced into the channel at a constant discharge of 0.15 cubic feet per second (0.004 m^3 s^{-1}) and sand was fed into the flow from a vibrating sand-feed device. During fifteen experiments the slope was changed from a minimum of 0.1% to a maximum of 2%. The rate of sand feed was regulated so that scour did not occur at the head of the channel, and the experiments were continued until the model channel had developed a stable configuration.

As anticipated, the rate at which sand was fed into the channel (sediment load) was closely related to slope (Fig. 2). There are three segments to a line drawn through the points. A lower segment for slopes less than about 0.2% for which there is a relatively slow increase of sediment load with slope; an intermediate segment between slopes of about 0.2% and 1.3% and, finally, an upper segment where a doubling of slope causes an approximate doubling of sediment load. Similar changes in the rates of increase of flow velocity and of tractive force occur at these slopes. Thus, there seem to be two threshold values of slope at which significant changes in hydraulics of flow and sediment transport occur, and these changes are also reflected in alterations of channel morphology (Fig. 3). At slopes less than about 0.2% the channel is straight (sinuosity equal to 1.0); at slopes in excess of 0.2%, however, the channel pattern changed as alternate bars formed, and a sinuous thalweg developed. Thalweg sinuosity increased with increased slope and sediment load to a maximum of 1.25 at slopes between 1% and 1.3%.

As the slope was increased beyond 1.3%, another change in channel morphology occurred. The alternate bars began to erode, and for slopes of 1.6% and greater a braided channel pattern developed (Fig. 3). The change from meandering thalweg to braided channel was not as abrupt as the change from a straight to a meandering thalweg channel. Nevertheless, within a narrow range of slope (1.4% to 1.6%) the channel was completely transformed, as was the sediment transport rate.

Water was introduced into the model channel at an angle in order to initiate meanders; nevertheless, at slopes less than 0.2% the channel remained straight. Thus, as Ackers and Charlton suggested[3], there is a threshold value of slope below which a meandering thalweg will not develop, although our experiments were designed to force development of a sinuous pattern. Furthermore, our experiments confirm the existence of an upper slope threshold between meandering thalweg and braided channels, as suggested by others[1,2].

Our meandering thalweg channels were similar to those developed during earlier attempts to study model meandering channels[5–8]. Fig. 1 shows, however, that, although the thalweg meandered, a truly meandering channel did not form, for the alternate bars were always submerged and did not form point bars. Freidkin[7] notes, in fact, that in order to obtain photographs of his "meandering channels", the discharge was reduced until the water level had decreased sufficiently to expose the alternate bars.

In summary, in the conditions imposed during experimentation (one type of sediment and constant water discharge) the complete range of previously described thalweg patterns formed as slope and sediment load increased. From the geological point of view the experiments can be considered to represent a fluvial system that at constant discharge is receiving a progressively larger sediment load. As the sediment load increases, the gradient is increased by aggradation. Depending on the initial pattern, a channel may change from straight to meandering thalweg or from meandering to braided as the threshold sediment loads are exceeded. We conclude therefore that two threshold values of sediment load exist at a given discharge. When these values are exceeded, channel patterns change and the rate at which slope increases with sediment load decreases (Fig. 2).

Considerably more remains to be done, but the results of

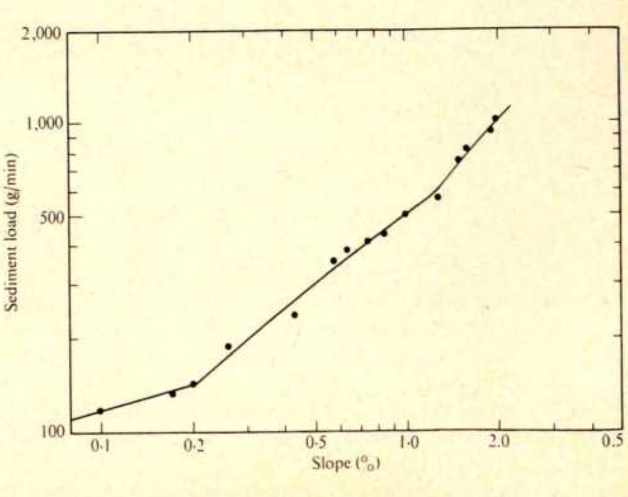


Fig. 2 Relation between rate at which sand was fed into experimental channels and slope of surface on which channels were formed. Breaks in slope of line indicate threshold values of sediment load and/or slope at which channel pattern changed.

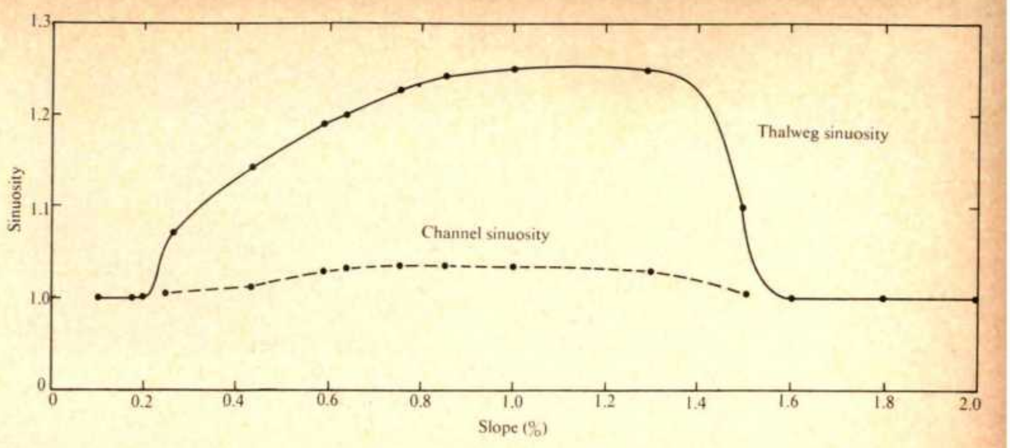


Fig. 3 Relation between thalweg sinuosity (distance along deepest parts of channel divided by length of flume), channel sinuosity (distance along midline of channel divided by length of flume) and slope.

these experiments raise questions concerning some previous explanations of diverse channel patterns. For example, a deflexion or perturbation of flow is not always sufficient to induce meandering because a meandering thalweg would not develop at low sediment loads and slopes, although the water was introduced into the flume through an initiating bend. Further, although the water was introduced directly into the channel during other experiments, a meandering thalweg always developed at moderate sediment loads and slopes.

In addition, high sediment loads do indeed appear necessary for the development of a braided channel, but bank erodibility is not in itself a sufficient reason. The banks of the model channel eroded during every experiment. That is, at low slopes the width of the channel almost doubled, but the channel remained straight. Therefore although the banks must be erodible for a channel to adjust to altered conditions, bank erodibility in itself is not the cause of meandering or braiding. Rather we consider bank erosion in these experiments as simply the response of the channel to altered slopes and sediment loads.

Except within the ranges of sediment loads and/or slopes where thalweg sinuosity increases rapidly (0.2% to 0.4%) and where the change from a meandering thalweg to a braided pattern occurs (1.4% to 1.6%), a considerable increase of sediment load and/or slope may have a minor effect on the thalweg pattern and on the nature of sediment transport and deposition within a channel. If a river falls near one of the threshold zones on Fig. 3, however, a minor change in sediment load and/or slope could completely alter the channel morphology as well as the character of the sedimentary deposit produced by the river.

We thank the NSF for support, and R. B. Johnson and J. A. Campbell for reviewing the manuscript.

S. A. SCHUMM
H. R. KHAN

Department of Geology and Engineering Research Center,
Colorado State University,
Fort Collins, Colorado 80521

Received July 5, 1971.

[1] Leopold, L. B., and Wolman, W. G., *US Geol. Surv. Prof. Paper*, 282–B (1957).
[2] Lane, E. W., *US Army, Corps of Engineers, Missouri River Division Sediment*, Series 9 (1957).
[3] Ackers, Peter, and Charlton, F. G., *Inst. Civil Eng. Proc.*, Supplement 1970 (XV), Paper 7362S (1970).
[4] Fahnestock, R. K., *US Geol. Surv. Prof. Paper*, 422–A, 57 (1963).
[5] Tiffany jun., J. B., and Nelson, G. A., *Amer. Geophys. Union, Trans.*, **20**, 644 (1939).
[6] Quraishy, M. S., *Current Science*, **13**, 36 (1944).
[7] Freidkin, J. F., *A Laboratory Study of the Meandering of Alluvial Rivers*, US Waterways Experiment Station (Vicksburg, 1945).
[8] Ackers, Peter, *J. Hydraulics Div., Amer. Soc. Civil Eng. Proc.*, **90**, 1 (1964).

Aerodynamic Noise produced by a Gliding Owl

THE flight of owls has been reported by ornithologists to be unusually quiet[1]. This conclusion is generally based on the success of the owl's hunting technique and on the relative inability of the observer to distinguish the owl aurally from other birds.

As part of an investigation of aerodynamic noise and

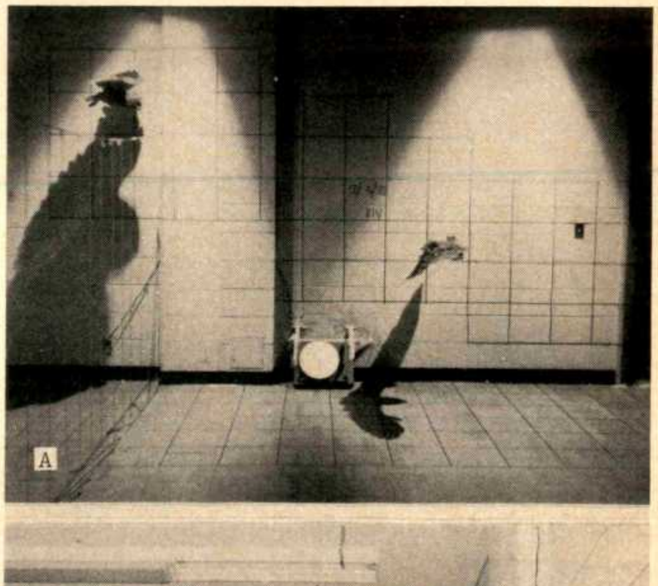


Fig. 1 Reverberation chamber. *A*, Double exposure of the owl during a gliding flight, showing a typical flight path of the bird. The bird glides from the string barrier down to the lower perch which is in the right-hand corner of the room (not shown). The patterns on the wall and the ground were used to determine the gliding velocity of the owl. The average gliding velocity was 24 foot s^{-1}.

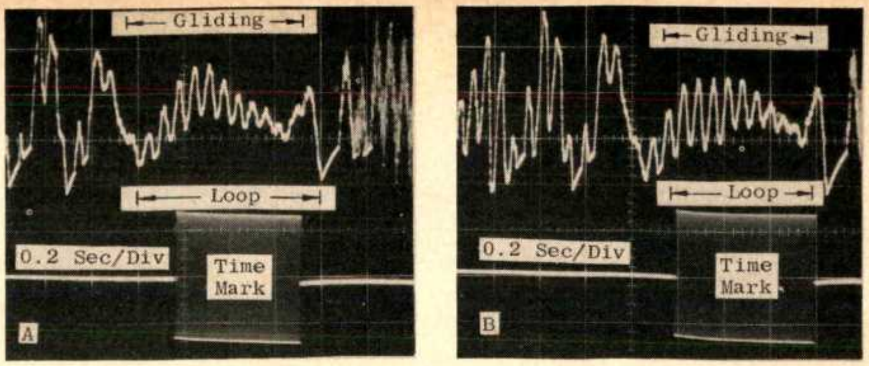


Fig. 2 *A* and *B*, Noise signals of two different test flights. The upper trace on the oscilloscope photograph shows the unmodified noise signal as sensed by the microphone, the lower trace indicates the gliding phase as judged by the observer in triggering a "time mark". The noise produced during the "gliding" of the owl is thereby identified. "Loop" indicates the noise signal which was frequency analysed.

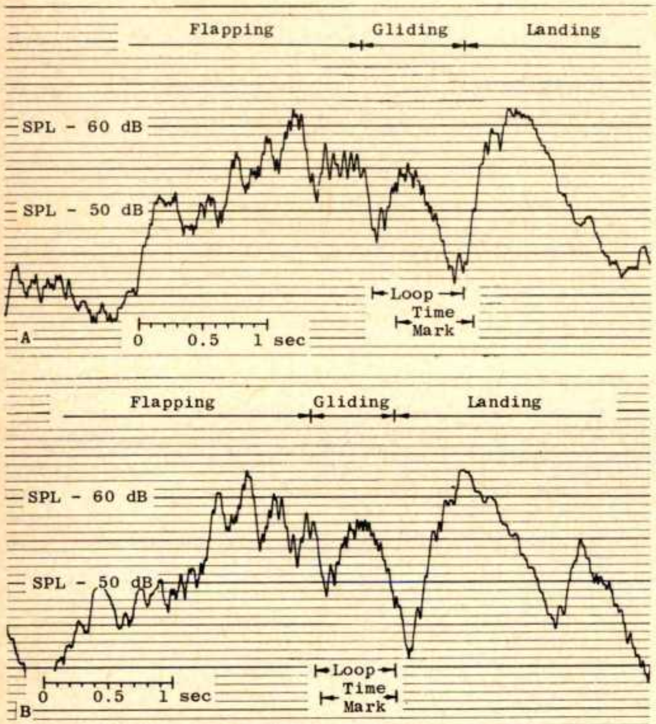


Fig. 3 *A* and *B*, Time history of the overall sound pressure level (OSPL) in dB, ref. 2×10^{-4} μbar, of the noise produced by the owl during the two test flights mentioned in Fig. 2. The recordings were made directly with a sound level recorder during the tests. The notation of the different flight phases is the same as in Fig. 2. The peak of the OSPL during the "gliding" is obviously due to the relatively high level of the low frequency components (fundamental frequency 15 Hz).

noise reduction methods, we conducted a series of tests at the University of Tennessee Space Institute, Aeroacoustic Laboratory, to determine the level and the characteristics of the noise produced by owls during flight, especially during gliding approaches. The owl used in these tests was the Florida barred owl (*Strix varia alleni*). (See Table 1 for measurements.)

We used a reverberation chamber[2] to measure the total sound produced by the flying owl and the spectral distribution of the overall noise signal. The chamber (see Fig. 1) is a room of 240 m^3 volume with reverberation times varying between approximately 0.8 s and 0.4 s in the frequency range 100 Hz to 10 kHz. The general procedure involved the recording of the unmodified noise signal and simultaneously the overall sound pressure level (OSPL) in dB, ref. 2×10^4 μbar, of the noise produced by the flying owl. The noise signal was then frequency analysed in terms of one-third octave frequency bands.

It was particularly important that we obtained noise samples from only the gliding phase of the observed flights. In all tests, the owl flew from an upper perch in one corner of the room to a lower perch in the diagonal opposite corner (see Fig. 1). A flight generally consisted of an initial flapping phase followed by a gliding phase and a short flapping during touch down. Because of the size of the test room the duration of the gliding phase of all flights was relatively short, usually in the order of 0.6 to 0.8 s. By introducing a light string barrier mounted near the middle of the room, the test flight configuration, however, became very reproducible. The owl was forced to flap up to the string barrier and to a gliding descent with nearly constant speed down to the lower perch. The average value of all measured gliding velocities was 24 foot s^{-1}.

We report here acoustic data for two representative flights. The measurements were made with a single condenser micro-

Table 1 Data of the Florida Barred Owl

Weight	1.52 pounds
Wing area *	344.8 $inch^2$
Wing span	38.1 inches
Wing sweep angle	32.7 deg. forward
Chord at half span	11.0 inches
Wing loading	4.41×10^{-3} pounds/$inch^2$ = 0.635 pounds/$foot^2$
Wing aspect ratio	4.21

* Wing area includes the body area intercepted by the wing.

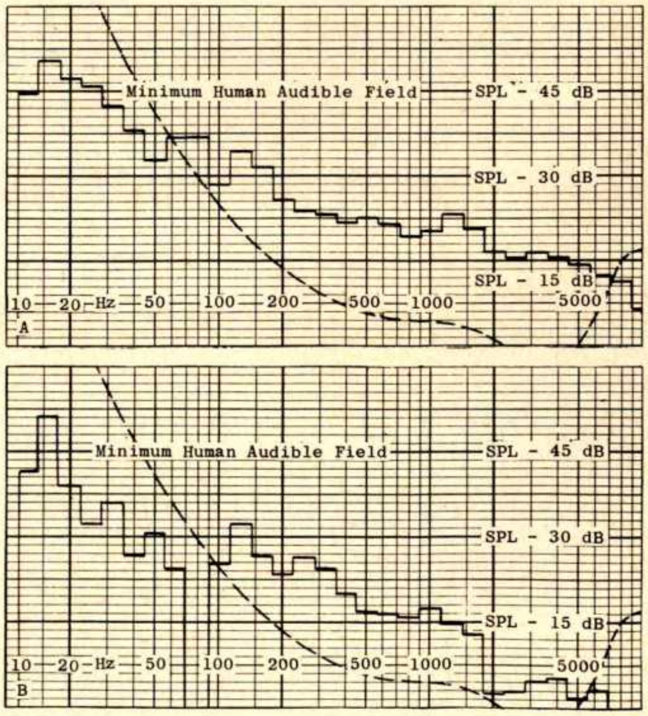


Fig. 4 *A* and *B*, Spectral distribution of the sound pressure level of a gliding owl. The one-third-octave band frequency analysis was made by using a magnetic tape reproduction of the gliding noise signal which is indicated in Fig. 2 by "Loop". The spectral distribution is compared with the normal human minimum audible field.

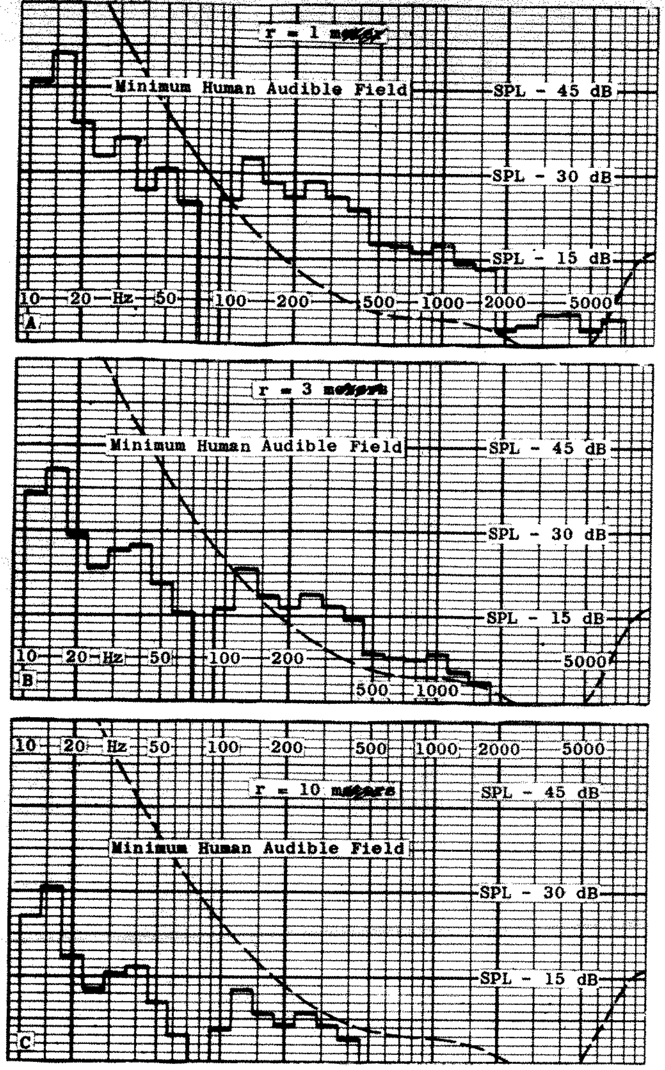


Fig. 5 *A*, *B* and *C*, Spectra of the sound pressure level of a gliding owl for distances of (*A*) 1 m, (*B*) 3 m and (*C*) 10 m. The data results from the second test flight (Figs. 2*B*, 3*B* and 4*B*). The calculation of the spectra shown is based on the assumption that the OSPL measured in the test room equals the free field sound pressure level at the reverberation radius. From the comparison with the normal human minimum audible field it becomes evident that the gliding owl is inaudible for a human observer beyond a distance of approximately 3 m.

phone with a sensitivity of 5 mV/μbar and a flat frequency response within the range 2 Hz to 10 kHz. The microphone was positioned slightly above the floor and at a lateral distance from the flight path larger than the reverberation radius of the moving noise source. During a flight test two recordings were obtained, a direct recording of the unmodified noise signal as sensed by the microphone made with an FM magnetic tape recorder (Fig. 2), and a graphical recording of the time history of the overall sound pressure level made with a sound level recorder (Fig. 3). The oscilloscope photographs show the unmodified noise signal in the upper trace and a "time mark" on the lower trace indicating the gliding phase of the flight as judged by the observer in the test room. In identifying the noise produced only during the gliding of the owl the observer's reaction time in triggering the time mark has to be taken into account. This leads to a corrected "gliding" as indicated in Figs. 2 and 3.

A one-third-octave band frequency analysis was also made by using only a reproduction of the gliding noise signal on a magnetic tape loop. The measured spectra were corrected for the ambient noise level observed during the tests. In Fig. 4 the resulting spectral distribution of the sound pressure level is shown and is compared with the normal human minimum audible field. Below about 150 Hz, the sound field in the chamber may no longer be considered as perfectly uniform because the relative magnitudes of the measured sound levels at lower frequencies are influenced to a certain degree by discrete room resonances.

In order to arrive at an estimate of the sound pressure levels which would occur at various distances in an acoustic free field, the data obtained in the reverberation chamber were used to calculate the sound field radiated by a spherical source of the same intensity. This is based on the assumption that the OSPL measured in the reverberation chamber equals the free field sound pressure level at the reverberation radius given by

$$r = \sqrt{\frac{0.163\ V}{16\,\pi\ T}}\,[\mathrm{m}]$$

where V is the volume of the chamber measured in m^3, and T is the reverberation time in seconds. For the chamber used in the test, r was approximately 1 m. The calculated spectra for distances of 1, 2 and 3 m are shown in Fig. 5; absorption effects in humid air of 50% relative humidity at 20° C are taken into account based on the results obtained by Harris[3]. It becomes evident from these results that a gliding owl would be inaudible for a human observer beyond a distance of approximately 3 m.

The source of the periodic component with a fundamental frequency of 15 Hz which is apparent in all gliding noise signals (see Fig. 2) could not be clearly identified, but it may be caused by the aeroelastic properties of the owl feathers[4].

This is part of a research work which has been supported by the US Air Force. We thank Dr B. H. Goethert, director of the Space Institute, for his interest in this work and for many helpful discussions; Dr R. A. Kroeger and Dr T. C. Helvey, who conducted the aerodynamics and bionics studies of the project; Mr G. O. Schrecker and Mr J. Goodman for technical assistance.

H. D. GRUSCHKA
I. U. BORCHERS
J. G. COBLE

University of Tennessee Space Institute,
Tullahoma,
Tennessee 37388

Received August 24, 1971.

[1] Graham, R. R., *J. Roy. Aero. Soc.*, **38**, 837 (1934).
[2] Gruschka, H. D., *Progress Report on NASA Project NGR 43–001–075* (1970).
[3] Harris, C. M., *J. Aeronaut. Soc. Amer.*, **40**, No. 1 (1966).
[4] Kroeger, R. A., Gruschka, H. D., Helvey, T. C., *et al.*, Final Report WADC F33615–70–C–1762 (1971).

BIOLOGICAL SCIENCES

Is the Local Orientation of Anadromous Fishes determined by Pheromones?

IN the study of the navigating ability of anadromous fish a distinction is made between long range navigation and navigation in local waters[1]: here I am concerned with the latter ability. My results support White's[2,3] suggestion that the standing fish population in a river conditions the water in such a way that individuals on their way back from the sea recognize the stream in which their relatives are living. The attractant may be substances in the mucus excreted.

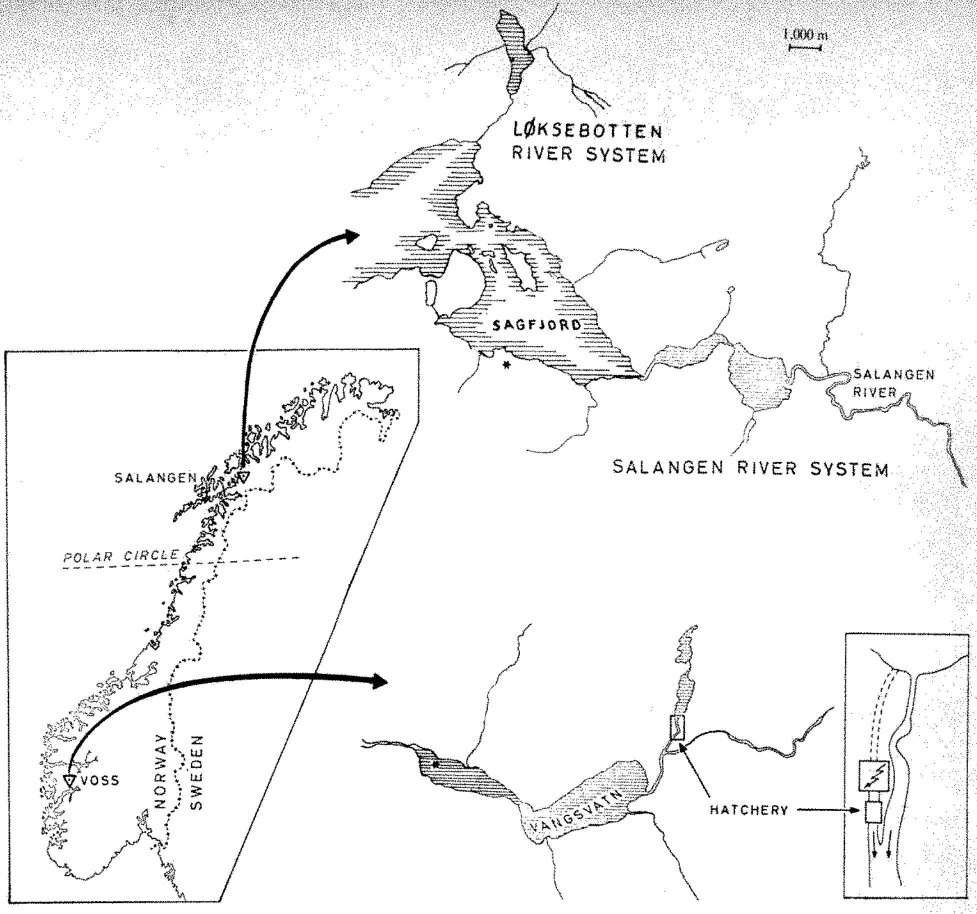


Fig. 1 Map of investigation areas.

The char (*Salmo alpinus* L. = *Salvelinus alpinus* (L.)) in the Salangen river system in North Norway is divided into a stationary and a migratory population[4]. The stationary population stays in fresh water during the whole life span and reproduces every year (up to thirteen times) after reaching maturity at an age of 2–8 yr. Smolt of the migratory population descend to the sea, when 3–4 yr old, and return to their native river after about 1 month in coastal waters. From then on, each individual migrates to the sea once every summer during the rest of its life. Most of the char of the migratory population become mature after their second or third sojourn in the sea and will then reproduce up to seven times in successive autumns.

The Salangen fjord with its inner bay, Sagfjord, receives two river systems—the Salangen river system and the Løksebotn river system (Fig. 1). Char were tagged in the Salangen river system and at a station in Sagfjord marked on the map with an asterisk. Table 1 shows that individuals of the migratory population tagged at stations in the Salangen river system return exclusively to this system after their yearly sojourn in the sea. None were recaptured in the Løksebotn river, although large quantities of char descend and ascend both rivers during the same period (May–August). On the other hand, char tagged as smolt from the same catches at the station in Sagfjord were recaptured annually in both the Løksebotn and the Salangen river systems indicating yearly mixing and subsequent separation of the populations in the sea.

In the autumn of 1964, artificially fertilized eggs of the migratory population at Salangen were transported by air to South Norway, and arrived at the hatchery at Voss less than 24 h after fertilization. The embryos were hatched and the young reared in the hatchery until spring 1968 when they had passed their fourth winter. Then 317 individuals were taken back by air and released in the estuaries of the Løksebotn and Salangen river systems. Of the 174 released in the Løksebotn estuary, thirty-one (18%) were recaptured—ten in the Løksebotn river and twenty-one in the Salangen river system, the estuaries of which are about 10 km apart.

Considering the distance, the result indicates that the fish preferentially return to the stream of their parents. This conclusion is supported by the 143 fish released in the Salangen estuary. Of these, twenty-six were recaptured in the Salangen river, and only one in the Løksebotn river. The result could be explained if the eggs were conditioned by the water from Salangen river in which fertilization and transportation took place. A more reasonable hypothesis, however, is that the released fish were attracted to the Salangen river system by an attractant released by their relatives in that system.

The hypothesis is supported by a few casual observations. Offspring of the migratory population from Salangen were reared for three years in the Voss hatchery (see Fig. 1). In the spring of 1962, 200 immature individuals of smolt size were tagged and released, at a station marked with an asterisk, in Vangsvatn (lake) below the hatchery. A fortnight later (late

Table 1 Tagging Experiment 1962–64 (Young Char, Smolt and Post-smolt)

Total No. tagged in fresh water (smolt tagged 1964 in fresh water and sea)		Recaptured 1963–68					
Stations	No.	Sea	Salangen river system	Løksebotn river system	Other river systems	Total No.	%
Salangen river system	627	77	172	—	1	250	40
	(73)	(11)	(27)	—	—	(38)	(52)
Sagfjord	(166)	(10)	(55)	(20)	—	(85)	(51)

Table 2 Tagging Experiment 1962 (Young Char)

Young char tagged in Salangen river system		Recaptured 1963–65					
			Salangen river system				
Stations	No.	Sea	Lakes and estuary	River	Other river systems	Total No.	%
Lakes	313	34	58	—	—	92	29
River	112	13	10	14	1	38	34

May) during a period of 1 week, a great number of these fish were observed under the floor of the hatchery, standing at the outlets from the tanks containing the rest of the population (2,000 fish). Individuals from the large population of the local stationary char were not observed. This observation is in agreement with the results of another transfer experiment[5] and can be explained in the same manner provided that the hatchery to which the transferred sockeye returned in mature stage was still populated by relatives.

In 1957 the Russians started to introduce pink salmon (*Oncorhynchus gorbuscha*) into four rivers at the Kola peninsula, and in 1960 thousands of mature individuals were caught along the Norwegian coast and around Iceland and Scotland[6]. Coincident with natural spawning[7] and continued release of pink fry[7,8] in the Kola rivers, the catches of mature pink in Norwegian waters decreased to zero in 1961–62. This development was to be expected if a population had to be built up before the salmon could recognize the area in which they belonged. In this case pink fry distributed in coastal waters may attract the spawners to the estuaries. The attraction of eelings (*Anguilla anguilla*) to fresh water increased when adult eels were present in the water, but no conclusions were drawn from this observation[9].

The experiments of Donaldson and Allen[10] with silver salmon (*Oncorhynchus kisutch*) show clearly that the fish return to the river where they were reared and not to the river where they were born. This seems at first sight contradictory to our results. But both findings can be understood if the fish are conditioned to react to different attractants present in the waters. These attractants are derived partly from their own bodies and partly from the native population. When the fish return from the sea their behaviour will then first be determined by the dominant attractant. In the transfer experiment reported above, the char reared at Voss were exposed to both their own attractant and the attractant of the local stationary race in the lakes above. When released in Salangen their behaviour could be determined only by their own race-specific attractant, for this was the only one present.

Table 2 shows that none of the 313 char of the migratory population tagged as young in the Salangen lakes were recaptured in post-smolt stages in the river above the lakes, whereas 14 of the 112 char tagged in the river were recaptured there. This indicates that char in the river and in the lakes belong to different populations. To test this possibility 599 char in smolt or post-smolt stage, derived from a population in the upper lake and reared at Voss, were released in the river and at the spawning place of the parents (autumn 1967, spring 1968). Of the 396 fish released at the spawning place, forty were recaptured in the lakes and only one in the river when they returned from the sea. Of the 233 fish released in the river, eleven were recaptured there and only three in the lakes. This result does not support the view that there are local races of char in the Salangen river but, on the other hand, does not exclude this possibility. The explanation may be that experimental fish are conditioned to the attractant of the local school of fish into which they are introduced.

HANS NORDENG

Zoological Laboratory,
University of Oslo,
Box 1050,
Oslo 3

Received March 23; revised May 28, 1971.

[1] Hasler, A. D., *Underwater Guideposts: Homing of Salmon* (University of Wisconsin Press, Madison, Milwaukee and London, 1966).
[2] White, H. C., *Trans. Amer. Fish. Soc.*, 360 (1934).
[3] White, H. C., *Rep. Biol. Board Canada 1933*, 41 (1934).
[4] Nordeng, H., *Nytt. Mag. Zool.*, **10**, 67 (1961).
[5] Harden Jones, F. R., *Fish Migration* (Edward Arnold, London, 1968).
[6] Berg, M., *Acta Borealia A. Scientia*, No. 17 (1961).
[7] Azbelev, V. V., *Nauchno-teknicheskij Bjulletin PINRO*, **1** (19), 16 (1962).
[8] Bakshtansky, E. L., *Int. Council for the Exploration of the Sea, Salmon and Trout Committee*, No. 105 (1962).
[9] Miles, S. G., *J. Fish Res. Board Canada*, **25**, 1591 (1968).
[10] Donaldson, L. R., and Allen, G. H., *Trans. Amer. Fish. Soc.*, **87**, 13 (1958).

Expression of Differentiated Functions in Mouse Neuroblastoma mediated by Dibutyryl-cyclic Adenosine Monophosphate

CELLS derived from the mouse neuroblastoma (C1300) grown in culture are predominantly round and highly refractile. A small number of the cells are flatter and possess one or more extensions ("neurites") which may be as long as 3 mm[1–3]. The proportion of these morphologically differentiated cells increases when the medium lacks serum[4] or when 5-bromodeoxyuridine is added[5]. The cells also contain enzymes of neural function[2,6] and are capable of generating action potentials *in vitro*[7,8]. We now show that differentiated characteristics of neuroblastoma cells can be induced by $N^6,O^{2'}$-dibutyryl adenosine 3′ : 5′-monophosphate (dibutyryl-cyclicAMP).

The C1300 tumour was obtained as a solid implant in strain A/J mice from the Jackson Laboratories, and the cells adapted to growth in Dulbecco's modified Eagle's medium containing 15% calf serum, in 10% CO_2–air at 37° C. Clones were obtained as previously described[9], in the absence of feeder layers, and one, NB60, was used in the experiments below.

Clone NB60 extended neurites when serum was removed from the medium, as reported by others[4] (Fig. 1*A* and 1*B*). Extension was complete within 24 h and was readily reversed on readdition of serum. In medium containing both serum and 1 mM dibutyryl-cyclicAMP, neurite extension was also induced (Table 1, Fig. 1*C*). Higher concentrations of dibutyryl-cyclicAMP were only slightly more stimulatory, while 0.1 mM had only a small effect. Neurite induction by dibutyryl-cyclicAMP was reversed by feeding the cells with fresh, drug-free medium. Adenosine cyclic 3′ : 5′-monophosphate (cyclic-AMP) at 1 mM was not as effective as the dibutyryl derivative in inducing neurite extension; higher concentrations of cyclicAMP did not induce more cells to extend neurites. AMP (and ADP and ATP, data not shown) induced modest degrees of neurite extension and, in the concentrations used (1 mM), inhibited growth and were toxic to the cells. Sodium butyrate induced no increase in cells possessing neurites.

Cycloheximide, added to serum-free medium, was reported as not preventing neurite extension, suggesting that the specific protein components of the microtubules found in neurites are

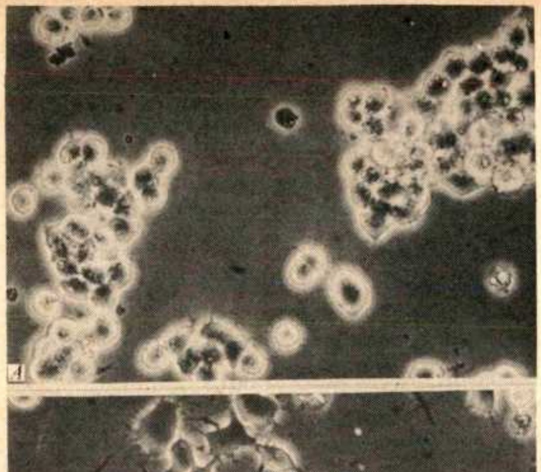


Fig. 1 Morphological differentiation of neuroblastoma clone NB60 *in vitro*: *A*, cells in the presence of serum; *B*, cells in the absence of serum for 48 h; *C*, cells in the presence of serum and 1 mM dibutyryl-cyclicAMP for 48 h.

preformed in uninduced cells[4,10]. In the conditions described here, cycloheximide (10 µg/ml.) consistently caused a moderate decrease in the percentage of cells, in serum-free medium, with visible neurites (Table 1). In contrast, in the presence of serum, 1 mM dibutyryl-cyclicAMP, and 10 µg/ml. cycloheximide, induction of neurite extension was inhibited.

Theophylline, which inhibits cleavage of cyclicAMP by phosphodiesterase (see ref. 11), induced a small percentage of the cells, grown in the presence of serum, to extend neurites, and this effect was additive with induction by dibutyryl-cyclicAMP (Table 1).

Table 1 Induction of Morphological Differentiation in Neuroblastoma Clone NB60 by Dibutyryl-cyclicAMP

Conditions	Cells with neurites (%)
A. +serum	12
−serum	89
+serum+1 mM dibutyryl-cyclicAMP	60
+serum+1 mM cyclicAMP	44
+serum+1 mM sodium butyrate	11
+serum+1 mM AMP	23
+serum+10 µg/ml. cycloheximide	7.7
−serum+10 µg/ml. cycloheximide	54
+serum+1 mM dibutyryl-cyclicAMP +10 µg/ml. cycloheximide	8.5
B. +serum+1 mM dibutyryl-cyclicAMP	57
+serum+1 mM theophylline	25
+serum+1 mM dibutyryl-cyclicAMP +1 mM theophylline	73

Cells were collected from suspension culture by centrifugation, treated with 0.02% EDTA in calcium-magnesium-free phosphate-buffered saline to disaggregate clumps, recentrifuged, and seeded on to 60 mm plastic tissue culture dishes (Falcon) containing 5 ml. of Dulbecco's medium with 15% calf serum. After incubation for 24 h, the medium was replaced with fresh medium containing appropriate additions, in the presence or absence of serum, as indicated in the experiments. Cells were examined under phase optics and scored as positive when at least a single extension of about one cell diameter or greater was seen[5]. More than 500 cells were counted per plate. The results are from typical experiments. Reproducibility among experiments was ±10%.

In the presence of serum and dibutyryl-cyclicAMP, the growth rate of neuroblastoma cells in culture was greatly reduced when compared with cells in serum alone (doubling times of 170 and 22 h, respectively). Because morphological differentiation of neuroblastoma cells has been suggested to be a function of their growth rate[4], neurite extension produced by dibutyryl-cyclicAMP might be attributed to inhibition of growth. The possibility, however, that this inhibition is not the cause of neurite extension but the result of induction of differentiated functions incompatible with rapid growth is supported by the following observations. (1) In the absence of serum, during the period of most active neurite extension, cells divide at a rate equivalent to control cultures, and only subsequently does growth rate decline (see refs. 4 and 10, and unpublished observations); and (2) cyclicAMP, AMP, ADP, ATP, and sodium butyrate inhibited growth substantially but were only poor inducers of neurite extension. Thus, inhibition of cell growth is not a sufficient cause of neurite extension.

Table 2 Acetylcholinesterase Activity of Neuroblastoma Clone NB60 *in vitro*

Conditions	Specific activity of acetylcholinesterase 48 h	96 h
+serum	2.1 (1.0)	7.5 (3.6)
−serum	10.4 (4.9)	23.2 (11.1)
+serum+1 mM dibutyryl-cyclicAMP	6.8 (3.2)	48.3 (23.0)

Cultures were grown as described, the cells removed from the plates with EDTA, washed, sonicated, and assayed by the method of Ellman[12]. Specific activity is expressed as nmol product formed/min/mg protein. Enzyme activity with butyrylthiocholine was subtracted from the rates obtained with acetylthiocholine. The inoculum had a specific activity of 5.4. The values are the average of at least three determinations. The numbers in parentheses represent the increase in activity compared to the value at 48 h in the presence of serum, taken as 1.

The activity of acetylcholinesterase, an indicator of biochemical differentiation, was measured in cells of neuroblastoma clone NB60 in several conditions (Table 2). Both addition of dibutyryl-cyclicAMP and removal of serum increased enzymatic activity. The increase in specific activity in the control plates when the cells reached stationary phase (96 h) agrees with results previously reported for neuroblastoma[6].

As described here, the morphological and biochemical differentiation *in vitro* of a neural cell tumour is induced by dibutyryl-cyclicAMP. It has recently been reported that dibutyryl-cyclicAMP causes the reversal of abnormal morphological and growth characteristics of fibroblastic tumour cell lines *in vitro*[13,14]. The cells become elongated with narrow processes and oriented in parallel arrays; this reversal, as indicated in one report[14], requires protein synthesis. Thus, dibutyryl-cyclicAMP may have a generalized effect on various tumour cells, a manifestation of which is the reversal of abnormal growth, and the induction of differentiation in neuroblastoma. The determinants of expression of differentiation in neuroblastoma are of interest because human malignant neuroblastomas occasionally differentiate *in vivo* and give rise to benign ganglioneuromas[15].

This work was supported by a grant from the National Institute of Allergy and Infectious Diseases, US Public Health Service, the American Cancer Society, and by post-doctoral research fellowships from the Damon Runyon Memorial Fund for Cancer Research (P. F.) and the National Cancer Institute (D. J. S.).

PHILIP FURMANSKI
DAVID J. SILVERMAN
MARTIN LUBIN

Department of Microbiology,
Dartmouth Medical School,
Hanover,
New Hampshire 03755

Received April 7; revised June 18, 1971.

[1] Klebe, R., and Ruddle, F., *J. Cell Biol.*, **43**, 69A (1969).
[2] Augusti-Tocco, G., and Sato, G., *Proc. US Nat. Acad. Sci.*, **64**, 311 (1969).
[3] Schubert, D., Humphreys, S., Baroni, C., and Cohn, M., *Proc. US Nat. Acad. Sci.*, **64**, 316 (1969).
[4] Seeds, N. W., Gilman, A. G., Amano, T., and Nirenberg, M. W., *Proc. US Nat. Acad. Sci.*, **66**, 160 (1970).
[5] Schubert, D., and Jacob, F., *Proc. US Nat. Acad. Sci.*, **67**, 247 (1970).
[6] Blume, A., Gilbert, F., Wilson, S., Farber, J., Rosenberg, R., and Nirenberg, M., *Proc. US Nat. Acad. Sci.*, **67**, 786 (1970).
[7] Nelson, P., Ruffner, W., and Nirenberg, M., *Proc. US Nat. Acad. Sci.*, **64**, 1004 (1969).
[8] Harris, A. J., and Dennis, M. J., *Science*, **167**, 1253 (1970).
[9] Puck, T. T., Marcus, P. I., and Cieciura, S. J., *J. Exp. Med.*, **103**, 273 (1956).
[10] Kates, J. R., Winterton, R., and Schlessinger, K., *Nature*, **229**, 345 (1971).
[11] Pastan, L., and Perlman, R. L., *Nature New Biology*, **229**, 5 (1971).
[12] Ellman, G. L., Courtney, K. D., Andres, V., jun., and Featherstone, R. M., *Biochem. Pharmacol.*, **7**, 88 (1961).
[13] Hsie, A. W., and Puck, T. T., *Proc. US Nat. Acad. Sci.*, **68**, 358 (1971).
[14] Johnson, G. S., Friedman, R. M., and Pastan, I., *Proc. US Nat. Acad. Sci.*, **68**, 425 (1971).
[15] Cushing, H., and Wolbach, S. B., *Amer. J. Pathol.*, **3**, 203 (1927).

Leukaemia Virus-induced Alteration of Leucocyte Migration *in vitro*

INFECTION of susceptible mice by a leukaemia virus generally suppresses the immune response at both the cellular and humoral levels[1-8]. Although the mechanism of such immunodepression is not fully understood, it is widely recognized that normal functions of the reticulo-endothelial system are affected by the leukaemic process. Earlier studies in this laboratory of the effects of leukaemia virus on cellular immunity included the macrophage migration inhibition assay with lymphoid cells from normal and virus infected mice sensitized to mycobacterial antigens[9]. It was found, however, that spleen cells from leukaemia virus-infected mice failed to migrate normally *in vitro*, even in the absence of antigen. Studies with normal mice showed that cells from lymphoid tissues other than the spleen, such as the thymus, bone marrow and superficial lymph nodes, as well as from the peripheral blood, migrated readily from capillary tube cultures[10]. The effects of leukaemia virus infection on the *in vitro* migration pattern of lymphoid cells from various organs were determined by comparison of infected and non-infected cells.

Young adult Balb/c mice were infected with Friend leukaemia virus (FLV), which induces a rapid leukaemia-like erythroblastic disease. Graded concentrations of the virus preparation were used, ranging from 10 to 1,000 ID_{50} per inoculum[5-7]. At various times after infection the animals, together with an equal number of control mice of the same age, were killed and their spleens and other lymphoid tissues were excised. Dispersed cell suspensions were prepared by "teasing" the tissues in cold Hank's solution. The cells were washed several times at 4° C by serial centrifugation and placed in individual capillary tubes, approximately 1–2 mm in diameter and sealed at one end with melted paraffin wax[10]. The tubes were centrifuged for 5–10 min at 500*g* and then cut with a glass file at the interface between the cells and the medium[9,10]. The bottom portions of the tube, with the packed cells, were placed in Sykes-Moore chambers containing 1 ml. medium 199 containing 20% foetal calf serum[10]. The chambers were incubated at 37° C for 18 to 24 h and the area of migration of cells out of the capillary tubes was determined by planimetry or by projecting the image of the migrating cells on a sheet of paper. The outline of the image was cut and the paper weighed. The area of migration inhibition or enhancement was expressed as a percentage of the migration area of experimental cultures relative to the average migration of cells from control cultures.

Table 1 Migration of Lymphoid Cells from FLV Infected Mice *in vitro* from Capillary Tube Cultures

Time after infection* (days)	Lymphoid cell migration *in vitro* (area in mm²)†			
	Spleen	Lymph nodes	Bone marrow	Thymus
Normal controls	15.2±4.6	11.3±2.6	18.9±3.1	25.6±6.8
Infected 1	14.8±6.3	12.5±3.1	19.6±3.1	29.2±5.3
2	15.1±3.9	10.9±2.7	16.5±4.2	26.5±3.2
3	12.2±2.6	11.8±2.1	17.3±3.9	23.5±3.2
4	9.3±3.1	—	19.1±4.0	—
5	8.0±2.9	12.5±3.1	16.8±3.9	27.8±6.8
7	3.1±2.5	11.7±2.5	12.1±3.8	25.2±5.3
10	<2	12.5±3.0	15.3±4.1	—
14	<2	10.2±4.2	11.3±2.6	25.8±7.5
21	<2	6.3±2.1	10.3±1.6	18.3±3.5
28	<2	2.1±1.0	5.2±3.9	16.5±4.9

* Mice were infected intraperitoneally with 500 LD_{50} FLV and the lymphoid cell suspensions were tested on the day indicated for *in vitro* migration.

† The average area of cell migration of 3–5 cultures of cells derived from the lymphoid tissue on day indicated after infection with FLV.

Table 1 shows that the area of migration of spleen cells from infected animals decreased considerably with time after infection. When infection occurred 2–3 days before death, the area of migration was 10 to 20% smaller than that of the controls. After 4–7 days, the area of migration was 70–80% smaller than that of the controls (Fig. 1). This inhibition of *in vitro* migration of spleen cells was directly related to the dose of infecting virus. The decrease of migration activity was less pronounced and occurred more slowly when lower dilutions of virus were used for infection. Smaller doses of FLV produced a slower development of splenomegaly and blood cell dyscrasia.

Migration of cells from the superficial and deep lymph nodes, the thymus and the bone marrow was not depressed during the first 7–10 days after infection (Table 1). After 2–3 weeks, however, the lymph node and bone marrow cells migrated less actively than the same cells from control mice. The thymus cells were affected least; moderate inhibition occurred only when mice were infected 3–4 weeks earlier (Table 1).

The mechanism whereby spleen cells from FLV infected mice rapidly lose their capacity to migrate *in vitro* is not clear. This effect cannot have been due merely to the presence of leukaemia virus in the lymphoid cell suspensions, because addition of spleen cells from 14 day infected mice to spleen cells from control animals gave the expected degree of migration. Furthermore, incubation of concentrated FLV splenic homogenates with normal spleen cell suspensions for 1–8 h at 37° C before culture in capillary tubes did not affect the subsequent cell migration.

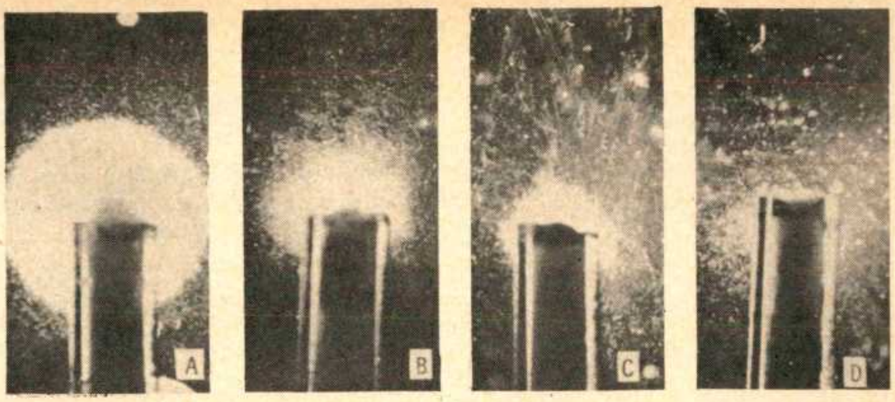


Fig. 1 Typical areas of leucocyte migration from capillary tube cultures containing spleen cells from normal mice (*A*) or mice infected with FLV either 4 (*B*), 6 (*C*) or 9 (*D*) days earlier.

Macrophages are highly motile on glass surfaces and they are thought to be the chief type of cell to migrate rapidly from capillary tubes. Yet other lymphoid cells, including typical lymphocytes, are also highly motile[10]. Histological examination of splenic tissue from infected mice showed a marked increase in the number of leukaemic or undifferentiated cells. Cells morphologically similar to macrophages were also present. The marked infiltration of obvious leukaemic cells did not occur until about 7–10 days after infection. Nevertheless, significant inhibition of leucocyte migration occurred with spleen cells from mice infected 3–5 days earlier, before splenomegaly became apparent. Thus it seems evident that a functional capability of splenic leucocytes, presumably macrophages, was affected by the FLV infection. We therefore attempted to determine whether glass adherent macrophages could be concentrated from the spleens of infected animals by the glass adsorption procedure and shown to migrate *in vitro*. Spleen cell suspensions from normal and 7-day infected mice were passed through glass bead columns and the fraction containing mainly macrophages was eluted with EDTA. There was no significant increase in the migration capacity of cells in the fraction containing the glass-adherent cells when the spleens were derived from the infected mice. A similar fraction from spleen cell suspensions from normal mice had the same migration capacity as the unfractionated cell suspension. Thus, it seems likely that the decrease in motility of spleen cells from infected mice was not due merely to "dilution" of macrophages with non-migrating cells.

It seems from our results that infection of mice with a typical leukaemogenic virus not only causes a rapid diminution of antibody forming capacity but that it also suppresses the *in vitro* migration activity of macrophages which is involved in cellular immunity and antigen handling. Although the significance of this loss of cell motility *in vitro* is not clear, it seems to be related to the leukaemic process.

We thank Mrs Leony Mills and Mrs Vivian Simmons for technical assistance and Dr Maria Sanz for assistance in some experiments. This work was supported in part by research grants from the US National Science Foundation, the American Cancer Society and the Damon Runyon Cancer Fund, Inc.

HERMAN FRIEDMAN
WALTER S. CEGLOWSKI*

Department of Microbiology,
Albert Einstein Medical Center,
Philadelphia,
Pennsylvania 19141

Received April 19; revised June 14, 1971.

* Present address: Department of Microbiology, College of Life Sciences, Pennsylvania State University, University Park, Pennsylvania.

[1] Salaman, M. H., *Antibiotica Chemotherapia*, **15**, 393 (1969).
[2] Notkins, A. L., Mergenhagen, S. E., and Howard, R. J., *Ann. Rev. Microbiol.*, **24**, 525 (1970).
[3] Siegel, B. V., and Morton, J. I., *Immunology*, **10**, 559 (1966).
[4] Salaman, M. H., and Wedderburn, N., *Immunology*, **10**, 445 (1966).
[5] Ceglowski, W. S., and Friedman, H., *Proc. Soc. Exp. Biol. Med.*, **126**, 662 (1967).
[6] Ceglowski, W. S., and Friedman, H., *J. Nat. Cancer Inst.*, **40**, 983 (1948).
[7] Ceglowski, W. S., and Friedman, H., *J. Immunol.*, **101**, 594 (1968).
[8] Old, L. J., Clarke, D. A., Benacerraf, B., and Goldsmith, M., *Ann. NY Acad. Sci.*, **88**, 264 (1960).
[9] Friedman, H., and Ceglowski, W. S., *Proc. Soc. Exp. Biol. Med.*, **136**, 154 (1971).
[10] Friedman, H., Sanz, M., Combe, C., Mills, L., and Lee, Y., *Proc. Soc. Exp. Biol. Med.*, **132**, 849 (1969).

Cytogenetic Analysis of a Chinese Hamster–Mouse Hybrid Cell

THE usefulness of cell fusion in the genetic analysis of somatic cells stems largely from two observations. First, Harris and Watkins[1] demonstrated that not only like cells, as shown by Okada[2], but also cells of widely different types, for example, man and mouse, could be fused together by inactivated Sendai virus. Second, Weiss and Green[3] found that in man–mouse hybrid cells most of the human chromosomes were rapidly eliminated. While most of the known human linkage relationships have been established by classical pedigree analysis[4], the "segregation" of chromosomes that occurs in man–mouse hybrid cells has enabled two further linkage assignments to be made: assignment of the gene controlling thymidine kinase production to an *E* group chromosome[5,6] and linkage of the genes controlling the production of lactate dehydrogenase B and peptidase B[7,8].

The hybrid cells used most often in genetic analysis have been man–mouse hybrids. Kao and Puck[9], however, have suggested that for optimal results cells with the greatest difference in growth rate should be used, on the assumption that the chromosomes of the slower growing parental cell would be preferentially eliminated. All the hybrid cells so far described seem to confirm the inverse correlation between growth rate and chromosome loss, but I have found an exception to this rule, in which the chromosomes of the more rapidly growing parental cell are preferentially lost from the hybrid. Some mechanism unrelated to the growth rate of the parental cells must therefore be responsible for the preferential loss of chromosomes.

The parental cells used were a Chinese hamster (DON)[10] cell (Wg 3-h) resistant to 3 μg/ml. 8-azaguanine, obtained from Dr A. Westerveld, and having a doubling time of about 12.5 h, and a mouse (3T3)[11] cell, resistant to 30 μg/ml. bromodeoxyuridine (BUDR)[12] and having a doubling time of about 24 h. The generation time of the mouse cell was thus approximately twice that of the Chinese hamster cell. The Chinese hamster cell was pseudodiploid with a sharp mode of twenty-two chromosomes, while the mouse cell had a mode of sixty-six chromosomes all acrocentric or telocentric (Fig. 1). The cells were fused essentially as described by Harris and Watkins[1].

In all, 5×10^5 cells of each type were mixed with 4,000 HAU of ultraviolet-inactivated Sendai virus. After fusion, the cells were incubated in Dulbecco's modification[13] of Eagle's medium[14], containing 10 µg/ml. of hypoxanthine and thymidine and 2 µg/ml. of aminopterin (HAT medium[15,16]). The cells were grown continuously in this selective medium. After 3 months chromosome preparations were made and examined. Two populations of cells were observed (Fig. 2*a*). In one population, consisting of 86% of the cells, the chromosome count ranged between seventy-five and eighty-nine with a mode at eighty-two; in the other, the chromosome count ranged between 124 and 148 without a distinct mode. Two published reports describe the chromosomes of hybrids between Chinese hamster and mouse cells: in one, the results suggest a preferential loss of mouse chromosomes[17], in the other the Chinese hamster chromosomes were preferentially lost[18]. In the second case, however, the Chinese hamster parental cell had a doubling time of 40.4 h compared with 14.5 for the parental mouse cell.

Karyotypic analysis of the hybrid cells reported here (Fig. 2) revealed the following points. (1) The cells with seventy-five to eighty-nine chromosomes contained approximately one mouse and one Chinese hamster chromosome complement. (2) Metaphases with 124–148 chromosomes were apparently derived from one Chinese hamster cell and either two mouse cells or one 2s mouse cell. These hybrid cells resemble those described by Koyama *et al.*[18]. (3) Both populations of hybrid cells showed preferential loss of Chinese hamster chromosomes in all cells examined. The number of identifiable Chinese hamster chromosomes varied between eight and thirteen. (4) There were some cells with eighty-eight chromosomes, this being the number to be expected from the sum of the two parental modes, but these cells nonetheless showed a loss of Chinese hamster chromosomes (Fig. 2*c*). Thus a hybrid cell may have a chromosome number corresponding to the sum of the two parental chromosome sets and have marker chromosomes of both parental cells yet still lack the full chromosome complements of both parental cells. (5) There was much karyotypic variation in the hybrid cells, as in the parental Chinese hamster cell. Of fifteen hybrid cells examined *seriatim*, no two contained the same complement of Chinese hamster

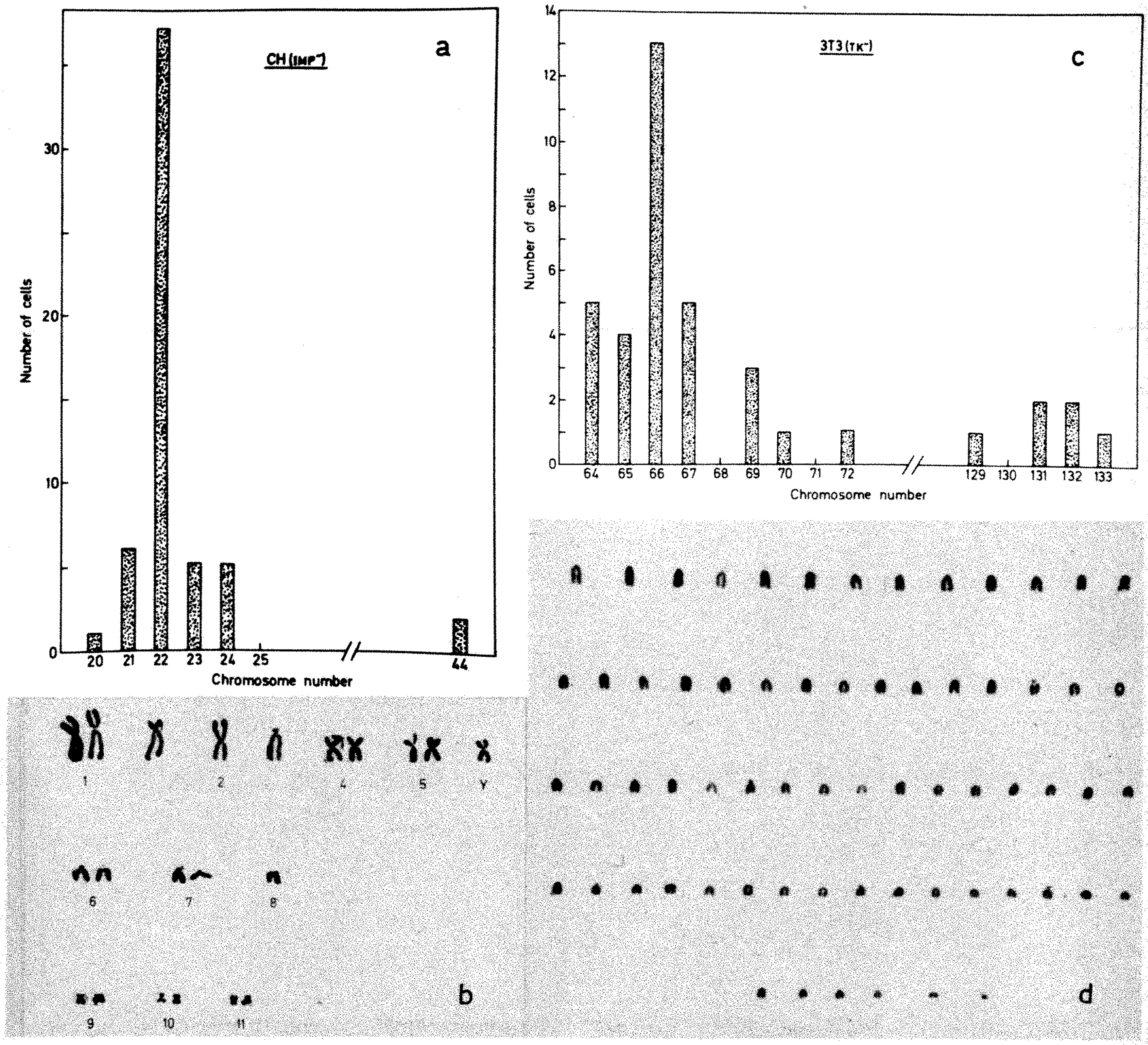


Fig. 1 Chromosome analysis of parental cells. *a*, Distribution of counts of Chinese hamster metaphases. *b*, Karyotype of Chinese hamster cell containing twenty-two chromosomes. *c*, Distribution of counts of mouse metaphases. *d*, Karyotype of mouse cell containing sixty-six chromosomes.

chromosomes (Fig. 2*d* and *e*). (6) Non-disjunction was a feature of both parental cells and was also characteristic of the hybrid cells; this could account for the chromosomal variation observed in the hybrid cell population. One example of this non-disjunction, illustrated in Fig. 2, was seen in the Chinese hamster marker chromosome intermediate in size between numbers 1 and 2: this varied from none (Fig. 2*b*) to four (Fig. 2*d*, line 3) per cell.

A characteristic pattern of chromosome behaviour in hybrids between cells of different species is the preferential loss of the chromosomes of one of the species. This was first shown by Weiss and Ephrussi[19], who observed preferential loss of rat marker chromosomes from rat–mouse hybrid cells. When the preferential loss of human chromosomes from man–mouse hybrid cells was demonstrated[3], it was immediately appreciated that such cells would be particularly suitable for genetic

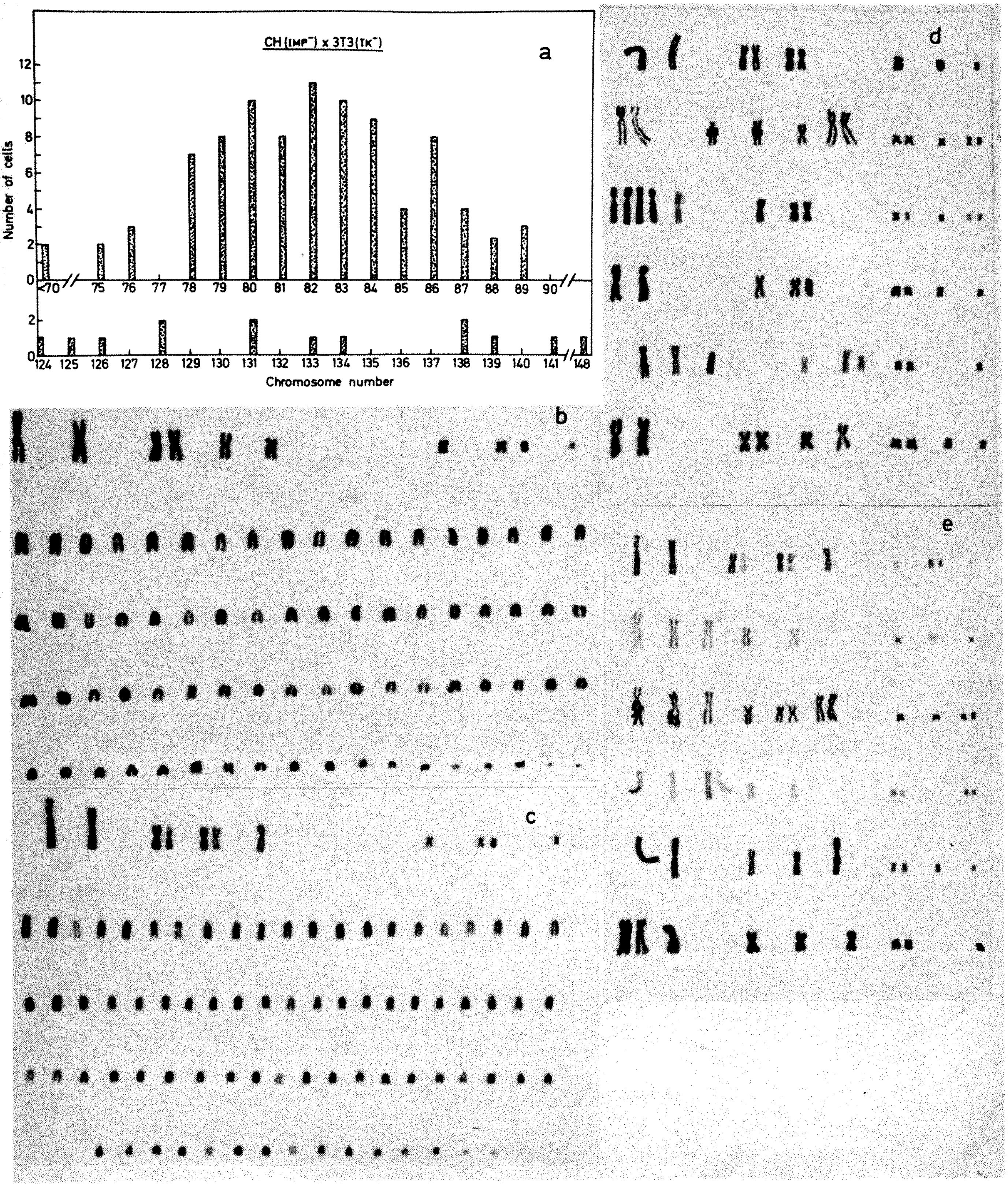


Fig. 2 Chromosome analysis of Chinese hamster–mouse hybrid cells. *a*, Distribution of counts of hybrid metaphases. *b*, Karyotype of Chinese hamster–mouse hybrid cell containing eighty-two chromosomes. *c*, Karyotype of Chinese hamster–mouse hybrid cell containing eighty-eight chromosomes. *d* and *e*, Identifiable Chinese hamster chromosomes in different hybrid cells.

analysis of human somatic cells. The chief limitation of this approach remains the relatively slow and unpredictable loss of chromosomes even in these cells. Kao and Puck[9] suggested that the difference in the growth rates of the parental cells determined the degree and direction of chromosome loss in the hybrid. If this were generally true, the usefulness of cell fusion for genetic analysis might be greatly enhanced. Unfortunately, as can be seen here, some other mechanisms must be sought to explain the pattern of chromosome loss. Perhaps different mechanisms operate in different cells. It is clear that the variation observed in the karyotype of the Chinese hamster–mouse hybrid cells reported here can be explained by non-disjunction, whereas this seems not to be true of man–mouse hybrids (my unpublished results with J. F. Watkins and D. R. Thompson). Similarly, the early losses of chromosomes in man–mouse and in Chinese hamster–mouse cell hybrids may be due to different causes.

I thank Drs A. Westerveld and M. Fried for the Chinese hamster and mouse cells, respectively, Miss L. Rolfe for technical assistance, and Mr S. Buckingham for photography. I am a research fellow of the Helen Hay Whitney Foundation.

STANLEY D. HANDMAKER

Sir William Dunn School of Pathology,
University of Oxford

Received March 26; revised July 30, 1971.

[1] Harris, H., and Watkins, J. F., *Nature*, **205**, 640 (1965).
[2] Okada, Y., *Exp. Cell Res.*, **26**, 98 (1962).
[3] Weiss, M. C., and Green, H., *Proc. US Nat. Acad. Sci.*, **58**, 1104 (1967).
[4] Renwick, J. H., *Brit. Med. Bull.*, **25**, 65 (1969).
[5] Migeon, B. R., and Miller, C. S., *Science*, **162**, 1005 (1968).
[6] Matsuya, Y., Green, H., and Basilico, C., *Nature*, **220**, 1199 (1968).
[7] Santachiara, A. S., Nabholz, M., Miggiano, V., Darlington, A. J., and Bodmer, W., *Nature*, **227**, 248 (1970).
[8] Ruddle, F. H., Chapman, V. M., Chen, T. R., and Klebe, R. J., *Nature*, **227**, 251 (1970).
[9] Kao, F. T., and Puck, T. T., *Nature*, **228**, 329 (1970).
[10] Ford, D. K., and Yerganian, G., *J. Nat. Cancer Inst.*, **21**, 393 (1958).
[11] Todaro, G. J., and Green, H., *J. Cell Biol.*, **17**, 299 (1963).
[12] Matsuya, Y., and Green, H., *Science*, **163**, 697 (1969).
[13] Vogt, M., and Dulbecco, R., *Proc. US Nat. Acad. Sci.*, **49**, 171 (1963).
[14] Eagle, H., *Science*, **130**, 432 (1959).
[15] Szybalski, W., Szybalska, E. H., and Ragni, G., *Nat. Cancer Inst. Mono.*, 7, 75 (1962).
[16] Littlefield, J. W., *Science*, **145**, 709 (1964).
[17] Scaletta, L. J., Rushforth, N. B., and Ephrussi, B., *Genetics*, **57**, 107 (1967).
[18] Koyama, H., Yatabe, I., and Ono, T., *Exp. Cell Res.*, **62**, 455 (1970).
[19] Weiss, M. C., and Ephrussi, B., *Genetics*, **54**, 1095 (1966).

Proposed Reaction Mechanism for the (Na^++K^+)-Dependent Adenosine Triphosphatase

ALTHOUGH a definitive description of the reaction mechanism of the ($Na^+ + K^+$)-dependent ATPase[1,2] must await determination of the three dimensional structure of the enzyme, some clues can be gained from studies of the amino-acids that seem to participate in the active site.

Of such amino-acids the participant best documented is glutamate: in the presence of Na^+ and Mg^{2+} the terminal phosphate of ATP is incorporated into the enzyme, and by isolating a derivative the phosphorylated group was identified as the γ-carboxyl of L-glutamic acid[3]. Certain misgivings[4] are based on the failure of hydroxylamine to inactivate the enzyme although it can release the phosphate (but see ref. 5); on the other hand, the possibility of phosphate migration during processing has been minimized by identification of an acyl phosphate after only mild treatment[6].

Cysteines also seem near the active site, and inhibition of the ATPase by N-ethylmaleimide (NEM) may be retarded by ATP[7]. Although there is a class of proteases in which sulphydryl groups are acylated during catalysis[8], such a role seems less attractive for a phosphatase. Perhaps the cysteines function in nucleotide binding, as suggested for a class of sulphydryls at the active site of myosin[9,10].

Mechanistic proposals often invoke a histidine, and in cases where firm data are available histidine frequently fulfils expectations: beyond demonstrations of its presence near the active sites of such enzymes as alkaline phosphatase[11] and myosin[10], histidine is a crucial component of the detailed catalytic mechanisms of several classes of esterases whose structure has been determined[8,12,13]. For this ATPase two lines of evidence suggest a histidine at the active site. (i) Plots of log V_{max} against incubation pH indicate an ionizable group with a pK of about 7.1 (ref. 14) to 6.7 (my unpublished results), consistent with the pK of the histidine imidazole. (ii) Photo-oxidation of a rat brain ATPase preparation in the presence of 5 μM methylene blue (Table 1) rapidly inactivated the enzyme (first order rate constant, $k = 0.41$/min), while 3 mM ATP markedly slowed the loss of enzymatic activity ($k = 0.13$/min). Further additions with ATP influenced inactivation little (Table 1), in contrast to their effect on NEM inactivation, as shown previously[7] and confirmed for this preparation (Table 1). This latter point is of interest because photo-oxidation is not entirely specific for histidine[16], and other amino-acids, notably cysteine, may also be destroyed. Unfortunately, the impurity of the enzyme preparation makes determination of destroyed amino-acids futile; since all reagents that attack histidine also attack cysteine[16] the limitations to this approach are obvious.

Table 1 Inhibition of the ($Na^+ + K^+$)-dependent ATPase by Photo-oxidation and by NEM

Additions	ATPase activity (% of control) After photo-oxidation	After NEM treatment
None	14±3	20±4
$MgCl_2$, 3 mM	11±2	18±3
NaCl, 90 mM	13±2	—
ATP, 3 mM	53±3	58±2
+$MgCl_2$	54±4	49±3
+NaCl	49±2	52±4
+KCl, 10 mM	54±5	40±4
+$MgCl_2$ and NaCl	50±2	39±2
EDTA, 1 mM	22±5	—

In the photo-oxidation experiments a rat brain ATPase preparation[15] was incubated for 5 min in an ice bath with 10 mM Tris-HCl (pH 7.8) and 5 μM methylene blue, plus the additions listed, either illuminated by a 100-watt tungsten bulb (at 8 inches), or kept in the dark (control). The preparations were then diluted 1 : 1 with buffer, homogenized, and incubated under dim illumination for 6 min at 37° in media containing 50 mM Tris-HCl (pH 7.8), 3 mM ATP, 3 mM $MgCl_2$, 90 mM NaCl, and 10 mM KCl (final concentrations). Concurrent incubations were performed in the absence of Na^+ and K^+, and the difference in activity, measured in terms of P_i production[15], was taken to represent the ($Na^+ + K^+$)-dependent ATPase. In the experiments with NEM, the ATPase preparation was incubated for 8 min at 37° with 50 mM Tris-HCl (pH 7.8) plus the additions noted, in the presence and absence of 1 mM NEM. To stop the action of NEM, 2-mercaptoethanol was added to a final concentration of 1 mM, and 4 min later the incubation was initiated by adding the components necessary for the standard incubation media (see above). Data are presented ± s.e.m.

The remaining amino-acid that may be implicated is serine, for during catalysis a serine is phosphorylated in alkaline phosphatase[17] and acylated in the serine proteases[12]. For the K^+-dependent phosphatase activity associated with the ATPase[18], and apparently reflecting the terminal hydrolytic process[19,20], kinetic studies of product inhibition indicate an enzyme-phosphate intermediate[21]. Moreover, with ^{32}P-nitrophenyl phosphate as substrate, K^+-stimulated labelling of the enzyme preparation occurred at an acidic pH, where dephosphorylation was slowed relative to phosphorylation[22]. To identify this phosphorylated product analogous experiments were performed, followed by acid hydrolysis and electrophoretic

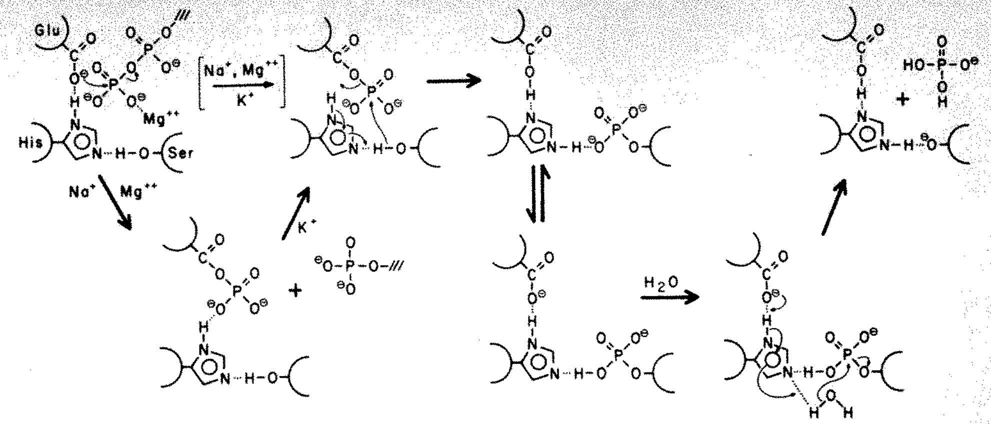


Fig. 1 Proposed reaction mechanism for the (Na⁺+K⁺)-dependent ATPase. For the ATP molecule only the terminal two phosphate groups are shown.

separation of the labelled products. At *p*H 5.0 K⁺ not only stimulated total ^{32}P incorporation, but also the fraction migrating with authentic phosphoserine (Table 2); 0.1 mM ouabain largely abolished the K⁺-stimulated increase. At *p*H 7.8 the Na⁺-stimulated increment in labelling was not associated with phosphoserine (Table 2), in accord with its sensitivity to hydroxylamine[22]. Although experimental uncertainties remain, particularly the possibility of phosphate migration during isolation, these data together with the kinetic studies suggest a K⁺-activated phosphorylation of an enzyme serine group, which is rapidly hydrolysed at neutral *p*H although demonstrable at acidic *p*H. By analogy, these data suggest for the overall ATPase reaction a K⁺-activated migration of phosphate from the glutamyl phosphate complex initially formed in the Na⁺-activated step. (Failure to demonstrate K⁺-stimulated labelling[22] with ^{32}P-ATP would be expected in light of the extremely slow hydrolysis[23] of ATP in the absence of Na⁺: apparently ATP must enter the reaction pathway through the Na⁺-activated formation of an acyl phosphate.)

Given this complement of amino-acids a reaction scheme can be visualized (Fig. 1) modified from that of the serine proteases[12,13] with their aspartate, histidine, and serine groups. First, ATP phosphorylates the enzyme to form the glutamyl phosphate complex in an Na⁺- and Mg²⁺-dependent step. In the absence of K⁺ this acyl phosphate is fairly stable, perhaps through hydrogen bonding; however, with K⁺ (through its effect on enzyme conformation) intramolecular migration occurs from glutamate to serine. This serine phosphate, through a "charge relay system" analogous to that described for the serine proteases[12], is quite labile at neutral *p*H and readily hydrolysed.

Table 2 K⁺-stimulated Incorporation of ^{32}P from ^{32}P-Nitrophenyl Phosphate

*p*H	Additions	Relative incorporation of ^{32}P Total labelling	Labelling in phosphoserine	% of total as phosphoserine
5.0	None	1.00	0.24±0.06	24
	KCl, 20 mM	1.53±0.16	0.54±0.08	35
	KCl+ouabain, 0.1 mM	1.02±0.12	0.29±0.05	28
7.8	None	0.68±0.09	0.12±0.03	18
	NaCl, 20 mM	1.43±0.10	0.16±0.04	11

The ATPase preparation[15] was incubated for 2 min at 30° C in media containing 50 mM Tris-maleate (*p*H 5.0) or Tris-HCl (*p*H 7.8), 1 mM $MgCl_2$, 1 mM ^{32}P-nitrophenyl phosphate (16.9 mCi/mmol), and the additions indicated. The reaction was stopped and the preparation washed with trichloroacetic acid as previously described[22], except that an additional wash with ethanol-diethyl ether (1 : 1) was included. Phosphoserine was isolated by a modification of the method of Schwartz and Lipmann[17]: the precipitated protein was suspended in 1 N HCl and heated in sealed-glass tubes for 16–20 h at 110° C; the hydrolysate was then lyophilized and the residue subjected to paper electrophoresis at 5,000 V for 75 min in 0.05 M ammonium formate (*p*H 3.3), together with authentic phosphoserine. Radioactivity in 0.5 inch strips cut from the paper was measured with a liquid scintillation counter. This procedure clearly separated phosphoserine from inorganic phosphate; together these represented essentially all the radioactivity (recovery of the applied radioactivity was greater than 80%). Data are presented, ±s.e.m., relative to the total incorporation of ^{32}P at *p*H 5.0 in the absence of additions, defined as 1.0; such controls were run with all experiments, and this incorporation averaged 0.07 nmol/mg protein.

The energy flow through the system accompanies the transitions from pyrophosphate (ATP) to acyl phosphate to ester phosphate to inorganic phosphate, with major liberations of free energy at the final two steps. Coupling these free energy changes to cation translocation is unspecified, although opportunities for protein conformational changes are clear (it should be noted that such conformational changes are distinct from the cooperative allosteric effects among cation sites[15,20,24], presumably representing subunit interactions[15,25], which may be associated chiefly with regulatory processes[26]). Finally, it can be seen that the phosphorylated enzyme bears an extra negative charge, which may be pertinent to the stoichiometric transport of 3 Na⁺ and 2 K⁺ for each ATP hydrolysed: while two Na⁺-sites balance two K⁺-sites the added negative charge would neutralize an endogenous positive group, freeing a third site for Na⁺.

This work was supported by a US Public Health Service grant.

JOSEPH D. ROBINSON

Department of Pharmacology,
State University of New York,
Upstate Medical Center,
Syracuse,
New York 13210

Received April 16; revised July 9, 1971.

[1] Skou, J. C., *Physiol. Rev.*, **45**, 596 (1965).
[2] Albers, R. W., *Ann. Rev. Biochem.*, **36**, 727 (1967).
[3] Kahlenberg, A., Galsworthy, P. R., and Hokin, L. E., *Science*, **157**, 434 (1967).
[4] Schoner, W., Kramer, R., and Seubert, W., *Biochem. Biophys. Res. Commun.*, **23**, 403 (1966).
[5] Charnock, J. S., Opit, L. J., and Potter, H. A., *Biochem. J.*, **104**, 17c (1967).
[6] Alexander, D. R., and Rodnight, R., *Biochem. J.*, **119**, 44P (1970).
[7] Skou, J. C., and Hilberg, C., *Biochim. Biophys. Acta*, **110**, 359 (1965).
[8] Drenth, J., Jansonius, J. N., Koekoek, R., Sluyterman, L. A. A., and Wolthers, B. G., *Phil. Trans. Roy. Soc.*, B, **257**, 231 (1970).
[9] Stracher, A., *J. Biol. Chem.*, **239**, 1118 (1964).
[10] Stracher, A., *J. Biol. Chem.*, **240**, 958 (1965).
[11] Tait, G. H., and Vallee, B. L., *Proc. US Nat. Acad. Sci.*, **56**, 1247 (1966).
[12] Blow, D. M., Birktoft, J. J., and Hartley, B. S., *Nature*, **221**, 337 (1969).
[13] Hartley, B. S., *Phil. Trans. Roy. Soc.*, B, **257**, 77 (1970).
[14] Hexum, T., Samson, jun., F. E., and Himes, R. H., *Biochim. Biophys. Acta*, **212**, 322 (1970).
[15] Robinson, J. D., *Biochemistry*, **6**, 3250 (1967).
[16] Valee, B. L., and Riordan, J. F., *Ann. Rev. Biochem.*, **38**, 733 (1969).
[17] Schwartz, J. H., and Lipmann, F., *Proc. US Nat. Acad. Sci.*, **47**, 1996 (1961).

[18] Uesugi, S., Dulak, N. C., Dixon, J. F., Hexum, T. D., Dahl, J. L., Perdue, J. F., and Hokin, L. E., *J. Biol. Chem.*, **246**, 531 (1971).
[19] Askari, A., and Koyal, D., *Biochem. Biophys. Res. Commun.*, **32**, 227 (1968).
[20] Robinson, J. D., *Biochemistry*, **8**, 3348 (1969).
[21] Robinson, J. D., *Biochim. Biophys. Acta*, **212**, 509 (1970).
[22] Robinson, J. D., *Biochem. Biophys. Res. Commun.*, **42**, 880 (1971).
[23] Fujita, M., Nagano, K., Mizuno, N., Tashima, Y., Nakao, T., and Nakao, M., *J. Biochem.*, Tokyo, **61**, 473 (1967).
[24] Robinson, J. D., *Arch. Biochem. Biophys.*, **139**, 17 (1970).
[25] Monod, J., Wyman, J., and Changeux, J.-P., *J. Mol. Biol.*, **12**, 88 (1965).
[26] Robinson, J. D., *J. Theoret. Biol.*, **19**, 90 (1968).

The Role of Distortion in the Lysozyme-catalysed Hydrolysis of Glucosides

BLAKE *et al.* proposed that during cleavage of oligosaccharides, the saccharide bound in subsite D of the active site of lysozyme is distorted into a half chair conformation[1,2]. In agreement with this mechanism, Dahlquist *et al.*[3,4] concluded that considerable carbonium ion character is involved in the hydrolysis of the synthetic substrate phenyl-4-*O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (NAG-GLU-φ). Lowe and Sheppard[5] found, however, in their study of the hydrolysis of NAG-GLU-φNO_2 that participation of the acetamido group is important in the lysozyme mechanism.

The hydrolysis of natural oligosaccharides by lysozyme is complicated by non-productive binding and trans-glycosylation, making a study of any one feature of the mechanism difficult without the use of synthetic substrates[6]. We report here the results of a nuclear magnetic resonance (NMR) study of the conformation of the glucose ring of NAG-GLU-φNO_2 when bound in the active site of lysozyme (Rand-Meir *et al.*[6] have shown that the productive mode of binding of NAG-GLU-φ-NO_2 to lysozyme is at least competitive with, if not identical to, binding in subsites C, D and E). The chemical shift of the anomeric proton H_1 of the glucose ring (Fig. 1) and its apparent coupling constant to proton H_2 can be obtained from the NMR spectrum of NAG-GLU-φNO_2 (Fig. 2); in the fast exchange limit the coupling constant so obtained is the weighted average of the coupling constants for the free and bound substrate. The dihedral angle dependence of such coupling constants is well known[7-10] and the dihedral angle of the glucose ring obtained in this way is a sensitive indicator of its conformation.

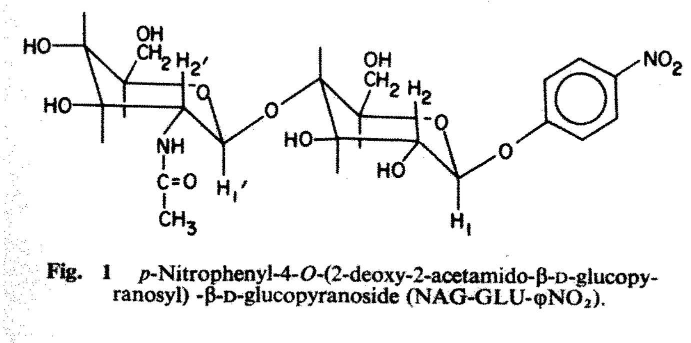


Fig. 1 *p*-Nitrophenyl-4-*O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl) -β-D-glucopyranoside (NAG-GLU-φNO_2).

NAG-GLU-φNO_2 was prepared by a modified method of Rand-Meir *et al.*[6], and the lysozyme was obtained from P.L. Biochemicals, lot LY4. All solutions were made up in 0.1 M citrate buffers in D_2O [*p*H (meter reading) $\simeq 5.5$] containing 12% dioxane. NMR spectra were obtained on a Varian HA-100 spectrometer, using both conventional and Fourier transform time averaging techniques. The measured coupling constants were derived from the spectra by curve fitting programs using linearized least squares techniques[11].

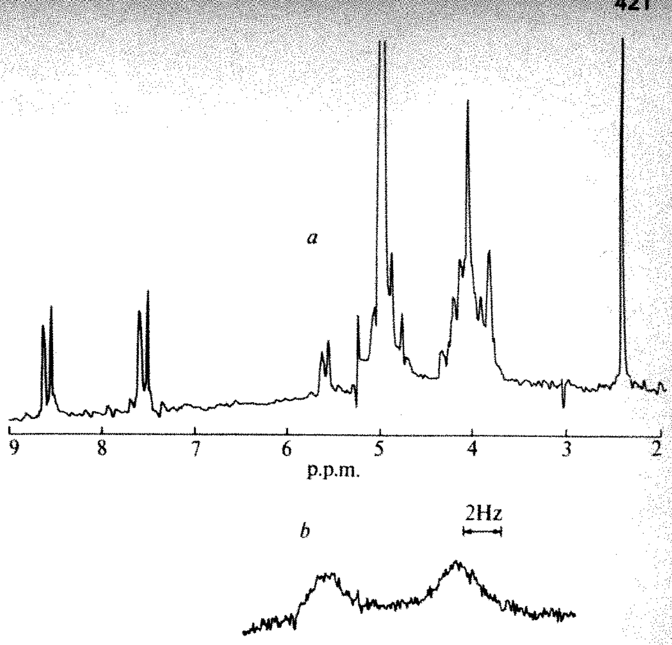


Fig. 2 *a*, 5.1×10^{-3} M NAG-GLU-φNO_2, $pH = 5.5$, 0.1 M citrate buffer in D_2O and 12% (v/v) dioxane at 32° C; 500 scans with Fourier transform (VFT-100), reference HMDS. *b*, 2.6×10^{-2} M NAG-GLU-φNO_2, H_1 region expanded, twenty-five scans with C-1024 time averaging computer.

The NMR spectrum of NAG-GLU-φNO_2 is shown in Fig. 2. The conclusion that the doublet near $\tau = 5.5$ p.p.m. arises from H_1 (the doublet from H_1' is near the HDO peak) was made on the basis of a comparison with the spectrum for GLU-φNO_2.

Upon the addition of lysozyme, the acetyl resonance shifts up field and broadens. The lifetimes for the exchange of the substrate between solution and the active site of lysozyme can be calculated from the linewidth of the acetyl resonance as a function of enzyme concentration[12]. The calculated lifetimes satisfy the fast exchange conditions. The value of the dissociation constant and bound chemical shift obtained from the observed shifts are very similar to those obtained previously[6]. The doublet corresponding to H_1 also broadens, but never so much that the values of J_{H_1,H_2} could not be obtained. The values of J_{H_1,H_2} obtained are given in Table 1.

Table 1 Observed and Predicted Coupling Constants between H_1 and H_2 of NAG-GLU-φNO_2

[EI]/[I] *	J_{H_1,H_2} (observed)	J_{H_1,H_2} (predicted)
0.0	7.0 ± 0.1	7.0
0.1	6.7 ± 0.2	6.5
0.14	7.2 ± 0.2	6.3
0.27	7.2 ± 0.2	5.9

* Calculated from the initial concentrations of enzyme and inhibitor on the basis of $K_D = 1.5 \times 10^{-2}$ M (Rand-Meir *et al.*[6]).

The coupling constant J_{H_1,H_2} for NAG-GLU-φNO_2 and GLU-φNO_2 free in solution is 7.0 ± 0.1 Hz. The relationship between the coupling constant and the dihedral angle between the H_1 and H_2 protons is given by[7-10]

$$J(\psi) = A\cos^2\psi + B\cos\psi + C$$

with the $\cos^2\psi$ term dominant. For the purpose of this discussion, we have assumed the simplified form where $A = 7.0$ and $B = C = 0$. The predicted coupling constant for a dihedral angle of $\psi \simeq 120°$ is 1.7 Hz.

The observed coupling constant is a weighted average of that for free and bound NAG-GLU-φNO$_2$

$$J_{\text{obs}} = P_F J_F + P_B J_B$$

where J_F and J_B are the coupling constants, and P_F and P_B the relative concentrations of free and bound NAG-GLU-φNO$_2$, respectively. This suggests that if the substrate is bound with the glucose ring distorted into the half chair conformation, the observed coupling constant should decrease as P_B increases. Comparison of the observed results with the predicted results (Table 1) indicates that the quantity $P_B(J_B - J_F)$ is small

$$P_B(J_B - J_F) \ll 0.2 \text{ Hz}$$

For the largest value of P_B used,

$$P_B = \frac{[\mathrm{EI}]}{[\mathrm{EI}] + [\mathrm{I}]} \simeq 0.21$$

one obtains

$$J_B - J_F \ll 1 \text{ Hz}$$

Our results suggest that any change in the dihedral angle between H$_1$ and H$_2$ of the glucose ring of the bound substrate is small, indicating that the glucose ring is not distorted when NAG-GLU-φNO$_2$ is bound to lysozyme.

An alternative interpretation of these results is that only a small fraction of the bound substrate is distorted. This could be represented by a mechanism of the kind

$$\mathrm{E} + \mathrm{S} = \mathrm{ES}_1 = \mathrm{ES}_2$$

where

$$K_1 = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}_1]}, \quad K_2 = \frac{[\mathrm{ES}_1]}{[\mathrm{ES}_2]}, \text{ and } K_2 \gg 1$$

such that $K_D = \dfrac{K_1 K_2}{1 + K_2} \sim K_1 = 1.5 \times 10^{-2}$ M; or by a mechanism of the kind

$$\mathrm{E} + \mathrm{S} \overset{K_p}{=} \mathrm{ES} \text{ (productive)}$$

$$\mathrm{E} + \mathrm{S} \overset{K_u}{=} \mathrm{ES} \text{ (unproductive)}$$

with $K_u \ll K_p$ such that $K_D \simeq K_u$.

This dilemma is familiar. If one chooses a substrate which is slowly hydrolysed so that the nature of the bound substrate can be investigated, one cannot guarantee that the enzyme operates on the artificial substrate in a manner similar to that with the natural species. We have found that distortion does play a major role in the cleavage of the natural tetrasaccharide substrate from cell walls[13].

We thank Marcia Robbins and Joseph Maloney for their assistance and the US National Institutes of Health for their support.

B. D. SYKES
D. DOLPHIN

Department of Chemistry,
Harvard University,
Cambridge, Massachusetts 02138

Received April 6, 1971.

[1] Blake, C. C. F., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarma, V. R., *Proc. Roy. Soc.*, B, **167**, 365 (1967).
[2] Blake, C. C. F., Johnson, L. N., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarma, V. R., *Proc. Roy. Soc.*, B, **167**, 378 (1967).
[3] Dahlquist, F. W., Rand-Meir, T., and Raftery, M. A., *Proc. US Nat. Acad. Sci.*, **61**, 1194 (1968).
[4] Dahlquist, F. W., Rand-Meir, T., and Raftery, M. A., *Biochemistry*, **10**, 4214 (1969).
[5] Lowe, G., and Sheppard, G., *Chem. Commun.*, 529 (1968).
[6] Rand-Meir, T., Dahlquist, F. W., and Raftery, M. A., *Biochemistry*, **10**, 4206 (1969).
[7] Barfield, M., and Karplus, M., *J. Amer. Chem. Soc.*, **91**, 1 (1969).
[8] Karplus, M., *J. Amer. Chem. Soc.*, **85**, 2870 (1963).
[9] Karplus, M., *J. Chem. Phys.*, **33**, 1842 (1960).
[10] Karplus, M., *J. Chem. Phys.*, **30**, 11 (1959).
[11] Arley, N., and Randerbuck, K., *Introduction to Theory of Probability and Statistics*, ch. 12 (Wiley, New York, 1950).
[12] Sykes, B. D., *Biochemistry*, **8**, 1110 (1969).
[13] Sykes, B. D., Patt, S. L., and Dolphin, D. M., *Cold Spring Harbor Symp. Quant. Biol.*, **36** (1971).

In vitro Culture of Rabbit Ova from the Single Cell to the Blastocyst Stage

SEVERAL investigators have reported culturing two and four-celled mammalian ova to the blastocyst stage[1-5]. Although successful *in vitro* cultivation of eggs from the single cell to the blastocyst stage has been achieved in the mouse[6-8], few satisfactory methods have been devised for the culture of the ova, at this stage, of other mammalian species, apart from some experiments with human oocytes[9-10]. We now wish to report a higher rate of success with one-celled rabbit ova cultivated *in vitro* to the blastocyst stage, using a nutrient solution composed of several known synthetic media.

Preliminary trials showed that none of the usual chemically defined media were suitable for this work. The best results were obtained with the solution composed of HamF$_{12}$ solution[11] (Nissan 59% v/v); RPMI 1640[12] (Nissan 20% v/v); Eagle's solution[13] (Hanks' solution + Eagle's amino-acids and vitamins, Nissan 20% v/v); sodium caseinate solution (5 g milk casein dissolved slowly in 40–45 ml. of 0.1 N NaOH was centrifuged for 5 min at 3,000 r.p.m., and the supernatant was used as a sodium caseinate solution, 1% v/v).

Table 1 *In vitro* Development of Rabbit Ova from One-celled Stage

Culture system	No. of eggs tested	No. of embryos at final stage of development: 1∼2-cell	8-cell	Morula	Early expanding blastocyst	Expanding blastocyst
Earle's flask						
Open	24	5	4	9	4	2
Closed	27	3	2	3	4	15
Flat cuvette						
Closed	16	1	0	2	4	9

The medium was adjusted to *p*H 7.5 with NaHCO$_3$, supplemented with 20% v/v calf serum and completed by adding 60 mg of kanamycin sulphate/l.

Ova were flushed with the medium from the oviducts of a rabbit 17–19 h after copulation. In most cases, Earle's flasks (10 ml.) containing 6 ml. of medium were used for cultivation. Two ova were placed in every flask and cultured for up to 4 days at 37° C in a humidified incubator with a constant flow of 5% CO$_2$ in air. About half of the flasks were stoppered tightly with silicon bungs before being placed in the incubator. After incubation, ova were examined by phase contrast microscopy, and classified according to the final stage of development. The microcinephotographic microscope was also used, together with a specially designed cuvette (a flat slender-rectangular solid type, 3 mm internal depth, with 3–4 ml. volume) filled with the medium. The cleavage and behaviour of the embryonic cells could be recorded through the flat sides without distortion under the inverted microcinemicroscope kept at 37° C within the microscopic incubator.

Fifty-eight of sixty-seven ova incubated appeared to have undergone normal cleavage to eight cell, morula, or to the blastocyst. Most of them developed to the blastocyst (65%) rather than the morula (24%). Table 1 summarizes the results of the studies with either the open or closed system of cultivation.

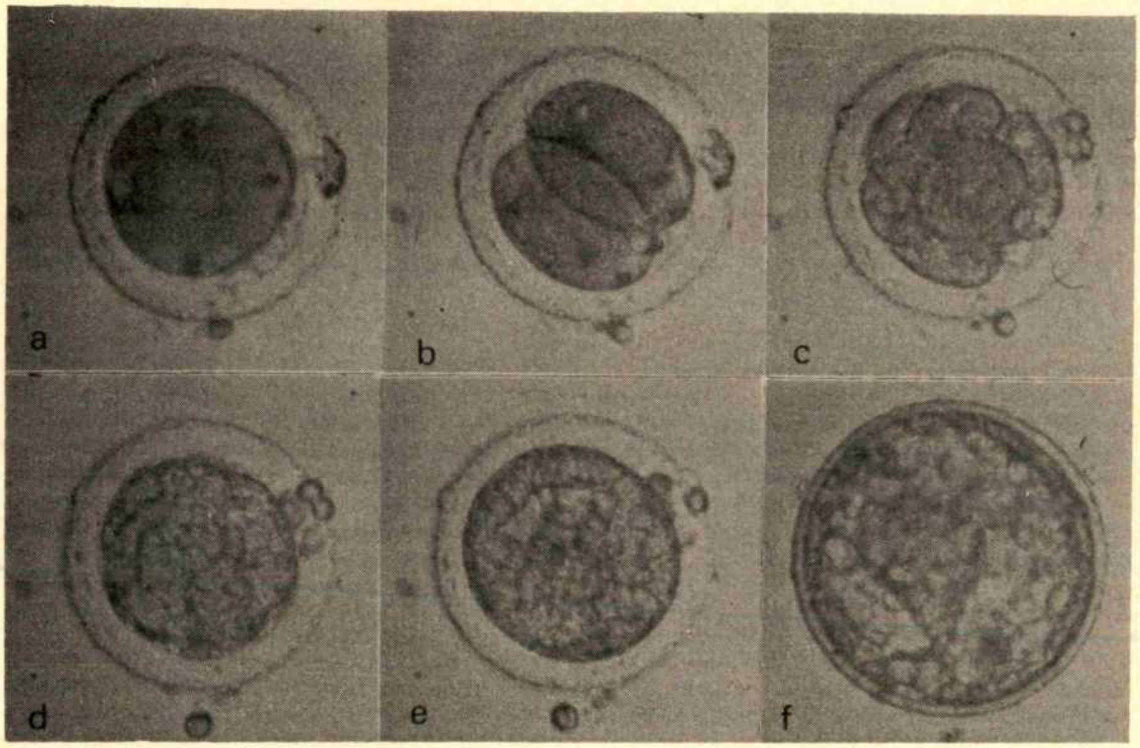


Fig. 1 The development of the rabbit ovum from one-cell to blastocyst stage *in vitro*. From a time-lapse film (16 mm) of an ovum isolated 18 h after mating (× *c*. 200). (*a*) 4.5 h after the start of culture; (*b*) two-cell stage, 6.5 h after (*a*); (*c*) eight-cell stage, 41 h after (*a*); (*d*) late morula to early blastocyst stage, 74 h after (*a*); (*e*) early blastocyst stage, 83.5 h after (*a*); (*f*) expanding blastocyst stage, 122 h after (*a*).

There was a difference in the proportion of ova developing to the blastocyst in the two culture systems; in the open system more embryos remained at the morula stage than in the closed system. There were, however, some cases of the development to the blastocyst stage in the open system. The most advanced development was obtained when we used a closed flask of cuvette without a gas phase. Four consecutive experiments yielded twenty-four expanding blastocysts (56%), eight early expanding blastocysts (19%) and five morulae (12%) from forty-three ova.

Time lapse microcinephotographs (1–8 frames/2 min on 16 mm film under phase contrast) of the embryo which developed from the one-celled stage to the expanding blastocyst (Fig. 1) show a regular pattern of cleavage as the culture passed (two-cell, 20–25 h; eight-cell, 38–45 h; morula, 58–67 h, and early blastocyst, 75–170 h after copulation). This pattern seems to parallel that which can be expected in oviducts and uteri.

We have confirmed by microcinephotography the presence of a rhythmical contraction and expansion, like those reported[14–16] in the mouse blastocyst, accompanying cleavage division of the cell mass in the embryo. The movement occurred at the transitional stage from late morula to early blastocyst and increasingly continued throughout the expanding blastocyst stage. The zona seemed to be thinner and elastic, its volume continuing to decrease, while the volume of the blastocoelic cavity continued to increase.

During the expanding stage, many intracellular particles moved up and down sometimes in a circular movement, within the blastocoelic cavity. The cell mass was finally fixed under the marginal expanding membrane of the blastocoelic cavity.

The composition of the culture medium *in vitro* is a critical factor in the cultivation of mammalian ova. Our medium can supply the necessary nutrients and environment to support a high rate of development of one-celled rabbit ova into early blastocysts. Furthermore, because a CO_2 incubator is not needed it seems to be convenient for the study of development of the rabbit ova *in vitro*. We think that this medium could also be used for the culture of the ova of other mammalian species *in vitro*.

This work was supported by a research grant from the Ministry of Education, Japan. We thank Mr S. Etoh and N. T. Takatsuna for microcinephotography.

Shyoso Ogawa
Kahei Satoh
Hajime Hashimoto

Department of Animal Reproduction,
College of Agriculture and Veterinary Medicine,
Nihon University, Setagaya-ku, Tokyo

Received June 2; revised July 5, 1971.

[1] Brinster, R. L., *Exp. Cell Res.*, **32**, 205 (1963).
[2] Brinster, R. L., *J. Reprod. Fert.*, **10**, 227 (1965).

[3] Biggers, J. D., Moore, B. D., and Whittingham, D. G., *Nature*, **206**, 734 (1965).
[4] Onuma, H., Maurer, R. R., and Foote, R. H., *J. Reprod. Fert.*, **16**, 491 (1968).
[5] Kane, M. T., and Foote, R. H., *Biol. Reprod.*, **2**, 245 (1970).
[6] Biggers, J. D., Gwatkin, R. B. L., and Brinster, R. L., *Nature*, **194**, 747 (1962).
[7] Whitten, W. K., and Biggers, J. D., *J. Reprod. Fert.*, **17**, 399 (1968).
[8] Mukherjee, A. B., and Cohen, M. M., *Nature*, **228**, 472 (1970).
[9] Edwards, R. G., Steptoe, P. C., and Purdy, J. M., *Nature*, **227**, 1307 (1970).
[10] Steptoe, P. C., Edwards, R. G., and Purdy, J. M., *Nature*, **229**, 132 (1971).
[11] Ham, R. G., *Proc. US Nat. Acad. Sci.*, **53**, 288 (1965).
[12] Moore, G. G., Gerner, R. E., and Addison, H. F., *J. Amer. Med. Assoc.*, **199**, 519 (1967).
[13] Eagle, H., *Science*, **130**, 432 (1959).
[14] Kuhl, W., and Friedrich-Freksa, H., *Zool. Anz., Suppl.*, **9**, 187 (1936).
[15] Borghese, E., and Cassini, A., in *Cinemicrography in Cell Biology* (edit. by Rose, G. G.), 274 (Academic Press, New York, 1963).
[16] Cole, R. J., and Paule, J., *Preimplantation Stages of Pregnancy*, Ciba Foundation Symp. (edit. by Wolstenholme, G. E. W., and O'Conner, M.), 82 (Little Brown, Boston, 1965).

Potentiation of Amphetamine-induced Arousal by Starvation

STARVATION and amphetamine both increase behavioural arousal, and have been postulated to act through adrenergic mechanisms in the brain stem. Amphetamine is believed to induce psychomotor excitation by releasing brain catecholamines, blocking their re-uptake from the synapse and mildly inhibiting the action of the catabolizing enzyme, monoamine oxidase[1–3]. The drug also accelerates the turnover of noradrenaline in the brain stem reticular formation[4]. Food deprivation increases electrophysiological arousal in the mesencephalic reticular formation and other areas[5] and Dell[6] postulated that behavioural arousal induced by starvation resulted from the release of catecholamines from the adrenal medulla and their consequent action on brain stem excitatory areas.

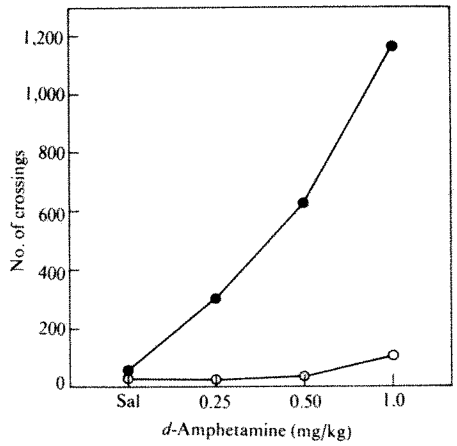


Fig. 1 The effect of 4 days of food deprivation (●) on amphetamine-induced psychomotor excitation. ○, *Ad lib.*

The similar action of these two different means of enhancing arousal raises the question of how the two states would interact if they occurred simultaneously. At one level, a simple summation of the two arousal states might result. At a more complex level there might be some form of synergistic action.

Sprague–Dawley male rats (Perfection Breeders, Douglasville, Pa.) weighing 250–275 g were used. Their locomotor activity was measured in wire mesh, stabilimeter activity cages[7] ($17.5\times20.0\times37.5$ cm) which were mounted on a central axis so that they tipped slightly and activated a sensitive microswitch as the rat moved from one end to the other. The number of crossings was recorded on electromagnetic counters in an adjacent room. Activity rooms were maintained at 22°–23° C with an 80 dB white noise background, and there was 12 h of light followed by 12 h of dark, the lights coming on at 0900 h. Activity was not recorded for 2 h each day (0900–1100 h), when weighing and maintenance of the animals were carried out.

Rats were habituated in the stabilimeter cages for 2 days, during which they had free access to food and water. Then they were divided into two groups which were matched on the basis of the activity on the second day. Food was removed from one group while the other continued to have free access to food. When the experimental group had been deprived of food for 96 h, all animals were injected intraperitoneally with either 0.9% saline or *d*-amphetamine sulphate (0.25, 0.50 or 1.0 mg/kg body weight), dissolved in 0.9% saline. Drugs and saline were injected at 1100 h in a volume of 1 ml./kg body weight, animals were then returned to the activity cages and crossings were recorded on a printing counter at 0.5 h intervals for 5 h (Fig. 1). Eight rats were tested once with each dose.

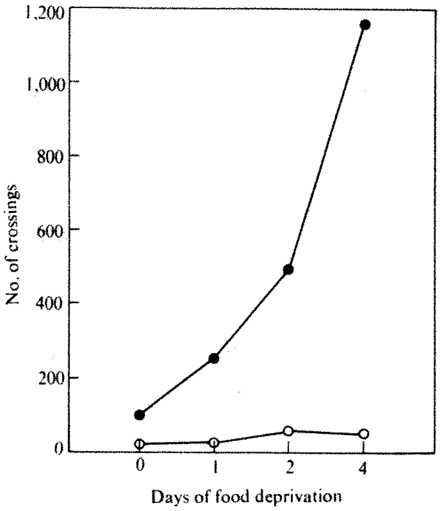


Fig. 2 Effect of duration of food deprivation on amphetamine-induced psychomotor excitation. ○, Saline; ●, *d*-amphetamine.

The same procedures were followed in the second experiment, except that the days of food deprivation were varied. Rats were compared in their response to *d*-amphetamine (1.0 mg/kg) and saline after 0, 1, 2, or 4 days without food. At each period of deprivation eight naïve rats were injected with amphetamine and eight with saline.

There was a significant difference in the activity of the starved and fed groups with all doses of drugs ($F=49.12$, df$=1, 56$, $P<0.01$). There was also a significant increase in activity with increasing dose ($F=17.41$, df$=3, 56$, $P<0.01$). A given dose of amphetamine had a significantly different effect on rats deprived of food compared with those fed freely; only the large dose (1.0 mg/kg) increased the activity of the latter ($t=3.73$, df$=14$, $P<0.01$), but after 4 days of starvation all doses produced significant increases in activity relative to the saline control group. Even the smallest dose (0.25 mg/kg) increased activity significantly ($t=2.64$, df$=14$, $P<0.02$). There was also a small but reliable difference between the starved and freely fed animals after injection with saline which is consistent with many reports of increased behavioural arousal during food deprivation.

The excitatory effects of amphetamine were proportional to the duration of starvation (Fig. 2, $F=12.89$, df$=3$, 28, $P<0.01$). Even after 24 h of food deprivation there was a significant enhancement of amphetamine-induced arousal ($t=2.73$, df$=14$, $P<0.02$). Fig. 3 shows that during each period of deprivation the increased response to amphetamine was due to a heightened peak response in addition to a prolonged action of the drug. The peak drug effect seemed to be more sensitive to deprivation than the duration effect, however, for the group deprived of food for 1 day showed a clear increased peak effect to amphetamine but only a negligible increase in the duration of responding.

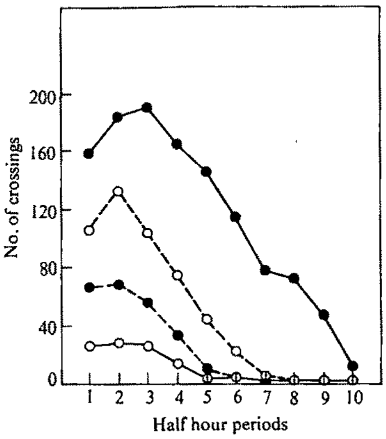


Fig. 3 Time-response curves for 1.0 mg/kg amphetamine after varying durations of food deprivation. ○—○, *Ad lib.*; ● - - - ●, 1 day deprived; ○ - - - ○, 2 day deprived; ●—●, 4 day deprived.

These experiments indicate that there is a substantial change in responsiveness to *d*-amphetamine during starvation. Taken together our results demonstrate that the arousal-inducing properties of starvation interact in a supra-additive summative manner with amphetamine-induced behavioural arousal. The mechanism responsible for the summative interaction of amphetamine and starvation is not known. Angel[8] found higher levels of cocaine in the brain in starved rats and interpreted this to indicate an increased permeability of the blood–brain barrier during starvation. That effect would be equally likely to occur, however, if the metabolism of cocaine was interfered with during starvation. In this regard Dixon, Shunttice and Fouts[9] have shown that during starvation liver metabolism of certain psychoactive drugs is impaired. It is possible that the present results have their basis in an impaired metabolism of amphetamine during starvation.

There has also been considerable work on the effects of stress on central biogenic amines. Although starvation is not typically viewed in this way, food deprivation is obviously a highly stressful experience. The increased sympathetic activity during hypoglycaemia is consistent with this model[6]. Stress significantly alters absolute concentrations, rates of synthesis and release of biogenic amines in the brain[10–13]. Furthermore, amphetamine toxicity is increased by some forms of stress[14,15]. Together these findings indicate that the mode of action of amphetamine and the central biogenic amines through which it is thought to exert its action may be changed in the central nervous system during stress. To the extent that starvation is a stressor, our results may be a reflexion of central neurochemical changes which occur during stress.

Whatever mechanisms underlie our findings, these experiments indicate that in psychopharmacological research the nutritive state of the animal is of considerable importance in determining the magnitude and the nature of the drug response.

This research was supported in part by a US Public Health Service grant from the National Institute of Mental Health.

BYRON A. CAMPBELL

Department of Psychology,
Princeton University,
Princeton, New Jersey 08540

HANS C. FIBIGER

Division of Neurological Sciences,
Health Sciences Centre Hospital,
University of British Columbia,
Vancouver, British Columbia

Received November 23, 1970.

[1] Glowinski, J., and Axelrod, J., *Pharmacol. Rev.*, **18**, 775 (1966).

[2] Glowinski, J., and Baldessarini, R. J., *Pharmacol. Rev.*, **18**, 1201 (1966).

[3] Carr, L. A., and Moore, K. E., *Science*, **164**, 322 (1969).

[4] Javoy, F., Thierry, A. M., Kety, S. S., and Glowinski, J., *Commun. Behav. Biol.*, **1**, 43 (1968).

[5] Hockman, C. H., *Electroenceph. Clin. Neurophysiol.*, **17**, 420 (1964).

[6] Dell, P. C., in *Neurological Basis of Behavior* (edit. by Wolstenholme, G. E. W., and O'Conner, M.) (Churchill, London, 1958).

[7] Campbell, B. A., in *Thirst* (edit. by Wayner, M.) (Pergamon, New York, 1964).

[8] Angel, C., *Dis. Nerv. Sys.*, **30**, 94 (1969).

[9] Dixon, R. L., Shunttice, R. W., and Fouts, J. R., *Fed. Proc.*, **103**, 333 (1960).

[10] Bliss, E. L., Ailion J., and Zwanzieger, J., *J. Pharmacol. Exp. Ther.*, **164**, 122 (1968).

[11] Gordon, R., Spector, S., Sjoerdsma, A., and Udenfriend, S., *J. Pharmacol. Exp. Ther.*, **153**, 440 (1966).

[12] Maynert, E. W., and Levi, R., *J. Pharmacol. Exp. Ther.*, **143**, 90 (1969).

[13] Corrodi, H., Fuxe, K., and Hokfelt, T., *Life Sci.*, **7**, 107 (1968).

[14] Weiss, B., Laties, V. G., and Blanton, F. L., *J. Pharmacol. Exp. Ther.*, **132**, 366 (1961).

[15] Goldberg, M. E., and Salama, A. I., *Toxicol. Appl. Pharmacol.*, **14**, 447 (1969).

Effects of 6-Hydroxydopamine on Sleep in the Rat

THERE is evidence that biogenic amines are involved in the mechanisms of sleep[1,2]. Although the exact relationship is not clear, recent results suggest that decreases in the concentration of catecholamines (noradrenaline or dopamine) in the brain cause increased desynchronized sleep (D) (sleep with rapid desynchronized cortical activity, also known as paradoxical or REM sleep), whereas increases in the concentration of catecholamines decrease D[3–5]. Not all the results, however, are in agreement[1,2,6].

6-Hydroxydopamine depletes catecholamines and large doses cause degeneration of catecholamine-containing neurones in the brain and peripheral sympathetic nervous system[8–15]. Because 6-hydroxydopamine does not cross the blood–brain barrier it must be administered intrathecally to be effective in the brain. The decrease in content of noradrenaline and dopamine in the brain has been found to last for at least 20 weeks[12,13,16,17], and we now report that, during this period, D is significantly increased.

Male Sprague–Dawley rats (190–200 g) were injected intracisternally with 6-hydroxydopamine hydrobromide (Regis Chemical Corp.) or saline, containing 0.1% ascorbic acid which was used as a solvent for the 6-hydroxydopamine. (This solution was kept ice-cold and was bubbled with nitrogen to prevent oxidation of 6-hydroxydopamine.) Each experimental

Table 1 Effect of 6-Hydroxydopamine (6-OHDA) on the Noradrenaline Content of the Rat Brain

Time after 6-OHDA (weeks)	Control (Percentage of control mean ± s.e.m.)	6-OHDA	P
1	100±4.5 (N=6)	27±1.4 (N=6)	<0.001
21	100±4.0 (N=9)	41±5.0 (N=8)	<0.001

In animals killed 1 week after the intracisternal injection assays were performed on extracts of homogenates of whole brain. In these assays the mean concentration of noradrenaline in controls was 764±34 ng per brain. In animals killed 21 weeks after the intracisternal injection assays were performed on extracts of homogenates of various brain regions. The levels in the whole brain were obtained by summing the values for each brain part in each animal. The mean concentration of noradrenaline in the controls was 904±38 ng per brain. N, Number of animals.

rat received two injections of 6-hydroxydopamine; the first contained 200 µg of the free base in 25 µl. of the solvent; the second, administered 72 h later, contained 300 µg in 25 µl. of solvent. Control rats received intracisternal injections of solvent according to the same schedule. The technique of intracisternal injection is described elsewhere[18].

Noradrenaline was determined in whole brain or various regions of the brain by a modification of the method of Anton and Sayre[19]. Experimental and control rats of one group were killed 1 week after the last intracisternal injection and noradrenaline was determined in the whole brain. The other animals were killed 21 weeks after the last intracisternal injection (when all sleep recordings had been completed) and noradrenaline was measured in various brain regions. The content of noradrenaline was significantly lower in brain of animals treated with 6-hydroxydopamine than in the controls (Table 1). The decrease occurred in all brain regions examined: cerebellum, cortex, hypothalamus, pons-medulla, corpus striatum and the remainder of the brain. The greatest depletion was in the cerebellum and cortex.

One week after the administration of 6-hydroxydopamine the content of noradrenaline in the whole brain was 27% of the matched control value, and 21 weeks after treatment the content of noradrenaline was 41% of the matched control (Table 1). If this represents a true change with time, it might reflect a regeneration of some noradrenergic neurones or a compensatory increase in the content of noradrenaline in the remaining noradrenergic neurones in the brains of animals treated with 6-hydroxydopamine. But other investigators have found no evidence of restoration of noradrenaline content in brain during similar time intervals[16,17].

Two to three weeks after the intracisternal injections, eleven animals (seven experimental and four control) were implanted with cortical, hippocampal and nuchal muscle electrodes according to the usual techniques for sleep recordings in the rat[20,21]. During a period of 3 months starting 2 weeks after surgery, sleep patterns of each animal were recorded in specially constructed recording chambers. Each animal had three 8 h "adaptation" recordings and then a further series of 8 h recordings (beginning at 0930 h) at 1 week intervals (Table 2). Two experimental and two control animals were also recorded over 24 h. Subsequently, three of the experimental animals and four of the controls were deprived of D for 72 h on small islands surrounded by water[22] and were recorded during 24 h of "recovery" immediately after removal from the island. All records were scored for waking (W), synchronized sleep (S), and desynchronized sleep (D), according to previously described criteria[20,21].

Results of the 8 h recordings are given in Table 2. Values for W, S and D in the control animals in this study agree closely with our usual results in untreated rats. 6-Hydroxydopamine produced a significant increase in D. This increase was a result of a significant increase in the length of D-period and a slight increase in the total number of D-periods. The increased D-time was obtained at the expense of decreased waking; S-time was almost identical in the experimental and control animals.

Table 2 Effects of 6-Hydroxydopamine on Sleep in the Rat

Treatment	No. of animals	No. of 8 h recordings	W-time $\left(\frac{\text{min}}{\%}\right)$	S-time $\left(\frac{\text{min}}{\%}\right)$	D-time $\left(\frac{\text{min}}{\%}\right)$	No. of D-periods	Length of D (min)	No. of awakenings
Solvent	4	12	$\frac{167.1\pm17.5}{34.6\%\pm3.3\%}$	$\frac{266.0\pm10.4}{55.2\%\pm7.7\%}$	$\frac{48.4\pm6.4}{10.1\%\pm1.4\%}$	24.4±3.0	1.9±0.04	37.8±2.3
6-OHDA	7	19	$\frac{143.1\pm4.7}{29.8\%\pm1.0\%}$	$\frac{268.7\pm5.9}{55.8\%\pm1.2\%}$	$\frac{69.7\pm6.2\dagger}{14.5\%\pm1.0\%}$	29.7±1.3	2.4±0.1‡	45.5±2.2*

W, Waking; S, synchronized sleep; D, desynchronized sleep. Values are given as means±s.e. Values for W, S and D under the horizontal lines are percentages of total recording time±s.e.

* $P<0.05$; † $P<0.02$; ‡ $P<0.005$; t test, two-tailed.

The data were also examined for the changes which may have occurred over the duration of the study (3 months). The only change noted was a slight decrease in D over the several months of the study in both experimental and control animals. This is consistent with the effects of ageing.

The 24 h recordings after 72 h of D-deprivation were almost identical for W, S and D in experimental and control animals; since the absolute amount of D was approximately equal in the two groups, the increase above baseline values was therefore smaller in the experimental animals. In the control, but not in the experimental animals, the length of D-period was greater than the comparable values obtained in baseline 24 h recordings. Recovery of the experimental animals appeared to be complete in 8 h, whereas the control animals showed a sustained increase in levels of D throughout the 24 h.

Our findings confirm that intracisternally administered 6-hydroxydopamine produces a marked decrease in concentrations of noradrenaline in rat brain and that this persists for 21 weeks. The increased D after 6-hydroxydopamine is consistent with our findings of increased D in conditions characterized by decreased catecholamines in the brain[3,4,23]. It is possible, however, that factors other than the decreased catecholamines in the brain may contribute to the effects on sleep patterns. For example, 6-hydroxydopamine has been found to produce a short-term decrease in levels of serotonin in rat brain[11,12,15], and our preliminary findings suggest that

6-hydroxydopamine may also produce a long-lasting decrease in levels of serotonin particularly in the hypothalamus (unpublished data).

6-Hydroxydopamine seems to increase both number and length of D but there is a much more prominent effect on the latter, suggesting that the mechanism controlling the length of D may be particularly influenced by catecholamines. The fact that total S was almost exactly equal in the control and experimental animals suggests that it may not be affected by brain catecholamines. The findings discussed here, as well as our data on the effects of alpha-methylparatyrosine[3,4], suggest that the catecholamines particularly affect the relative amounts of time spent in W and D. This would be consistent with the finding that agents such as amphetamines or the monoamine oxidase inhibitors, which increase concentrations or availability of catecholamines, have been found to produce decreased D and increased waking in several species[24–26]. The results are also consistent with the hypothesis that a biological function of D may be to increase the synthesis or availability of catecholamines or to increase sensitivity of catecholamine receptors[5]. This in turn may have a role in maintaining wakefulness or certain aspects of wakefulness[27].

This work was supported in part by grants from the US Public Health Service.

ERNEST HARTMANN
RICHARD CHUNG

Sleep and Dream Laboratory,
591 *Morton Street,*
Boston, Massachusetts 02124,
and Department of Psychiatry,
Tufts University School of Medicine,
Boston, Massachusetts

PAUL R. DRASKOCZY
JOSEPH J. SCHILDKRAUT

Neuropsychopharmacology Laboratory,
Massachusetts Mental Health Center,
Department of Psychiatry,
Harvard Medical School,
Boston, Massachusetts 02115

[1] Jouvet, M., *Science*, **163**, 32 (1969).
[2] Jouvet, M., *Res. Publ. Ass. Res. Nerv. Ment. Dis.*, **45**, 86 (1967).
[3] Hartmann, E., and Bridwell, T. J. (Report to the Association for the Psychophysiological Study of Sleep, Santa Fe, March 1970), Abst., *Psychophysiology* (in the press).
[4] Hartmann, E., Bridwell, T. J., and Schildkraut, J. J. (Report to the Association for the Psychophysiological Study of Sleep, Santa Fe, March 1970), Abst., *Psychophysiology* (in the press).
[5] Hartmann, E., *Intern. Psychiatry Clinics*, **7**, 308 (1970).
[6] Branchey, M., and Kissin, B., *Psychonom. Sci.*, **19**, 281 (1970).
[7] Marantz, R., and Rechtschaffen, A., *Percept. Motor Skills*, **25**, 805 (1967).
[8] Thoenen, H., and Tranzer, J. P., *Arch. Pharmakol. Exp. Pathol.*, **261**, 271 (1968).
[9] Tranzer, J. P., and Thoenen, H., *Experientia*, **24**, 155 (1968).
[10] Uretsky, N. J., and Iversen, L. L., *Nature*, **221**, 557 (1969).
[11] Bloom, F. E., Algeri, S., Groppetti, A., Revuelta, A., and Costa, A., *Science*, **166**, 1284 (1969).
[12] Burkard, W. P., Jalfre, M., and Blum, J., *Experientia*, **25**, 1295 (1969).
[13] Breese, G. R., and Traylor, T. D., *J. Pharm. Exp. Ther.*, **174**, 413 (1970).
[14] Malmfors, T., and Sachs, C., *Europ. J. Pharmacol.*, **3**, 89 (1968).
[15] Bartholini, G., Richards, J. G., and Pletscher, A., *Experientia*, **26**, 142 (1970).
[16] Uretsky, N. J., and Iversen, L. L., *J. Neurochem.*, **17**, 269 (1970).
[17] Evetts, K. D., Uretsky, N. J., Iversen, L. L., and Iversen, S. D., *Nature*, **225**, 961 (1970).
[18] Schanberg, S. M., Schildkraut, J. J., and Kopin, I. J., *J. Pharmacol. Exp. Therap.*, **157**, 311 (1967).
[19] Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exp. Therap.*, **138**, 360 (1962).
[20] Roldan, E., Weiss, T., and Fifkova, E., *Electroenceph. Clin. Neurophysiol.*, **15**, 775 (1963).
[21] Michel, F., Klein, M., Jouvet, D., and Valatx, J. L., *CR Soc. Biol.*, **155**, 2389 (1963).
[22] Morden, B., Mitchell, G., and Dement, W., *Brain Res.*, **5**, 339 (1967).
[23] Hartmann, E., *Psychopharmacologia*, **9**, 242 (1966).
[24] Rechtschaffen, A., and Maron, L., *Electroenceph. Clin. Neurophysiol.*, **16**, 438 (1964).
[25] Jouvet, M., Vimont, P., and Delorme, F., *CR Soc. Biol.*, **159**, 1595 (1965).
[26] Cramer, H., and Kuhlo, W., *Acta Neurol. Psychiat. Belg.*, **67**, 658 (1967).
[27] Jones, B. E., thesis, Univ. Lyon (1969).

Trace Metal Variations in Sea Water of the Menai Straits caused by a Bloom of *Phaeocystis*

PLANKTONIC marine organisms often accumulate trace metals greatly in excess of their metabolic requirements and to concentrations 10^2–10^6 times those in sea water[1]. But variations in the concentration of trace metals in sea water are not usually affected unless there is a large production of plankton as there is in bloom conditions[2].

During June 1969, anomalous metal concentrations in the Menai Straits were recorded which could not be explained by variations in run-off supply from the surrounding land. The occurrence of a heavy bloom of the flagellate *Phaeocystis* at this time suggested a possible biological control which was investigated the following year. *Phaeocystis* blooms regularly in the Liverpool Bay region during late spring. At this time, the organism occurs partially in a colonial bladder form, some bladders reaching such a size (up to 4 mm in diameter) that they are readily visible to the naked eye[3].

Sea water samples for metal analysis were collected from Menai Bridge Pier at approximately 4 day intervals throughout the period of *Phaeocystis* occurrence from May 21 to July 18, 1970. Particulate matter was separated by filtration through a membrane filter and dissolved Zn, Cu and Mn were determined by X-ray fluorescence spectrometry[4] after a preliminary concentration by extraction on a chelating-resin[5]. Particulate metal concentrations were also determined. The membrane filters were digested in concentrated nitric acid and the digest was evaporated to dryness. The evaporate was subsequently absorbed into a standard weight of cellulose powder and elemental analyses were carried out by X-ray fluorescence spectrometry[4].

Although inorganic particulate matter also contributes trace metals, particulate concentrations were consistently low (<2 mg/l.) throughout the investigation and measurements both before and after gave particulate metal values of <2 μg/l. in this amount of material.

The total flagellate counts throughout the investigation are shown in Fig. 1. Although quantitative data on the relative amounts of *Phaeocystis* and other flagellate species were not recorded, microscopic examination showed that from June 2 to July 3 flagellate counts were composed almost entirely of *Phaeocystis*. Two peaks of abundance are clear, each representing 10^8 cells/l. at their maximum.

The dissolved and particulate Zn, Cu and Mn concentrations recorded during this study are illustrated in Fig. 2, from which it is apparent that the phytoplankton bloom affects these three metals in quite different ways. Both particulate and dissolved Zn concentrations increased when *Phaeocystis* was present, but for both the increase was of much greater magnitude during the second peak than during the first. But the values of dissolved Zn (up to 45 μg/l.) recorded at this time were so large in comparison with the values just before and just after the bloom that Zn must have been released from *Phaeocystis* during filtration. Rupture of the outer membrane of colonies during filtration has been demonstrated; it releases the mucilaginous fluid which separates individual cells within a colony. Zinc therefore seems to be concentrated in the jelly-like material within the outer membrane. The release of larger amounts during the second peak indicates that there is a greater degree of colonization and a larger amount of mucilaginous material at this time.

Although the observed dissolved concentrations of Cu are relatively constant, the particulate Cu shows a double peaked variation which corresponds well with the changes in the number of flagellate cells. Copper is apparently accumulated almost solely within the flagellate cell itself.

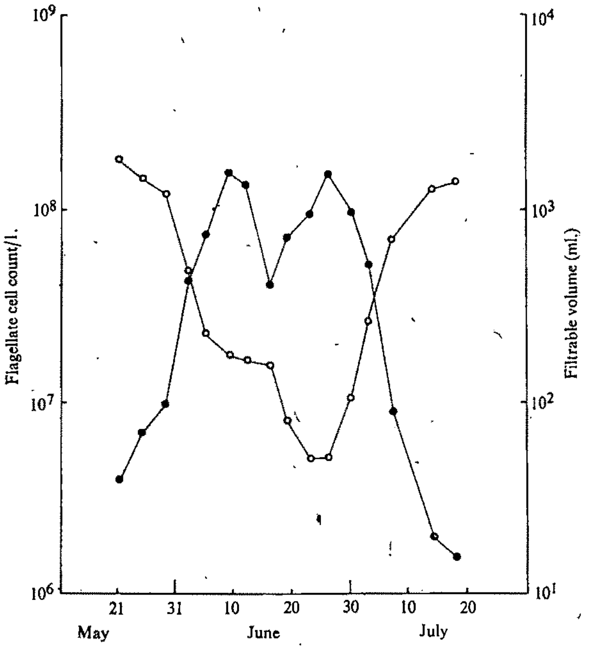


Fig. 1 Total flagellate cell count (●) at Menai Bridge Pier from May 21 to July 18, 1970. The peak values correspond to a bloom of the colonial flagellate *Phaeocystis*. From June 2 to July 3, the flagellates consisted almost entirely of this species. The other curve (○) represents the volume of water which could be filtered through a membrane filter, diameter 4 cm and pore size 0.45 µm, before it was completely blocked. The smaller volume obtained during the second peak in cell numbers indicates that there was a greater degree of colonization of *Phaeocystis* at that time.

Dissolved Mn has a minimum concentration which corresponds temporally to the second peak of *Phaeocystis*, and to a marked increase in particulate Mn. The particulate Mn concentration is very low during the first *Phaeocystis* peak but remains high after the rapid fall in cell numbers following the second peak. Such a pattern is explained best by a mechanism involving surface adsorption of Mn on the outer membrane of the colonies, the persistent high particulate Mn at the end of the investigation being the result of continued association of Mn with the disintegrating bladders. Such a mechanism again requires a greater degree of colonization during the second peak in flagellate cell numbers.

In spite of almost identical maxima in cell numbers for each of the two peaks of *Phaeocystis*, there is a clear difference in their affinity for Zn and Mn. The proposed explanations for these phenomena require a greater degree of colonization during the second peak. Changes in the filtration characteristics of the water during the investigation indicate that this was so.

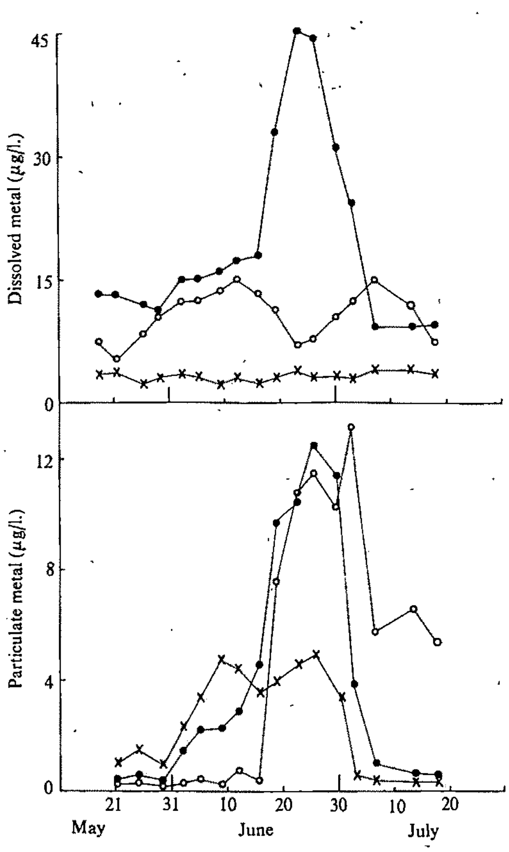


Fig. 2 Changes in concentration of the dissolved (upper curves) and particulate (lower curves) trace metals Zn (●), Cu (×) and Mn (○) in the sea water at Menai Bridge Pier from May 21 to July 18, 1970.

The rate of filtration of sea water containing *Phaeocystis* bladders is reduced rapidly as the bladder membranes spread over and block the filter surface. The remarkable plasticity of *Phaeocystis* bladders has been reported before[6]. As well as flagellate cell counts, Fig. 1 shows the volume of water which could be filtered through a single membrane filter (4 cm diameter) before the rate of filtration was reduced almost to zero; smaller volumes are indicative of the presence of a greater amount of membrane. Clearly the second peak represents a greater degree of colonization and thus a greater amount of bladder membrane and internal mucilaginous fluid.

Although the explanations of the causes of the variations reported here are only tentative, such marked changes can only derive from quite different interactions between *Phaeocystis* and the three metals. Clearly attempts to explain the accumulation of trace metals by marine organisms on the basis of the general chemical properties of the metals, such as the "Irving-Williams order", are not applicable to this species.

This work was partly supported by an NERC research grant. I thank Mr P. Tyler for flagellate counts.

A. W. MORRIS

Marine Science Laboratories,
Menai Bridge,
Anglesey

Received October 27, 1970; revised March 24, 1971.

[1] Goldberg, E. D., in *Chemical Oceanography* (edit. by Riley, J. P., and Skirrow, G.), **1**, 163 (Academic Press, London, 1965).
[2] Spencer, D. W., and Brewer, P. G., *Geochim. Cosmochim. Acta*, **33**, 325 (1969).
[3] Jones, P. G. W., and Haq, S. M. J., *Cons. Intern. Explor. Mer.*, **28**, 8 (1963).
[4] Morris, A. W., *Anal. Chim. Acta*, **42**, 397 (1968).
[5] Riley, J. P., and Taylor, D., *Anal. Chim. Acta*, **40**, 479 (1968).
[6] Kornmann, P., *Helgol. Wiss. Meeresunters.*, **5**, 218 (1955).

BOOK REVIEWS

Flying into Yesterday

Flights into Yesterday: The Story of Aerial Archaeology. By Leo Deuel. Pp. xvii+302+36 plates. (Macdonald: London, 1971.) £5.

THIS book is the first popular account of archaeological aerial photography to be written from a worldwide viewpoint: as such it is entirely successful. Dr Deuel sets out to tell the whole fascinating story of the development of aerial photography beginning with the exploits of the French balloonist, Nadar, over Paris in 1858 and ending with a look into the future. It was immediately before the First World War that the archaeological potential of the technique began to be realized but it was the war itself which was responsible for training a number of pilots in what was still a novel and largely unexplored approach to area surveying. Some of these men, returning after hostilities had ceased, were not slow to put their new found skills to archaeological use—men like Crawford in Britain, Wiegard working in Gaza and the Negeb, and Beazeley in Mesopotamia—these were the pioneers. But for sheer courage and vision few can compare with the exploits of Father Poidebard, a French Jesuit priest, who flew the Syrian Desert between 1924 and 1932 and published his results in a monumental work, *La trace de Rome dans le désert de Syrie,* two years later. This book is a model of its kind, showing with great clarity how an entire cultural landscape can be built up, painstakingly, from the air. Nor was Poidebard averse to landing his flimsy craft in the desert and taking out his spade to become a more conventional type of archaeologist.

All of these exploits Deuel lovingly describes, gradually extending the story to include chapters on more recent work in Italy and providing a substantial section on the fascinating and little appreciated aerial archaeology of America which owed much of its early impetus to the expeditions mounted by the famous pioneer flier Lindenbergh. It is this American section which will be the most unfamiliar to British archaeologists, and will help to redress the Old World-centred view of aerial photography.

The author pays a just tribute to British archaeologists like O. G. S. Crawford, Major Allen and Dr St Joseph. Nowadays, with archaeological sites being destroyed without record at an alarmingly accelerating rate, it is little short of a national disgrace that Britain has no adequate centralized, government-sponsored institute responsible for archaeological aerial recording. At present, Dr St Joseph's Committee for Aerial Photography at Cambridge and the valiant efforts of a seriously understaffed National Monuments Record, helped by the activities of amateur archaeologists, are all we can boast. Let us hope that Dr Deuel's book will be read by a few of our masters and shame them into a more responsible attitude towards Britain's rapidly disappearing heritage.

This book is clearly aimed at the general reader but its thorough treatment and broad approach will commend it to the specialist even though the writing is at times somewhat over expansive. B. W. CUNLIFFE

Theory in Science

Science as Metaphor: The Historical Role of Scientific Theories in Forming Western Culture. Edited by Richard Olson. Pp. xi+321. (Wadsworth: Belmont, California, 1971.) n.p.

IN recent years a number of attempts have been made to provide students of the history and philosophy of science with selections of readings from both primary and secondary sources. Some of these editions have been ill-conceived in both scope and depth, lacking unity of purpose, while exhibiting a poor selection of material, in spite, if not because, of their commercial possibilities.

The volume under present consideration, however, is to be recommended not only for the editor's intelligent choice of material but also because the scope of the work has been restricted to one of the most interesting and complex, yet poorly documented, aspects of the history of science. The central theme of this book is the influence of scientific theories on various other—often "extra-scientific" — disciplines. Several major scientific movements are considered and in each case readings are given to illustrate how parallel theories have been applied to such fields as law, theology, literature, social theory and so on. In discussing the influence of science on society, most writers take Social Darwinism as their paradigm, whereas this book is much wider and more exciting in its scope. The editor also avoids the pitfall of conflating technology with science.

Eight general subject areas are considered—the change in perspective accompanying the scientific revolution, the mechanical philosophy, Newtonian philosophy, Darwinism, energetics, Freudian theory (a valuable inclusion), positivism and behaviourism, and twentieth century physics. In the introduction to each section the editor presents a perspective of the subject under discussion. The readings themselves, varying in number from two to four, are reprints of short but substantial articles, often *in toto,* with footnotes omitted. The diversity of this selection is a further recommendation for the book, for example, Robert Lenoble's interesting piece on Mersenne's view of God as the Divine Engineer, Emile Zola's theory of the experimental novel, and an article on historical relativity. On the whole this is an admirable selection showing not only how scientific theories and methodologies have been applied in other contexts, but also how they have been misapplied.

The editor has contributed three original essays to this volume. In an introductory essay he stresses that scientific theories have an important place in the development of Western culture—Snow and Leavis please note. He then suggests it is by analogy that scientific theories are adopted for "extra-scientific" use. This discussion of the role of analogy is rather weak compared with the forceful views on methodology stated in some of the subsequent articles. The question of what may legitimately be viewed as the direct influence of science on other disciplines has not yet been satisfactorily answered. This important link is a methodological one, and something stronger than analogy is called for.

The volume concludes with a useful bibliographical essay which is preceded by the editor's analysis of what he considers to be a contemporary cultural lacuna, the reaction of certain "radical" groups against science. This is a fairly sympathetic although limited account of the "alienation" accompanying modern science.

In the preface Dr Olson states that the idea for the book grew out of an interdisciplinary course taught by himself and several other specialists at Crown College in California. This book should help the (intelligent) student, together with the scientist and the "literary intellectual" (to use Snow's pompous term), to appreciate not only that science is an integral part of our culture but also that scientific theories and scientific methodology have profoundly influenced other fields of human endeavour. G. N. CANTOR

Mathematical Past

The Development of the Foundations of Mathematical Analysis from Euler to Riemann. By I. Grattan-Guinness. Pp. xiii+186. (MIT: London and Cambridge, Massachusetts, February 1971.) £4.65.

THE place of history in mathematical education is controversial, and opinions differ widely. There is always a movement among students—with which, it would seem, the publishers of this work sympathize strongly—to demand a more historical treatment of their mathematics. This applies particularly to the elements of analysis which they tend to find difficult and resent having to learn anyway. My former colleague, Dr S. R. Tims, who was an outstandingly brilliant teacher of this subject, always used to claim that it was a mistake to teach the history of it, as students could find plenty of errors for themselves without putting ideas into their heads! I certainly look forward to confronting students with this book and seeing whether they really feel that it is more interesting (or even easier) than taking the subject straight.

The book places the development of nineteenth century analytical ideas in the context of the problems, starting with the vibrating string, in which they arose. Not surprisingly the central character of the book is Cauchy, whom the author effectively portrays in a not altogether amiable light. There is a deal of useful background information which enables the uninitiated reader to follow a lot of contemporary infighting which could otherwise be unappreciated. Perhaps the most important historical point made is that Cauchy had benefited (without a hint of direct acknowledgment) from a study of the writings of Bolzano. This assertion shows up neatly the nature of the difference between mathematical and historical proof, but clearly Dr Grattan-Guinness has made out a highly plausible case which could not be questioned by anybody less deeply immersed in the sources.

The author has clearly benefited from much recent historical work but it is perhaps a pity that by confining himself to a historical period, rather than being guided by the mathematical ideas, he is prevented from discussing the work of Abraham Robinson on non-standard analysis. Robinson's justification of the use of infinitesimals will certainly have to be taken into account in the definitive treatment of the ideas under discussion here, and yet this account, when it is eventually produced, will have again to go over a lot of the ground covered in this volume.

In spite of the claims on the dust cover the book appears to be intended by the author primarily for the benefit of his fellow historians. The historian's mastery of, and need to identify, his sources (as demonstrated by the copious footnotes) is to some extent at variance with providing a narrative which is easy to read. I wonder if the author has fallen stylistically between two stools. The presentation of the mathematical arguments (even when all allowances are made for the need to convey the flavour of past errors) seems somewhat uncomfortable for students of mathematics, and it is not clear that it will be found more helpful in putting the technical ideas across to possible historical readers with comparatively slight mathematical backgrounds.

D. B. SCOTT

Statistical Physics

Statistical Physics. By F. Mandl. Pp. xiii+379. (Wiley: London and New York, July 1971.) £2.75.

Statistical Physics. By A. Isihara. Pp. xv+439. (Academic: New York and London, June 1971.) $18.50; £8.65.

ALTHOUGH the two books reviewed here have the same title, they belong to two completely different worlds: Mandl's book is written for undergraduates and is clearly a textbook, while Isihara's book is clearly a reference book, even though the publishers in their blurb suggest that it might be adopted as the required text for certain advanced courses. Mandl's book covers both thermodynamics and statistical mechanics, while Isihara's book deals only with statistical mechanics; Mandl's book has a restricted coverage, while Isihara seems to include just about everything; finally, Mandl's book is cheap by present-day standards—and, I feel, excellent value for money—while Isihara's book is rather expensive and not such good value for money.

Mandl's book is a volume in the Manchester Physics Series which provides a set of textbooks for undergraduate degree courses in physics. In common with many modern books on the subject, statistical mechanics and thermodynamics are developed at the same time. This makes it necessary to stick to basic principles and to a restricted number of applications. One can hardly quarrel with the choice of material: I found in this book practically all the topics which I would like my own undergraduate pupils to know. As one has come to expect from the author, the book is carefully written and all steps are carefully and clearly presented. I shall certainly recommend this book to my pupils and see to it that it will be in my college library.

Mandl's book starts with the first two laws of thermodynamics, followed by a chapter on paramagnetism, including a section on negative temperatures. A second chapter on the second law includes a section on the third law, and is followed by a chapter on simple thermodynamic systems, and one on specific heats of solids. The next chapter is devoted to the perfect classical gas. A chapter on phase equilibria follows, and the book is concluded with chapters on perfect quantal gases, black body radiation, and systems with variable particle numbers. There are plenty of problems and hints for solving them. As every reviewer should find at least one point of disagreement, I shall mention the fact that the discussion of the relation between spin and statistics for composite particles is not quite correct and does not pay proper attention to the complications of the situation.

I cannot be so laudatory about Isihara's book. He has made important contributions to the subject, and many parts of the book are based on his research. This has as an important result that the book is foremost a research monograph, rather than a textbook. While I shall be glad to have it on my own bookshelves, I would not have bought it, as the contents to a very large extent can be found in research papers in the scientific literature—and more or less in the same form as they are presented here. I feel that the only people who will benefit from the book are those research workers who will themselves work in the fields covered by the author. In my opinion, the author would have produced a better textbook by restricting its scope and covering some topics in more depth and breadth: there are 132 sections in just over 400 pages, which means that the average length of a section is a mere three pages. The fifteen chapters in the book cover, respectively, kinetic theory, including elementary transport theory, principles of statistical mechanics, including ergodic and H-theorems, partition functions, including fluctuations, ideal bosons and fermions, including a very brief discussion of parastatistics and the de Haas–van Alphen effect, the linked cluster expansion, distribution functions, including hypernetted, Percus–Yevick, and Born–Green theories, Brownian motion, lattice statistics, including "melting" of DNA, phenomena near the critical temperature, including a one-page discussion of critical exponents, propagator methods for partition functions, propagator methods for distribution functions, transport phenomena in degenerate systems, including the Kondo effect, irreversibility and transport coefficients, including the master equation, second quantization, including spin and statistics, and Green functions, including superconductivity. As a result of the breakneck speed, the reader gets lost: to mention one instance, the author mentions that Fermi liquids should have a linear temperature dependence, but nowhere makes it clear why the quasi-particles are still fermions, or why the procedure for calculating the specific heat is the same as for a perfect gas and what is the meaning of the effective mass. Another objection I have against the book is the sloppy treatment of names and references: Birkoff instead of Birkhoff, Kammerlingh Onnes as well as Kamerlingh Onnes, Nozier or Nozière, instead of Nozières, Schöfield instead of Schofield, Soviet Phys.-JETP instead of Zh.Eksp.Teor.Fiz.

D. ter Haar

Interference and Waves

Interference of Electromagnetic Waves. By A. H. Cook. (International Series of Monographs on Physics.) Pp. viii+253. (Clarendon: Oxford; Oxford University: London, June 1971.) £5.

Interferometry is a discipline which, especially during the past two decades, has intruded more and more into science and engineering. Its classical beginnings were in the visible region of the spectrum, but it has spread over the whole electromagnetic gamut from X-rays to radio waves; it has even sidestepped into de Broglie waves.

Dr Cook's book declares its interest in the electromagnetic spectrum. He sets out, as he says in the preface, ". . . to deal with selected topics in the interference of electromagnetic radiation in a fundamental way while yet introducing them in an elementary manner". His choice of topics is, not unnaturally, coloured to some extent by his own experience. While in the Standards Division of the National Physical Laboratory (ultimately as its superintendent) he was concerned with extremely precise measurements which included the determination of the density of mercury, the measurement of the acceleration due to gravity and the establishment of the length of the standard yard in terms of the orange wavelength of krypton. In all of these interferometry was used as the method of choice for determining lengths with the utmost accuracy. Inevitably many exponents of interferometry could bridle at the fact that their own specialities have been left out. But, in the samples he has selected, Dr Cook has discussed the underlying principles with sufficient thoroughness to give the reader a clear notion of what goes on in virtually any interferometer. This he has done with the aid of mathematics which are merciful compared with those found in some other books on the same subject.

After introducing the concept of coherence, he devotes much of the book to the Michelson, Fizeau and Fabry–Perot interferometers and some of their applications. These include X-ray and microwave interferometry. Fourier transform spectroscopy is covered in this part of the book, as is the birefringence filter. A very useful chapter on the general theory of spectrometers is given.

The theory of "angular interferometers", covering Michelson's stellar interferometer and its radio counterpart, is presented in a very interesting chapter. It is a pity that, here, Homer has nodded; Dr Cook implies that the spacing of the fringes in the focal plane can be calculated from the separation of the outside mirrors and the focal length. This can only be done in "star-space"; in fact, the fringe spacing as calculated is wholly unrealizable. (I was "hung up" over this matter many years ago.) The difficulty with the early instruments at Mount Wilson was not the fineness of the fringes but their motion due to the "seeing"; Gebbie and Twiss sought to remedy this, to a first order, by an ingenious servo mechanism.

A short chapter on beats between independent sources reminds the reader firmly that times have changed since R. W. Wood wrote. Finally, the topic of intensity interferometers is discussed, another field of which the title seems at first sight to be a contradiction in terms. Unhappily, the curious jinx on the "angular interferometer" appears again; by an obvious slip of the pen "path difference" is used for "base-line". But, if the reader has absorbed Dr Cook's exposition of basic principles, he should, having read this far in the book, be in no danger of being misled.

These few quibbles—together with a few misprints—detract only negligibly from the value of a book written with clarity and authority. It will be a most useful introduction to a subject about which increasing numbers of scientists need to know.

J. Dyson

Palaeoecology Introduced

An Introduction to Quantitative Paleoecology. By R. A. Reyment. Pp. xii+226. (Elsevier: Amsterdam, London and New York, 1971.) n.p.

It is a pleasure to read such a clearly written and disarmingly simple little book whose contents quite belie the somewhat arid title. "How to enjoy using fossils and figures" might have been more apt as a description. The book is a short and very personal account of the way in which one palaeoecologist looks at his subject. He starts by introducing straightforward statistical concepts and then writes about such topics as the orientation of shells, the ways in which the environment can affect the size and shape of animals, the identification of predators, prey and population dynamics, the distribution of organisms in space, and some of the ways in which fossil assemblages can be analysed using statistical methods.

Part of the charm of the book lies in the twenty-eight case histories. In each example the problem is described, a model proposed, a statistical method and calculation provided, and the results discussed. Most of the examples are directly or indirectly the outcome of the author's own research and tend therefore to rely on ostracods and molluscs and be more concerned with younger than with older rocks. The last forty pages of the book give statistical tables, some short Fortran programs, references and an adequate index.

It does not really matter that the animal kingdom and the geological column have been selectively sampled. More than twenty of the more common statistical methods of particular interest to palaeoecology are described—more than sufficient for many of the present practitioners of the art. The author asks the question in the preface, "Am I kidding myself or is this result, or these data, really genuine? I have tried to do the same thing myself in the following pages but it is not always easy." It is pleasant to record that I believe that Professor Reyment has been successful not only with his data but with his approach. The book should appeal to a wide range of advanced students and thoroughly deserves to be successful.

G. Y. Craig

Rubber Text

Rubber Technology and Manufacture. Edited by C. M. Blow. Pp. xxii+527. (Butterworth: London, May 1971.) £9.

The first impression of a book which purports to cover the whole of rubber technology in just over 500 pages is inevitably one of superficiality. The wording on the dust cover implies that this book is "a definitive volume covering rubber technology and manufacture". The foreword says that it is intended (among other things) as a "guide" for undergraduates reading for the AIRI and for degrees in polymer science or technology. The latter is certainly a more realistic assessment, and in consequence some readers may feel that £9 is an excessive price to pay for what is merely an introduction to the subject. A selection of leading references is given for those who wish to follow up the various topics in greater detail, and this feature enhances the value of the book very considerably.

Most of the principal areas of rubber technology are dealt with, but rarely is space available to permit treatment in any depth. According to the foreword, the brief prepared by the editor stated that "the book is intended for graduates in chemistry, physics and engineering wishing to acquire a sound practical knowledge of rubber technology with emphasis on the processing, compounding and manufacturing of rubber products rather than on the chemistry and physics of rubbers, although the basic facts and theories of the latter must be adequately, though briefly, covered". In general, it seems that the objectives of this brief have been satisfactorily met. Subjects which have been deliberately omitted from the book include lattices, ebonite and adhesives. Engineering applications of elastomers have deliberately received "scant" treatment only, on the grounds that many conferences have been held and several books published on this subject in recent years.

The subject unfolds in a logical manner. Introductory chapters deal with the history of the rubber industry, an outline of rubber technology, and rubber physics. Then come chapters on raw polymers, vulcanization, compounding materials, reinforcement, machinery and production technology, compounding and manufacturing techniques. The book concludes with chapters on testing procedures and standards, and professional, trade, research and standards organizations.

Being the work of thirty-two separate authors, the book inevitably has some repetition and some unevenness of treatment. A stronger editorial hand could undoubtedly have been wielded. Thus, for example, the important phenomenon of swelling of elastomers is dealt with separately in several different places, and in no case is the treatment satisfactory. Furthermore, in discussing the determination of crosslink density by swelling, no mention is made of the important method of swollen compression modulus (which provides an effective procedure for eliminating the Flory χ-factor). Again, plastimeters are dealt with in two separate chapters, and space wasted through overlap could have been put to better use in amplifying some of the issues raised.

D. C. BLACKLEY

Safety in Numbers

Physical Biochemistry. By Edward Kensal Van Holde. Pp. ix+246. (Prentice Hall: Englewood Cliffs, N.J., July 1971.) $9.75 cloth. $4 paper.

THE achievements of molecular biology during the past two decades are too well known to need enumeration; it may well be asked, however, what contribution the physical chemist has made to all the excitement. Faced with the Augean stables of biological systems, the molecular biologist has been presented first with the problem of separating and assaying minute amounts of chemical species differing in but subtle ways. The solution of these problems would have been unimaginable without the efforts of a whole generation of physical chemists. But if you scratch a physical biochemist you are likely to find a polymer chemist or spectroscopist beneath the skin. Such preoccupations have of course led to many notable advances, but all too often the physical biochemist has left the dangerous waters of real biological systems to measure the size and shape and properties of proteins and nucleic acids, uneasily steering a course between the shoals of biological irrelevancy and sheer unanalysable complexity.

This brief and unpedantic book leads the embryo physical biochemist down similar traditional byways. After a quick review of classical thermodynamics it provides some insights into the implications of the Boltzmann distribution and the second law, an introduction to solution thermodynamics and a page or two on the nature of macromolecules. After this prolegomenon the author discusses chemical equilibria with a useful section on multiple equilibria and a mention of cooperative helix coil transitions. Then the traditional preoccupations return with a concern for the theory and practice of various methods of investigating the size and structure of macromolecules. Thus the last two sections deal with transport processes and with the interaction of radiation with biological matter. In passing, the principles behind various separation methods are discussed.

There is, oddly, little on the polyelectrolyte nature of biological macromolecules and nothing on surface chemistry or kinetics. The reader should know something about proteins and nucleic acids, but in case he doesn't, he is frequently referred to other volumes in this series (Foundations of Modern Biochemistry).

The style is lucid and informal with the occasional well chosen metaphor, but the high flown phrase is eschewed. The text is illustrated with pictures of pretty girls standing by pieces of apparatus and clear and elegant diagrams in green and grey. The reader should know about integral signs and partial differentials but may rest assured that, when the going gets too tough, rigorous analysis is neatly evaded by reference to higher texts. In the final section, quantum mechanics hovers in the background, letting drop the occasional arcane pronouncement. The text is not overburdened with references but there is a useful reading list at the end of each chapter together with a selection of numerical problems—without answers. I noticed a few misprints but no serious errors.

The biochemist endeavouring to understand the physical methods he encounters in his reading will find this book useful and it may be recommended to final year biochemistry students. The serious physical chemist will, however, prefer a more solid text.

E. G. RICHARDS

Chemistry Evolution

Biochemical Coevolution. By Kenton L. Chambers. (Proceedings of the Twenty-ninth Annual Biology Colloquium, April 26–27, 1968.) Pp. x+117. (Oregon State University: Corvallis, December 1970.) $5.

IN a respectable enthusiasm for the study of biochemical mechanisms, from the replication of DNA to the contraction of muscle, biologists have recently tended to neglect the precise and marvellous manifestations of evolutionary adaptation. It is therefore refreshing to find a book that points the way to studying these phenomena at the biochemical level. The book does not do more than point the way, and nobody should be misled by the title into supposing that its biochemical content is very great. Nevertheless it is a tantalizing introduction to a subject that must surely become more important during the next decade.

The book reports the proceedings of a symposium, and in common with others it harbours a motley collection of papers. In the first essay, P. R. Ehrlich manfully and almost successfully sets out to develop a theoretical framework on which the remainder can be hung. He has interesting things to say about the evolutionary interactions between plants and herbivores, predators and prey, parasites and hosts, flowers and pollinators. The second essay, by C. H. Muller, is a fascinating account of the airborne and waterborne toxins by means of which some plants inhibit the nearby growth of others. S. J. Karakashian writes the inconclusive history of the relations between a symbiotic alga, *Chlorella,* and its invertebrate "hosts". L. P. Brower, in the most thorough and closely reasoned contribution, summarizes his studies on the assimilation by Danaine butterflies of cardiac poisons from their food plants, and explores the effects of this assimilation on the interactions between the butterflies and their predators. Finally, C. H. Dodson describes his elegant work on the nature and species-specificity of the volatile substances by which orchids attract their insect pollinators.

In 1949, with characteristic prescience, J. B. S. Haldane drew attention to the unexplored territories of biochemical coevolution. More recently, the discoveries of enzyme polymorphism, and other aspects of protein evolution, have demanded a clearer knowledge of these territories. The studies reported in this book represent a few tentative forays, hopefully to be followed by more extensive colonization.

BRYAN CLARKE

CORRESPONDENCE

Doomsday Syndrome

SIR,—John Maddox's address to Section X of the British Association (*Nature*, 233, 15; 1971) shows him giving reasonable voice to the backlash against the "doomsday syndrome". He will find a warm welcome in many quarters for his suggestion that the whole thing has been a "wave of fashion". But there are some dangers in this particular role, and I would like to comment on them, in the light of my experience as the junior minister in the Labour government most concerned with environmental control.

Two phrases in the extract from Mr Maddox's address define his position. Talking about carbon dioxide in the atmosphere and the possible "greenhouse effect", he says: "In reality, nobody can be sure that the effect will be as predicted, and in any case the accumulation of carbon dioxide in the atmosphere is by no means the inexorable process that the doomsday men suggest. Other recent fears . . . are similarly unfounded."

Now to begin with, Mr Maddox implies that because nobody can be sure there will be a greenhouse effect, we ought all to forget about it. It seems to me that the contrary view is more rational, certainly more prudent; that we ought not to forget about it because nobody can be sure there will not be a greenhouse effect.

Nevertheless, Mr Maddox does not assert that the prediction is unfounded. But he does immediately go on to say that other fears are *similarly unfounded.* Into the logical gap between the statement of uncertainty and the statement of certainty a host of special interests will leap. Some of the oil firms are currently engaged in just this bad logic over lead in petrol; their scientific spokesmen are virtually saying that because there are holes in the anti-lead argument, therefore lead does no harm. *Non sequitur*.

When we were assembling the information which enabled Harold Wilson to appoint the new Secretary of State and the Royal Commission on the Environment and the Holdgate Unit, I found this attitude was widely held and powerfully defended by the more hidebound civil servants, both scientific and administrative. It is still *very* well represented in Whitehall, particularly in the more production-oriented sections such as the Ministry of Agriculture, the Department of Trade and Industry, and the former Ministry of Transport.

Another quarter where Mr Maddox's stand will be welcomed is among the already very powerful and well organized commercial interests whose testing and publication policy, as *Nature* has pointed out editorially before now, often leaves something to be desired.

To be sure the doomsday men and their disciples are overstating their cases; but this does not mean it is time for a general backtrack on the public consciousness and the legal and administrative measures of the last few years. The slogan "guilty until proved innocent" is no doubt a perfectionist one about new substances coming into use. But I would far sooner those in authority had it at the back of their minds than Mr Maddox's "other recent fears are similarly unfounded". It is the sounder slogan not only on ecological grounds, but on economic ones too: the true costs of a product, including certain, probable, and possible social costs, should be known before the product is marketed, so that they can be included in the price.

Yours faithfully,

WAYLAND KENNET

House of Lords

Working Europeans

SIR,—While I have full sympathy with the tenor of your editorial (*Nature*, 233, 152; 1971), I should like to suggest that a good deal more could be done through efforts of individual scientists in responsible positions, such as heads of departments or institutes. It is not really necessary to wait patiently till official agencies produce collaboration for us like a rabbit out of a hat. "When," you ask, "will the British Government agree that the Agricultural Research Council . . . should employ scientists from France or the Netherlands?" There is nothing now to stop them employing foreign nationals as research assistants at quite high salaries. In my department we had for some years a Swiss biologist whose salary was paid by the ARC, and we now have an Italian whose salary comes from the SRC. Both of these were on relatively short-term grants lasting only a few years, but we also have a Swiss citizen as a member of an MRC group, with a long term commitment. Again there is nothing to prevent integration at the level of university departments, at least at postgraduate level, where it is probably likely to be most useful. We have operated for the past few years an Anglo-Italian postgraduate course in epigenetics, with lecturing and laboratory work contributed jointly by our university staff and staff of the Laboratory for Molecular Embryology at Naples. The latter is funded by the Italian Council for Scientific Research, who have shown themselves very willing to meet the expenses of moving their teachers to Edinburgh and accommodating the students in Naples. The expenses on the British side, over and above the normal university contribution, are, it must be admitted, provided not by any governmental agency, but by the Leverhulme Trust. Such funds do, of course, have to be looked for. But they exist, and collaboration, at this perhaps rather minor level, can be what the Americans would call a grass-roots operation.

Finally, is there anything, except timidity, which stands in the way of the suggestion, which I have been urging for some years, that the research councils should appoint, to their main working committee which vets grant applications, one or two non-British European scientists? Some of us who find ourselves faced with steering the projects of our staff through committees of their colleague-rivals would welcome the judgment of respected outside opinion; and Europeans privileged to serve on such committees would gain valuable experience of how the really rather effective British system works.

Yours faithfully,

C. H. WADDINGTON

Institute of Animal Genetics,
West Mains Road,
Edinburgh EH9 3JN

Research Associations

SIR,—I wholeheartedly endorse the letter of Mr Jobling (*Nature*, 231, 477; 1971). The problems of research associations are similar in all countries and the same errors are being made in many places. My experience suggests that it is completely wrong to want the RAs to be commercially viable, because the services they render are for the most part of a general character and the earnings following from these are mostly invisible. As a typical example I would single out the laundry and dry-cleaning industries, which comprise almost exclusively small or very small firms. What they need is independent information on new products and machines, relevant abstracts from the literature, control of their technical and commercial operations and statistics. Such services may be vital to a firm and save it from bankruptcy, but in the opinion of the manager £10 would be a quite sufficient fee.

Moreover, the existence of a sound laundry and dry-cleaning industry backed by appropriate RAs is also essential to the benefit of the consumer. Difficult though it may be to calculate this in hard cash, the benefit may well be immense. There are examples of great countries which could serve as a warning in this respect.

Lastly, in order to communicate efficiently with government services, with other industries and with international organizations, RAs are invaluable.

All this leads up to the following conclusions. RAs should have a stable income from some system of government imposed compulsory fees (such as in France) to finance their normal routine work. Research proper should be financed by individual firms, organizations or governmental departments, on a project basis, and it could best be done in association with a university or a very large institute. Rotation of personnel between research and routine duties would ensure flexibility, feedback and awareness of practical problems.

Yours faithfully,

S. V. VAECK

Chief of the Laboratory,
Ministry of Economic Affairs,
17*A rue de la Senne*, *Brussels* 1000

Bradford's Law

SIR,—In considering the application of Bradford's Law of Dispersion[1] as a guide to acquisition policy in the research library or information centre it is pleasant to contemplate a bibliophilic Utopia of a complete collection in a library with unlimited space and acquisition funds. Utopias are rarely found, however, and the library *does* have limited resources. Given this restriction, the librarian or acquisitions specialist, in even the largest and most pecunious libraries, must make choices. These choices are rational only to the extent that the library collection maximizes the timely provision of requested documents to the satisfaction of the largest number of users.

In this light, A. Faser's letter[2] suggesting that a library is derelict in not purchasing a specialized journal of interest to only one user treats the occasional request with the same degree of importance as the on-going demand for the heavily used journals. An inventory policy in a department or food store, part-supply depot, manufacturing concern or library, based on ignoring frequency-of-demand distributions, leads to inefficient allocation of resources. Designers of sewer and flood control systems know they cannot design economic drainpipe and culvert systems of sufficient capacity to handle the runoff from the one-in-a-thousand chance that rainfall will exceed, say, 6 inches in any 1 h period. And mass merchandisers stock only a few or no items in the extremely low and high size ranges of shoes, hats and all attire in between.

Bradford's Law promulgates that a library can supply *most* of the requests for material with a relatively modest inventory of book and journal titles, geared to the *normal* pattern of demand. This demand pattern is one in which a relatively few items from among all possible items in the inventory satisfy a majority of the actual transactions. Progressively fewer transactions are satisfied from the balance of the inventory, or from further augmentation of the number of titles held. Abiding by the Bradford distribution, then, is an important factor in the library's overall success at demand-fulfilment.

The most efficient way for a library to exploit its collection and maximize utilization of its document file is to share its bibliographic resources with as many patrons as possible. It cannot *reasonably* be expected to serve *every* individual request. Carried to the extreme, if the only requests were one-time requests, there could not be an *economic* central library. The most efficient way of handling such a situation would be for each individual to have his own private collection.

Yours faithfully,

MELVIN WEINSTOCK

Institute for Scientific Information,
325 *Chestnut Street,*
Philadelphia,
Pennsylvania 19106

---

[1] Fairthorne, A., *J. Doc.*, **25**, 319 (1969).

[2] Faser, A., *Nature*, **227**, 101 (1970).

Ageing Ova

SIR,—The recent article[1] published in your journal, showing increased chromatid disjunction in mouse eggs which were some time in the oviduct, is the latest in a line of papers all demonstrating that delayed fertilization can result in abnormal development of the embryo. The author points out that this could explain the increased frequency of trisomy among the offspring of older women. It should also be pointed out that the rhythm method of birth control might increase the possibility of fertilization occurring some time after ovulation, and thus also increase the chance of producing a malformed embryo. This is not entirely speculation as Iffy[2] found that eighteen out of twenty-one cases of abnormal embryonic development could be related to post-17th day ovulation and fertilization, while Cross[3] suggested that increased practice of the rhythm method might be responsible for the higher frequencies of anencephalus and spina bifida among Roman Catholics. The results of this latest paper[1] suggest that a more searching investigation into this possible danger is called for.

Yours faithfully,

W. A. F. WATSON

Department of Genetics,
2 *Tillydrone Avenue,*
*Aberdeen AB*9 2*TN*

---

[1] Rodman, T. C., *Nature*, **233**, 191 (1971).

[2] Iffy, L., *Proc. Roy. Soc. Med.*, **56**, 48 (1963).

[3] Cross, R. G., *Brit. Med. J.*, **1**, 660 (1968).

Suckling Etymology

SIR,—Dr Spratling bemoans the misuse of suck and suckle (*Nature*, **233**, 73; 1971). The confusion can be blamed on the medieval yeoman, rather than on *Nature*[1]. Suckling, like its German and Dutch parallels Saugling and suigeling (=baby), is a noun with the suffix -ling, as in weanling, yearling, duckling, gosling, and, more recently, ratling. The English yeoman assumed that suckling had the suffix -ing, as in paddling and speckling, and coined a new verb "suckle". He gave no thought to whether it meant anything other than suck. Shakespeare does indeed use suckle in the sense nurse or nourish at the breast. The Authorized Version prefers the older Germanic idiomic "give suck" (*cf* German saugen geben, Dutch zuigen geven). The *Shorter Oxford English Dictionary* traces the "misuse" of suckler to mean a suckling calf back to 1473, whereas "correct" use to mean an animal that is suckling young dates only from 1850. The difficulty now, and undoubtedly the origin of the modern "error", is that a baby pig still in its mother's tender care can be called either a suckling or a sucking pig. The term piglet is, of course, briefer; the British Society of Animal Production recommends its use for a pig up to about 8 weeks of age, irrespective of time of weaning[2]. I would recommend that the mother be called a nurse-sow or nursing sow. German Ammenkuh, literally "foster-cow", can be approximated to "multiple suckling" without defining whether the cow or calf is suckling.

Equally to be bemoaned is the imprecise use of "feed". Traditional is: The farmer feeds cows. Or the passive: Cows are fed. The meaning is not identical with: Cows eat. The *Shorter Oxford* gives two innovations from 1883 that have since become established. First: Cholera feeds on impurities . . . Bacteriologists might object to the word "eat" but surely for animal nutritionists "Cows eat hay" is far better than "Cows feed on hay". Second: Mangel-wurzel . . . is fed to the cows. . . . This type has become far too common, even being telescoped into: The farmer feeds cows mangel-wurzels. The meaning has been reduced to "give" or "supply". Even St Matthew is no longer sacrosanct: we may now feed pigs pearls[3].

Yours faithfully,

J. C. Rigg

Centre for Agricultural Publishing
and Documentation (Pudoc),
Wageningen,
The Netherlands

---

[1] Cowie, A. T., Folley, S. J., Corss, B. A., Harris, G. W., Jacobsohn, D., and Richardson, K. C., *Nature*, **168**, 421 (1951).

[2] British Society of Animal Production, *The Description of Farm Animals in Scientific Publications* (West of Scotland Agricultural College, Auchincruive, Ayr, 1967).

[3] Matthew, S., Chap. 7, v. 6 (n.d.).

Retino-rectal Connexions?

Sir,—I notice that there are at least two errors in your correspondent's report of the Glasgow embryological meeting (*Nature*, **233**, 232; 1971). The first is obviously a trivial deletion: "leg disks" and not "legs" were bisected. The second is more profound as it has the optic nerve connecting with the rectum. This rarely occurs in Amphibia, to which I think he was referring. When it does it leads to an annoying condition known as tunnel vision.

Yours faithfully,

Anthony Robertson

Department of Theoretical Biology,
939 E. 57th Street,
Chicago,
Illinois

Announcements

University News

The title of honorary professor of thoracic surgery in the **University of Birmingham** has been conferred on **Mr J. Leigh Collis.**

Professor J. O'M. Bockris, University of Pennsylvania, has been appointed to a chair of chemistry in the School of Physical Sciences, **Flinders University of South Australia.**

Professor F. G. Heath, University of Manchester Institute of Science and Technology, has been appointed to the new second chair in the Department of Electrical and Electronic Engineering at **Heriot-Watt University.**

The following appointments have been made in the **University of London: Professor W. Brumfitt,** to the chair of medical microbiology tenable at the Royal Free Hospital School of Medicine; **Dr C. A. C. Mims,** to the chair of microbiology tenable at Guy's Hospital Medical School; **Dr J. Pitt-Rivers,** to the chair of anthropology tenable at the London School of Economics; **Professor J. G. Taylor,** to the chair of mathematics tenable at King's College.

Mr J. S. Ellis, director of postgraduate studies for the Wessex Regional Hospital Board, has been appointed to the chair of orthopaedic and accident surgery in the **University of Southampton.**

Appointments

Dr I. Steele Russell has been appointed director of the **MRC Unit on Neural Mechanisms of Behaviour,** in succession to **Professor G. C. Drew.**

Professor R. Baskett has been appointed a member of the **Agricultural Advisory Council,** in succession to **Sir Gordon Cox.** Professor Baskett will also succeed Sir Gordon as secretary of the Agricultural Research Council.

Sir Alan Wilson has been appointed chairman of the board of governors of the **National Institute of Agricultural Engineering,** in succession to **Major-General Cyril Lloyd.**

Miscellaneous

Professor L. J. Bruce-Chwatt, London School of Hygiene and Tropical Medicine, and **Professor A. Corradetti,** Istituto Superiore di Sanita, Rome, have been awarded jointly the **Darling Medal and Prize of the World Health Organization,** for services to research on and control of malaria.

Professor Luigi Broglio, University of Rome, has received the **1971 Daniel and Florence Guggenheim International Astronautics Award.**

Two **A. E. Bennett research awards,** one in basic and one in clinical science, are offered by the **Society of Biological Psychiatry** for the purpose of stimulating research in biological psychiatry by young investigators. The awards, each worth $750, will be made for papers describing work currently in progress, which shows particular independence of thought and originality. Further information can be obtained from Dr Sabit Gabay, chairman of the Committee on Research Awards, Biochemistry Research Laboratory, Veterans Administration Hospital, Brockton, Massachusetts 02401, USA.

International Meetings

October 18, **Phloem Transport,** London (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1).

October 26–28, **Chemistry and Biological Activity of Cannabis,** Stockholm (Dr J. Lars G. Nilsson, Apotekarsocieteten, Stockholm, Sweden).

November 10, **Are Consumers Offered the Food Products They Want?,** London (Dr N. C. Robson, 5 Crest Road, Bromley, Kent BR2 7JA).

November 11–13, **Mammary Neoplasia,** Cherry Hill, NJ (Mrs Diane Meredith, Executive Secretary, Institute for Medical Research, Copewood Street, Camden, NJ 08103, USA).

December 3, **Alcohol in Nutrition,** London (Dr J. D. Sutton, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT).

British Diary

Tuesday, October 12

Communications in Control (5.30 p.m.) Dr R. H. Barker, Institution of Electrical Engineers, at Savoy Place, London WC2.

Pigmentation of Plastics (7.30 p.m.) Mr J. E. Todd, Oil and Colour Chemists' Association, at the Griffin Hotel, Boar Lane, Leeds.

The Interface Between Biological and Mechanical Systems (7.30 p.m.) Mr S. V. Hayes, Society of Environmental Engineers, at the University of Birmingham.

The Mechanism of Nerve Conduction (5.30 p.m.) Professor A. F. Huxley, FRS, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Sixth Form Pupils from Schools in London and the Home Counties. To be repeated on October 13, 18 and 19.)

Wednesday, October 13

A Blood Analyser Using the PDP-8/L (5.30 p.m.) Mr E. T. Oram, joint IEE/IERE Medical and Biological Electronics Group, at the Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

A Review of Printing Inks and Printing Processes (4.30 p.m.) Mr F. Lewis, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1. (Student Lecture.)

Comparison of PCM and FDM/FM Microwave Radio Relays (6.30 p.m.) Mr S. G. Allen, Institution of Electronic and Radio Engineers, at the Civic Centre, Chelmsford, Essex.

Conversations with Computers (7 p.m.) Dr C. R. Evans, Institution of Electrical Engineers, at the Culham Laboratory, Culham, Abingdon, Berks.

Cost Effectiveness and Profitability in the Paint Industry (2.15 p.m.) Mr D. E. Eddowes, Oil and Colour Chemists' Association, at the South Bank Polytechnic, Borough Road, London SE1.

On-Line Sampling and Analysis (5.30 p.m.) Institution of Chemical Engineers, Yorkshire Branch, at the College of Technology, Queens Gardens, Hull, Yorkshire.

The Role of Data Links in Air Traffic Control Systems (5.30 p.m.) Mr B. D. Parker, Institution of Electrical Engineers, at Savoy Place, London WC2.

Thursday, October 14

Computer Control (7.30 p.m.) Institution of Chemical Engineers, at the Grammar School, Brigg, Lincs.

Computers and the Professional Engineer (5.30 p.m.) Professor J. F. Coales, FRS, Institution of Electrical Engineers, at Savoy Place, London WC2.

Corrosion and the Automobile (7 p.m.) Mr H. L. Quick, Oil and Colour Chemists' Association, jointly with the Institute of Metal Finishing, at the British Rail School of Transport, London Road, Derby.

New Approaches to Safety (6 p.m.) Mr J. R. Melluish, Institution of Chemical Engineers, North Western Branch, at ICI Offices, The Heath, Runcorn, Cheshire.

Personalized Marketing (6 p.m.) Mr W. A. Coom, Oil and Colour Chemists' Association, at the St Enoch Hotel, Glasgow.

Problems of Diagnosis of Tumours of the Lung (5.45 p.m.); **Unilateral Exophthalmos** (7.15 p.m.) British Institute of Radiology, at 32 Welbeck Street, London W1.

Rapid Transport and Suburban Electric Railways (7.30 p.m.) Mr B. J. Prigmore, Institute of Electrical Engineers, at High Wycombe College of Technology and Art, High Wycombe, Bucks.

Friday, October 15

The Importance of Electrokinetics in Electrode-position (6.30 p.m.) Professor G. D. Parfitt, Oil and Colour Chemists' Association, at the Chamber of Commerce and Industry, 75 Harborne Road, Birmingham.

The Limitations of Error Detection Coding at High Error Rates (5.30 p.m.) Mr J. D. Ralphs, Institution of Electrical Engineers, at Savoy Place, London WC2.

What Shall We Make?: The Chemist's Problem in Drug Research (6.30 p.m.) Dr B. W. Langley, Society of Chemical Industry, Fine Chemicals Group, at 14 Belgrave Square, London SW1.

Saturday, October 16

Excavations at Swanscombe in 1970 and 1971 (3.30 p.m.) Dr John Waechter, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Monday, October 18

Syndicate Studies: an Assessment of Their Value in Undergraduate Courses (5.30 p.m.) Mr G. J. Edwards, Institution of Electrical Engineers, at Savoy Place, London WC2.

Reports and Publications

not included in the Monthly Books Supplement

Other Countries

Honouring Excellency: International Science Schools for High School Students. Pp. 88. (Sydney: The Science Foundation for Physics within the University of Sydney, 1971.) [76

Dreissigster Jahresbericht der Schweizerischen Gesellschaft für Vererbungsforschung, Société Suisse de Génétique (SSG) 1970, mit Unterstitzung der Julius Klaus-Stiftung für Vererbungsforschung Sozialanthropologie und Rassenhygiene in Zürich. Herausgegeben von Ernst Ochler. (Separatabdruck aus Archiv der Julius Klaus-Stiftung für Vererbungsforschung Sozialanthropologie und Rassenhygiene, Band XLV, 1970.) Pp. 111. (Zürich: Art. Institut Orell Füssli, AG., 1970.) [86

Mezquites y Huizaches: Algunos Aspectos de la Economia, Ecologia y Taxonomia de los Generos, *Prosopis* y *Acacia* en Mexico. Por F. G. Lorence, J. S. Poillon y M. del C. A. Moreiras. Pp. 192. Mesas Redondas Sobre Problemas de Ecologia Humana en la Cuenca del Valle de Mexico—Auditorio del Instituto Mexicano de Recursos Naturales Renovables 9 al 13 de Noveimbre de 1970. Pp. 206. (Mexico, DF: Ediciones del Instituto Mexicano de Recursos Naturales Renovables, A.C., 1970 y 1971.) [96

Arquivo do Instituto Gulbenkian de Ciencia. B: Estudos de Economia e Financas. Vol. 5, No. 1: On Reurns to Education. By M. D. F. Leite. The Taxation of Income from Land. By M. de Carvalho. Pp. 1–32. Vol. 5, No. 2: Demographic Forecasting Models for Rural-Urban Migration and Zipfian Distributions. By Roland Pettersson. Pp. 33–74. (Lisboa: Instituto Gulbenkian de Ciencia, Centro de Economia e Financas, 1970.) [96

Pesticide Biochemistry and Physiology, Vol. 1. No. 1 (March 1971). Edited by R. D. O'Brien, Pp. 1–122. Price for institutional subscribers $30. Price for private subscribers certifying that the journal is for personal use only $15. (New York and London: Academic Press, 1971.) [106

Smithsonian Contributions to Zoology. No. 69: Biostatistical Programs in BASIC Language for Time-Hired Computers: Coordinated with the Book "Quantitative Zoology". By James A. Peters. Pp. 46. $0.50. No. 62: A Revision of the Leptophlebiidae of the West Indies (Ephemeroptera). By William L. Peters. Pp. 48. $0.55. No. 79: Western Atlantic Shrimps of the Genus *Metapenaeopsis* (Crustacea, Decapoda, Penaeidae), with Descriptions of Three New Species. By Isabel P. Farfante. Pp. 37. $0.45. No. 88: Pogonophora of the Northwest Atlantic—Nova Scotia to Florida. By E. C. Southward. Pp. 29. $0.40. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [106

US Department of the Interior: Geological Survey. Bulletin 1211-D: Geologic Reconnaissance of a Possible Powersite at Takatz Creek, Southwestern Alaska. By James E. Callahan. Pp. iii+18+1 plate. Bulletin 1312-L: Geochemical and Geophysical Reconnaissance of Parts of the Yakutat and Mount Saint Elias Quadrangles, Alaska. By E. M. McKevett, Jr., and George Plafker. Pp. 12+1 plate. Professional Paper 562–1: Transport and Dispersion of Fluorescent Tracer Particles for the Flat-Bed Condition, Rio Grande Conveyance Channel, Nearer Bernardo, New Mexico. By R. E. Rathburn, V. C. Kennedy and J. K. Culbertson. Pp. vi+56. $0.65. Professional Paper 700A: Geological Survey Research 1970. Pp. viii+426. $4. (Washington, DC: Government Printing Office, 1970 and 1971.) [106

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A. & Canada
Nature (Friday)	£14	$48
Nature + Nature Physical Science	£24	$83
Nature + Nature New Biology	£24	$83
All three editions	£29.50	$108
Annual Index	£1	$3

* Rates for shorter periods *pro rata* (minimum three months)

(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 29242

American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004

All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, October 8 1971

NATURE Volume 233
OCTOBER 15 1971

DDT may be Good for People

DR N. E. BORLAUG is entirely right to protest, as he did in Washington last week, that too zealous a campaign to regulate the use of DDT, and in particular a ban on it, will do much more harm than good. He might have added that it is an immense irony that a comparatively simple chemical widely regarded twenty years ago as a great benefaction has almost become a symbol of all things fearsome. Part of the trouble is that DDT and the other organochlorine pesticides such as dieldrin and aldrin were the centrepiece of Miss Rachel Carson's book *Silent Spring*. In some atavistic way, however, pillorying this chemical serves to satisfy a great many vague but common fears—that governments are incompetent in regulating the uses of innovation, that greedy manufacturers are urging farmers to use more of these materials than is strictly necessary, thus demonstrating yet another way in which the search for private profit will undermine society, and also, given the apparent ubiquity of DDT in the environment, that it is never possible in the modern world to escape from the secret threat of technology, the centre of fashionable paranoia. It is regrettable that in the past few years, professional scientists have done as much as anybody to lend support to what has become a thoroughly irrational protest against the persistent pesticides. It would be a great public service if some members of the profession were to respond to Dr Borlaug's plea by helping to put the dangers of DDT in some kind of a perspective.

If many facts are still uncertain, it is at least possible to say what the uncertainties consist of. Since DDT was first introduced to agriculture and public health immediately after the Second World War, there have been many occasions on which this and other persistent pesticides have been misused. Sometimes excessive doses have been used, so that animals have been killed off as well as insects. Sometimes, so little has been understood of the natural pressures to which a pest is subject that DDT and its relations have killed not the pests at which they were directed but their natural predators, with entirely unpleasant results. And there are always circumstances in which the organochlorine pesticides can be concentrated at the top of a food chain, thus imposing a strain on species quite unrelated to pests.

For reasons like this, there is a strong case for better methods of regulating the application of pesticides so as to ensure that their use in agriculture or public health does not bring about unwanted side effects. Even if this should imply that the modern pesticides are too potent to be left in the hands of farmers and others without professional training, as the United States Administration's new proposal for legislation would suggest, that would not be an insuperable obstacle to the successful use of these materials. It is, however, quite another matter to suggest a more general restriction of the use of DDT and related chemicals as pesticides. That might be throwing out the baby with the bath water.

In spite of a decade of research, there is at present only enough evidence to justify an agnostic position on DDT. Nobody should complain, for the problems have turned out to be unexpectedly difficult. It is, after all, worth remembering that the persistent pesticides are by their nature chemically inert, so that analysis must depend on physical methods such as chromatography, mass spectrometry and spectral absorption. The most headway has been made with gas–liquid chromatography, but only recently has it been appreciated that the polychlorinated biphenyls are easily confused with the pesticides proper. To be sure, discriminating practitioners know that a sequence of otherwise inexplicable peaks in a chromatography plot may be indicative of a mixture of biphenyls and pesticides, but less careful workers, or earlier workers, have often ignored such evidence. The result is that many of the measurements in the early sixties of concentrations of DDT in the environment may be misleading. Although there is no doubt that substantial quantities of the pesticides such as DDT are to be found in the flesh of fish and seabirds and in the fatty tissues of mammals, human beings included, there is at present no means of being sure whether the organochlorine pesticides are distributed on a truly world-wide basis or whether their spread is more a regional or even a local phenomenon, a measure of agricultural practice in the neighbourhood. In the past few years, it does appear that concentrations of DDT in human fatty tissue are more than twice as great (twelve parts per million) in the United States as they are in Britain. For what it is worth, the reports of DDT in the tissues of Antarctic penguins, portentous though they may be, refer to proportions which are smaller by a factor of ten or more. What this implies is that there may be a high degree of correlation between the use made of DDT in some agricultural region and the proportions which find their way into the fatty tissue of the people eating the food produced there. Before too many breasts are beaten on the altar of global pollution, it would be helpful if there were a better understanding of the distribution of these pesticides.

The great uncertainty which persists about the effects of DDT on human beings is another question that needs to be resolved. In these heady days of anxiety about the environment, it tends to be forgotten that only twenty-five years ago DDT was welcomed because it was effective against insects and much less harmful to people than other insecticides then in use. To be sure, DDT itself is poisonous in sufficiently large doses and its relatives are usually more toxic still, so that there is already a long and depressing record of death by accidental poisoning. On the other hand, no serious harm seems to have come to those who work in factories where pesticides are manufactured and who have avoided acute toxic doses. Careful studies have shown, with samples which are admittedly small, that working in pesticide factories brings neither an increased risk of liver disease nor an increased risk of cancer. So what of the hazards of the small quantities of these pesticides now stored in fatty tissues ? The simplest statement of the position is still that of the Mrak Commission which said in December 1969 that "the only demonstrable effect of this exposure in man is the tissue residue". For practical purposes this view was confirmed last month by the United States Environmental Protection Agency's DDT Advisory Committee (see *Nature*, **233**, 299 ; 1971) which said that "DDT has a very low acute toxicity to man and his domestic animals, and exposure to high doses for short periods of time does not appear to cause any irreversible damage". The same committee goes on to say that the risk of carcinogenesis is low—the only suggestions so far of such effects are a series of experiments with laboratory rodents. Clearly, the danger that DDT and its relatives may damage human beings is exceedingly small in spite of the profligacy of the use of DDT in the years immediately after the Second World War.

So what is the case for banning the use of DDT ? Other species than human beings are the only causes that carry weight. Careful experiments with birds in captivity have shown that DDT can make eggshells thinner and thus impede the hatching of young birds. Such effects may well be responsible for the reduction of pelican populations in the Mississippi Delta and off the west coast of the United States in the past few years, but it remains a sobering truth that more eagles have been shot by farmers than can possibly have been killed by DDT. Outside the United States, it remains to be seen whether changes in the sizes of bird populations are to be explained by pesticides in the diet, by other causes such as the urbanization of the countryside or simply by changes in the vigilance of bird watchers. On balance, however, it does appear that the damage so far done to wild life, while important and regrettable, is not disastrous and is easily attributable to extravagant use of DDT in the United States in the late fifties and early sixties. To be sure, there are more remote dangers to be considered—the possibility that pesticides might affect the metabolism of phytoplankton and thus exert an influence on the productivity of the oceans, but more needs to be known of the processes involved before anything but a hypothesis can be put forward.

In all the circumstances, there is a danger that speculation, sometimes quite proper speculation, about the effects that pesticides might have will be dignified by the status of received doctrine. The difficulty is that DDT and many of its works are economically important and, as Dr Borlaug points out, have a specially important part to play in helping to make sure that food production in the world will rise more quickly than the population. This is why it is important that the present wave of sentiment in favour of a ban on the use of DDT and such materials should be replaced by a proper and necessary case for regulation and supervision of the ways in which these pesticides are used. At the same time, of course, it is essential that there should be a better understanding both of the mechanics of the distribution of DDT, the mechanism by which it is accumulated in human tissue and the damage which it is likely to do to human beings. One absorbing question, for example, is why there should be such a large spread of DDT concentrations in human fat about the average value. University research could have an important part to play in furthering this understanding, for governments and industry have axes of their own to grind. But professional scientists everywhere could help by insisting that the present wave of discussion about DDT and other pesticides is conducted with a better sense of proportion than in the past.

Where are the Bounds of Decent Practice ?

THERE have in the past few days been allegations that the experiments carried out in the past eleven years at the University of Cincinnati have offended against one of the essential principles of medical research—that human beings should not be used as experimental subjects without volunteering for the role and without being fully aware of all the circumstances. In all the discussions that there have been in the past few years of the problems of medical ethics in laboratory situations, this principle has been repeatedly affirmed. It is fair to say, of course, that the principle by itself is insufficient. What precisely is intended by the injunction that a potential experimental subject should "understand"? How can laymen hope to appreciate all the subtleties of a medical experiment, even if they grasp the essential objectives ? And there are also difficulties in knowing what should be done in circumstances in which people may be tempted to volunteer in ignorance or under pressure (as when participation in an experiment carries with it the promise of early parole from prison). It would be in the public interest that there should be an investigation into the circumstances in which the Department of Defense let a contract to the College of Medicine at the University of Cincinnati and the circumstances in which this has been administered in the past eleven years. Whether Senator Kennedy's congressional committee is the best forum is another matter. The hope now is that extraneous issues will not confuse the overriding question of how human experimental subjects should be dealt with and whether on this occasion they have been dealt with wrongly.

One obvious irrelevancy is the involvement of the Pentagon. No doubt there are many people who will say that it is in all circumstances reprehensible to use animals, human or otherwise, in experiments which are preparations for some nuclear battlefield. This, however, is entirely beside the point. So long as the military forces of the United States, the Soviet Union and Western Europe are organized so as to defend themselves against nuclear attack, it is clearly unreasonable to expect that the services responsible will refrain from taking steps to know what doses of radiation soldiers can withstand. Those who say that the College of Medicine at the University of Cincinnati should not have accepted a contract from the Department of Defense on grounds like these have no logical alternative but to spend their energies on a refutation of the defence policy to which the governments of the principal powers are committed. But that is a wider issue, not relevant to what is said to have been happening at Cincinnati.

It is also beside the point to complain that therapy should also be used as an occasion for research. In medical research, there is also a long and heroic tradition of willingness on the part of one generation to endure discomfort voluntarily for the sake of a better understanding of some condition or disease. Patients with terminal disease have frequently been concerned with such experiments and, such is the inherent altruism of many people, have participated willingly. But it is clear that the dignity of everybody concerned, and that of the profession of medical science, demands that there should be a full disclosure of all the circumstances to those who are asked to participate. If one objective of a study is to make it easier to know what doses of radiation can be tolerated, by troops or by civilians, it is obviously incumbent on the people in charge to help their patients to understand by giving not merely a laconic description of the purpose of the experiment but also an account of the ways in which the information is likely to be valuable. If the Pentagon is a sponsor of the work, this should be spelled out.

From the reports so far available, it is not clear how far these conditions have been met, although some of those concerned have said that patients were told that "this research will be valuable if somebody is injured on the battlefield". What will need to be determined now is whether it is a sufficiently large step. An especially taxing feature of the Cincinnati case is the allegation by the *Washington Post*—it is no more than that—that many of the patients in the experimental series were in some way or another less able than they might have been to appreciate what was being asked of them. Circumstances like these are necessarily hard to reconcile with the essentials of what should be the code that governs the relationship between a patient–subject and his doctor–experimenter, but the only workable rule is that if a prospective subject is thought to be incapable of understanding fully what is expected of him, he should not be involved. As yet, there is nothing to suggest that the people at Cincinnati have not behaved with great propriety. It is, however, essential for everybody, and for medical research as such, that this should be demonstrated clearly and publicly.

One Way to Europe

WITH the British political parties preoccupied with their invariably introspective party conferences at British seaside towns (Brighton this year), and with the question of whether the British Government will eventually accept the terms of membership offered by the European Communities reduced to questions of which party leader will be able to carry how many of his supporters with him, the initiative for taking a few practical steps towards Europe has fallen to others. And as luck will have it, there is an obvious and urgent task for universities in Britain and on the mainland. Will they dare ?

In the past few months, there have been several declarations of how necessary it will be, once the European Communities are enlarged, for there to be a coordination of European policies on research and development. Because development is necessarily linked closely with industry and with commercial interests, the best place to start devising machinery for collaboration is in basic research, in the universities and government laboratories. This point was put forcefully by Sir Brian Flowers, chairman of the Science Research Council, at the British Association meeting in September. One difficulty is that the coordination of research between European countries entails the free movement of people between one laboratory and another. But there are at present practical difficulties which prevent this happening. And so is there not a case for a serious attempt by European universities to work out among themselves some method of doing this job ? On this occasion, as luck will have it, they could act more quickly than their governments.

---

100 Years Ago

Extract from Helmholtz on the Axioms of Geometry.

With all due deference to so eminent a man as Helmholtz, I must hold that his article includes an *ignoratio elenchi*. He has pointed out the very interesting fact that we can conceive worlds where the Axioms of our Geometry would not apply, and he appears to confuse this conclusion with the falsity of the axioms. Wherever lines are parallel the axiom concerning parallel lines will be true, but if there be no parallel lines in existence, there is nothing of which the truth or falsity of the axiom can come in question. I will not attempt to say by what process of mind we reach the certain truths of geometry, but I am convinced that all attempts to attribute geometrical truth to experience and induction, in the ordinary sense of those words, are transparent failures. Mr. Mill is another philosopher whose views led him to make a bold attempt of the kind. But for real experience and induction he soon substituted an extraordinary process of *mental experimentation*, a handling of ideas instead of things, against which he had inveighed in other parts of his " System of Logic." And the careful reader of Mr. Mill's chapter on the subject (Book II. chapter 5) will find that it involves at the same time the assertion and the denial of the existence of perfectly straight lines. Whatever other doctrines may be true, this doctrine of the purely empirical origin of geometrical truth is certainly false.

W. STANLEY JEVONS

From Nature, **4**, 482, *October* 19, 1871.

OLD WORLD

TRADE UNIONS

Enter the APST

ONE of the first bodies to apply for registration under the Industrial Relations Act at the beginning of October was the Association of Professional Scientists and Technologists, which was formed recently by the Council of Science and Technology Institutes. When the council itself was set up in 1968 by five professional scientific institutes (including the Institute of Physics and the Royal Institute of Chemistry), its principal aim was to put forward the views of its member institutes on such subjects as the training of technicians and teachers; but when it became clear in the summer of 1970 that the government's legislation on industrial relations could pose problems for professional scientists, the council set up a working party to examine the desirability of forming an independent protective association.

Although the Industrial Relations Act does cater for professional bodies to a certain extent—they can be included on a special register and thus enjoy some of the rights of trade unions, for example—many of the professional organizations find their hands tied in other ways. Activities by the science institutes in the financial interests of their members are restricted to varying extents by their charters or articles of association, and separate salary negotiations for scientists in different disciplines would anyway be unacceptable both to employers and to employees.

The association is at present run by staff from the council's member institutes and the principal permanent staff will not be appointed until early 1972 when the association hopes to have a permanent headquarters and a healthy membership. The council says that between 5,000 and 10,000 members will have to be recruited during the next month or two so that the association can make itself felt before employers become too involved with the intricacies of the Industrial Relations Act. The annual subscription for a full member will be £5.

Dr Louis Cohen, secretary of the Institute of Physics, said this week that the association would probably attract principally those scientists who work in industry; he estimates, for example, that there are 4,000 to 5,000 potential members of the association in the Institute of Physics.

The Council of Science and Technology Institutes is conscious that, especially outside the industrial sector, some groups of scientists (for example, those in the civil service) already have effective negotiating bodies and that "there is no question of trying to usurp their position in any way". Mr William McCall, general secretary of the Institution of Professional Civil Servants, says that competition for members between the association and the institution is unlikely and that it would anyway be self-defeating.

EUROPEAN LABORATORY

Vain Effort?

A CAMPAIGN to avert the threatened closure of the European Space Research Institute (ESRIN) is gathering momentum as a result of a conference of the Plasma Physics Division of the European Physical Society held at ESRIN in September. A united front was formed against the proposed closure under the leadership of Professor B. Lehnert of the Royal Institute of Technology, Stockholm, chairman of the Plasma Physics Division.

The decision to close the laboratory was made at a meeting of the council of the European Space Research Organization in July. The chairman, Professor G. Puppi of Bologna, proposed a plan for ESRO which was adopted by the council and which recommended that "circumstances enforce consideration of closing down ESRIN" but "the council would welcome any alternative viable solution". This decision is to be ratified at a further meeting of ESRO in November.

Professor Lehnert said this week that one particularly distressing feature of the affair was the lack of warning given to the scientific community of the closure. The presentation of the *fait accompli* has undermined the morale of the laboratory and, even if the laboratory can still be saved, the damage is probably irreparable. Lehnert, on behalf of the Plasma Physics Division, has this week sent a letter to ESRO protesting against the decision. He stresses that the laboratory has made several contributions to plasma physics and "during the existence of ESRIN, members of the [Plasma Physics] Division have established contacts with the staff and realize the high value of the scientific work performed at ESRIN". The letter continues, "The ESRIN laboratory must be considered an essential and important part of activities within ESRO and not as a luxury. We consider the proposal to relinquish ESRIN a retrograde step in European space research. Any apparent saving in the budget will be nullified by a decrease in the efficiency of ESRO and by increased pressure on national research budgets." It is very difficult to argue with this logic and an ESRO spokesman did not attempt to deny it when he said this week that ESRIN could not carry on in its present form because of financial problems. He said, "ESRO is entering the applications field—air traffic control, meteorology and telecommunications—and some of the money now being spent on scientific programmes must go. The programmes least connected with essential ESRO activities—the ones at ESRIN and the rocket sounding programme—are being run down."

The reason for the precipitate decision to close the laboratory has aroused speculation in the European press in recent weeks. An article in *Die Welt* on September 30 says that the decision was forced upon ESRO by a French threat that they would withdraw from ESRO if the laboratory was not closed. Professor Lehnert said that he did not know why the decision had been taken but he was certain that pressure was being exerted on ESRO from a member country. It is known that the French feel that the science budget of ESRO should be limited.

The efforts to save the laboratory within ESRIN seem doomed but the scientists hope that the laboratory can be maintained in its present form under the auspices of some other European or Italian organization. An ESRO spokesman said on Monday that the director general, Dr A. Hocker, is in touch with Italian organizations to find a solution. He said, "The Italian authorities accept the idea that the present ESRIN activities cannot continue permanently within ESRO and the director general is actively negotiating to see what activity could be carried out at ESRIN."

A problem with transferring the laboratory to Italian hands is the current unrest among Italian scientists. At present there are unfilled positions in Italian national laboratories and another addition to their number will probably not be welcomed by the Italians. A more feasible possibility, if the laboratory is to be maintained in its present form, is for another European organization to take ESRIN under its wing. EURATOM seems the obvious candidate although CERN is a possibility. A difficulty that the scientists are facing in their campaign is that ESRIN is not unique in Europe. There are several national laboratories that rival ESRIN in quality of work and so the motivation for keeping the laboratory as a viable entity is not so strong as it might be.

It is still possible that ESRIN will remain within ESRO but with a different purpose. The ESRO office in Paris said on Monday that the director general is now negotiating with the Italian authorities to see what activity could be carried out at ESRIN instead of plasma physics. There is no information on what this new purpose might be but a spokesman said that it would be more closely related to the activities of ESRO than previously.

INSECTICIDES

Pyrethrin Prospects

One of the most promising developments in pesticides in these days of great public concern for the environment has been the development and marketing of pyrethrin analogues. Pyrethrum, a natural pesticide from the ground flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum*, has been known since the early nineteenth century; six esters are found in the pyrethrum of which the most important and plentiful are known as pyrethrins I and II. They are used against houseflies, mosquitoes and cockroaches, mainly in the domestic field; their great advantage is that pyrethrin I has a high kill rate and pyrethrin II a high knockdown rate and that, once used, they degrade quickly in the light, leaving no appreciable toxic residues.

Pyrethrum has never been cheap; today a kilo of 100 per cent active pyrethrum costs about £38 compared with about £0.2 for DDT. Work was begun years ago to find a synthetic analogue but only recently have really significant advances been made. Since 1962, work on pyrethrin analogues has been carried out at Rothamsted supported by the National Research Development Corporation and two analogues, resmethrin and bioresmethrin, have recently been produced which are a considerable advance on the analogues previously available, notably tetramethrin and bioallethrin.

The new synthetic products have a kill rate many times higher than the natural product. Their knockdown rate is, however, lower although high when compared to other insecticides. This has been overcome by mixing them with the natural pyrethrin and a synergist which increases their effect.

The synthetic products appear to have all the advantages of the natural pyrethrins, without the drawback of the natural pyrethrins of causing nasal irritation if present in the air in sufficient quantities (pyrethrins are often used in aerosols). Indeed, the synthetic pyrethrins seem to be safer; the acute oral toxicity (LD50, rat) of bioresmethrin is approximately 8,500 mg per kg of body weight, that of resmethrin approximately 2,000 and that of natural pyrethrin approximately 600. In feeding trials conducted by Cooper McDougal and Robertson, one of the two companies marketing bioresmethrin in Britain, no ill effects have so far appeared.

Where the potential of synthetic pyrethrins may lie is in agricultural applications where cost has so far prevented the use of natural pyrethrins. At present synthetic pyrethrins are even more expensive than the natural product (about £40 a kilo for resmethrin and about £65 for bioresmethrin), but their relative non-toxicity, allied to their high kill rate, could make them a viable substitute for organochlorine and organophosphorus insecticides, many of which tend to break down less quickly than the pyrethroids.

The only known drawback to pyrethrin, both natural and synthetic, is that it is highly toxic to fish. This may not limit its use near rivers, however, as both water and light produce rapid breakdown, and tests may well prove that the quantities in which it will be used will present no danger to life in nearby water, should it be used for agricultural applications.

At present, however, the analogues are competitive with pyrethrum for a wide variety of largely domestic uses, and will be used overseas to replace other insecticides in household aerosols.

The development of synthetic pyrethrins has led to speculation that the market for the natural product may disappear, and Coopers admit that that was their first reaction, but the present need to mix synthetic with natural pyrethrins to achieve rapid knockdown, plus the expected expansion of the market, may well mean that the natural product is safe for some time to come. Kenya produces a large share of the world's natural pyrethrum and a spokesman at the Kenya High Commission said this week, "We think that synthetic pyrethrum will have some effect."

Ironically it was a world shortage of pyrethrum, caused by a fall of exports, that brought about the rapid commercial exploitation of the analogues, although Kenya claims that its output is now rising again. But whether the market for natural pyrethrum disappears—and experience with other synthetic substitutes for a natural product, for example, quinine, suggests it might—the future for the pyrethrum analogues looks bright.

COAL

Robens Recollects

Lord Robens's first public address since leaving the Coal Board surveyed the position of coal during this century and speculated on the uses of coal in future years. His address, the twentieth, and possibly the last, Coal Science Lecture of the now defunct Coal Utilization Research Association, was given on Monday to 400 scientists—most of them employed in coal research.

Lord Robens declared that more efficient use of man's resources must be made. In particular, it is now becoming clear that coal, which was considered the poor relation of gas and oil until very recently, has an important part to play in the future energy equation of Britain. Coal is Britain's only indigenous energy source and, although the rich veins are being fully mined, a large supply of low quality coal is, as yet, unexploited.

One of the chief efforts of the coal board to this end has been directed towards the efficient burning of low grade coal and coal dust by fluidized combustion. In this process a mixture of coal and ash constitutes the fluid bed and the NCB effort has been devoted to obtaining a high efficiency of heat exchange between this and immersed boiler tubes. The practicability of such systems has been established in the laboratory and future effort will be devoted to making the system work on a large scale. A further advantage of the fluidized bed combustion is, as Lord Robens pointed out, its ability to suppress the emission of sulphur into the atmosphere when limestone is mixed with the fuel. This aspect of the process has interested scientists in the United States where there are large undeveloped supplies of low grade fuels and Lord Robens issued a warning that this might yet be another technique developed in Britain but exploited in the USA.

One of the problems that will face Britain towards the end of the century will be the gradual decrease in supply of North Sea gas. Lord Robens pointed out that efforts should be made to supplement this by synthesizing a gas that can be blended with supplies from the North Sea. At present the Gas Council is devoting considerable research effort to synthesizing gas from oil and Lord Robens stressed that in view of the uncertain future of oil—which is obtained mostly from politically unstable countries—efforts should be made to obtain such a gas from coal. Historically the way to achieve this has been by coking but this process is not efficient. Also the gas obtained has a low calorific value that is not commensurate with natural gas. The British effort towards achieving this end is at present concentrated solely in a combined Gas Council–NCB working party.

The NCB was, in Lord Robens's view, caught unawares in the early 1960s by the full implications of the Clean Air Act. The board was faced with having to develop in a short time either a smokeless solid fuel or appliances that would burn bituminous coal smokelessly. The board's quandary was finally resolved when it was decided to proceed with making smokeless fuels but Lord Robens stressed that this was no easy decision and, as he aptly put it, "the choice between the two approaches was by no means a simple black and white one." He added that it took ten years to solve all the technical problems of making smokeless fuels, after a smaller research effort into the design of appliances to burn bituminous coal smokelessly.

NEW WORLD

Fighting the Great ABM Battle Again

by our Washington Correspondent

A REPORT sharply critical of the professional conduct of some of the scientists who took part in the 1969 debate on the Safeguard anti-ballistic missile system has triggered off a controversy in the scientific community that could seriously damage relationships between scientists and Congress. Whatever the quality of the report and the validity of its conclusions (both of which are being vigorously contested), there is little doubt that it will be remembered for the impression it has generated—that prominent participants in the ABM debate sought to mislead Congress and the public on technical matters. Also at issue are the ethics involved when a professional society is seen to ally itself with participants on one side of a highly political debate by impeaching non-members for their alleged professional misconduct.

The basis of the controversy is a report written by an *ad hoc* committee of the Operations Research Society of America (ORSA) and published last week in the society's journal. Based on a study instigated at the request of Professor Albert Wohlstetter, the chief conclusion of the committee is that "when prominent experts outside the Administration supported their opinions on Safeguard deployment with arguments or results of an operations-research nature, these analyses were often inappropriate, misleading or factually in error".

The six-member committee also says that opponents of the Safeguard system were more unethical than their colleagues who supported the Administration. In particular, the report singled out for criticism Dr Jerome Weisner, Dr George Rathjens and Dr Steven Weinberg, all from Massachusetts Institute of Technology.

For their part, however, the MIT scientists charge the committee with lack of professional ethics because it took a narrow and biased view of the debate and failed to comment on the arguments and alleged mis-statements of supporters of the ABM. The MIT group also suggests that the analysis may have been instigated for personal rather than for altruistic reasons, and points out, in a rejoinder to the report, that whatever the findings of the *ad hoc* committee, arguments put forward by opponents of the ABM have been vindicated by subsequent events.

The report concentrates chiefly on the arguments which surrounded the possible vulnerability of US Minutemen silos to pre-emptive attack by the Soviet SS-9 rockets, paying little attention to the fierce debate over the ability of Safeguard to do the job specified for it and ignoring almost totally the discussions on whether or not the system could easily be overwhelmed or made useless by destruction of the Missile Site Radar (MSR) which guides the

THE Operations Research Society of America was launched into the ABM debate by a letter sent in November 1969 by Professor Albert Wohlstetter to Dr Tom Caywood, then president of the society. The letter listed points of difference between calculations performed by Wohlstetter and by opponents of the ABM, in particular by Rathjens, Weisner and Weinberg. Rathjens, Weisner and Weinberg responded to an invitation to cooperate by saying: "Any general inquiry that would widen the public's understanding of the issues would be welcomed by us but we feel that for the Operations Research Society of America to carry out an inquiry into the ABM debate along the lines suggested by Professor Wohlstetter would be absurd". The scope of the inquiry would be too narrow, the MIT scientists suggested, and "could well appear to the nation as an ugly resurgence of those attacks on civil liberties and dissent that were far too common fifteen years ago".

One member of the committee, Dr Howard Berger, a former president of the Military Operations Research Society, had had a serious disagreement with Rathjens when both were employed at the Institute of Defense Analysis. According to Rathjens, the disagreement led to his relieving Berger of responsibility for a project, and eventually to Berger's resignation from the institute. Caywood said last week that he was aware of some disagreement between Berger and Rathjens when he formed the committee.

The *ad hoc* committee presented its report to a meeting of the ORSA council in May this year, at which it was adopted by a vote of 11 to 1, with only Professor John Little of MIT dissenting. Subsequently, however, five members of the council submitted a minority statement for inclusion in the journal alongside the report, pointing out that they consider it inappropriate for an organization such as ORSA to take on the quasi-judicial function of investigating the professional behaviour of individuals. The ORSA council is therefore now itself divided on the issue of whether it should have carried out and published the report, and to add to the internal troubles of the organization, Professor Philip Morse, also of MIT and a founder member of ORSA, wrote a letter to the *Boston Globe* last week in which he strongly protested against the report and threatened to resign from the society. The burden of his argument is that, by denouncing by name individuals who are not members of the society, the report suggests ORSA is "on the side of ex-Senator Joe McCarthy, is promilitary and supports the assumption that experts always know best".

When the report was published last week, Rathjens, Weisner and Weinberg sent out a rejoinder in which they said that the report had substantiated virtually every criticism raised in their original letter to Caywood. The rejoinder was not, however, sent to members of the committee, or to Wohlstetter, who complained in an interview that this was simply another instance of the MIT scientists' "form of arrogance and elite contempt of the lay public".

Professor Little has taken the different view that, since the society should not have attempted an analysis of the debate and since it should not have placed itself in a quasi-judicial position, Rathjens had every right not to take part.

intercepting rockets to their targets. The committee says that it chose to study these areas because of their importance to the debate and because the calculations are simple enough to allow major points of difference in the assumptions underlying the various calculations to be singled out.

The ORSA report chooses to analyse in particular the arguments put forward by Rathjens and by Wohlstetter, which respectively came up with predictions that 75 and 95 per cent of the US Minutemen force could be destroyed in their silos by the stock of SS-9 rockets thought to be available to the Soviet Union by the mid-1970s. Rathjens argued on the basis of his calculations that the threat was not sufficient to justify deployment of Safeguard, while Wohlstetter suggested that if 95 per cent of the US retaliatory force of intercontinental ballistic missiles (ICBMs) could be knocked out by a pre-emptive strike, then they needed protecting.

The *ad hoc* committee found in effect that Wohlstetter's calculations were correct, and that he had presented them in a professional manner, but that Rathjens's performance was less than adequate. In particular, the report suggests that Rathjens assumed that the SS-9s would have less than their possible complement of independently targeted warheads (MIRVS), that he misread a chart presented in testimony to the Senate Armed Services Committee by Deputy Secretary of Defense David Packard, and that all the MIT scientists, together with Dr Wolfgang Panofsky, Director of the Stanford Linear Accelerator Center, ignored the possibility that some of the SS-9 rockets that may fail during launch or in flight could be replaced by other rockets by an intricate system of reprogramming. The committee finds that if all these alleged errors in calculation are taken into account, a preemptive strike by SS-9s could be expected to dispose of 95 per cent of the undefended Minuteman silos by 1976, if the trends in Soviet arms deployment evident in 1969 were maintained.

Rathjens assumed in his calculations that the SS-9s would each have four MIRVS, each with a yield of one megaton, while Wohlstetter based his calculations on the assumption that the missiles would each carry three five-megaton warheads. The basis of Rathjens's assumption is claimed to be data supplied by Deputy Secretary of Defense Paul Nitze in 1967, and he pointed out in a letter published in the *New York Times* in June 1969 that he assumed the SS-9 payload before the Administration suggested that each would have three five-megaton warheads. In any case, Rathjens points out that the payload likely to be employed on the SS-9 is not the most sensitive assumption in the calculation, and that he is not even now convinced of the Soviet Union's ability to deploy large yield warheads on its missiles. Nevertheless, in the rejoinder to the ORSA report, Rathjens does not answer this particular criticism of his calculations.

As for the suggestion that he misread the chart submitted by Packard, Rathjens admits that he assumed the chart was calibrated in statute rather than in nautical miles and that this misreading led to his adoption of an unduly high value for the blast resistance of the Minuteman silos. But as he pointed out in a letter to Wohlstetter in June 1969, this error in fact was offset by an error in the accuracy of the SS-9, which also occurred from misreading the Packard chart in statute miles.

The chief point of disagreement between the opponents and proponents of Safeguard in 1969 was whether or not the SS-9s could be reprogrammed so that those which failed in launch or in flight could be replaced by fresh rockets. The OSRA report suggests that if Rathjens's calculations are altered to include the correct value for blast resistance of the Minuteman silos and a substitution is made to include three five-megaton instead of four one-megaton warheads in the SS-9, the number of Minutemen assumed to survive a first strike is reduced from 25 per cent to 16 per cent. But if the attacking missiles can be reprogrammed to replace some failures, only five per cent of the Minuteman silos would remain intact.

Why did the opponents of the ABM not consider reprogramming ? According to the MIT rejoinder to the ORSA report, they did not consider sophisticated reprogramming to be technically feasible, particularly reprogramming to compensate for failure of a single reentry vehicle that had gone astray. The ORSA report points out, however, that failures occurring during launch and during early flight could be compensated for relatively easily with a significant improvement in the efficiency of the strike.

The rejoinder in fact does not attempt to rebut the ORSA report point by point. In fact, Weisner, Rathjens and Weinberg point out that they feel no obligation to do so but instead attack the report for not tackling all of the relevant issues. In particular, they suggest that the ORSA committee should have looked at the arguments concerned with the vulnerability to attack of the missile site radar, especially since the Administration is now admitting the limitations of the system and is looking into designs for an improved defence of missile silos, they chide the ORSA committee for not investigating the possibility that Safeguard may be overwhelmed, they point to a statement attributed to President Nixon that the Safeguard system could "provide a virtually infallible defence" against Chinese attack, and ask why it was not examined, and finally they suggest that an examination of the shifting justification of Safeguard could well have found a place in the report.

There is little doubt that opponents of the ABM employed incorrect assumptions at times and came up with incorrect conclusions as a result (in fact the rejoinder admits as much), but Weisner and his colleagues point out that their calculations have been proved essentially correct by events. For example, they point out that the threat suggested by the Administration from the SS-9 has not materialized—the Administration was predicting a force of 420 SS-9 missiles by early 1972, but according to testimony given by Dr John Foster to the Senate Armed Services Committee on April 19 this year, the USSR has about 300 SS-9s and the rate of deployment was slowed down. A recent study carried out by TRW Inc., a California firm, suggests that the re-entry vehicles on the SS-9 may not be accurate enough to pose much of a threat to Minuteman silos, at least not a sufficient threat for a first strike capability—and the vulnerability of the missile site radar is now recognized and the Department of Defense is pressing ahead with design of a system specifically designed for protection of Minuteman (the Hardsite system).

Whatever the merits of the case that the committee has argued—and it cannot be denied that it has provided some evidence of errors—the report is likely to achieve little more than to cast doubt on the validity of testimony of future witnesses before Congressional committees. Moreover, in presenting what seems to be a one sided investigation, at least as far as examining and reporting on statements and arguments from both sides is concerned, the report has caused internal strife in the Operations Research Society of America itself (see box). In any case, there seems to be little point in digging over past debates when a more productive exercise would be to examine the validity of present assumptions about the ABM system.

CANCER

Defence Therapy

TERMINAL cancer patients at the University of Cincinnati College of Medicine are being exposed to large doses of radiation in a study paid for by the Department of Defense. One aim of the study is to determine the likely effect on troops of radiation from nuclear weapons, but members of the Cincinnati team claim that the treatment is given primarily for therapy and that it does not harm the patients. Since the details of the study were disclosed last week in the *Washington Post*, however, they have raised some doubts about the ethics of undertaking this type of research.

Total body radiation therapy has been used at the University of Cincinnati for the past fifteen or sixteen years, according to Dr Edward Gall, one of the Cincinnati team, but the investigative part funded by the Department of Defense has a history of eleven years. Dr Gall claims that the Pentagon became interested in the radiation therapy when some of the results were first described, and although the programme has been expanded the therapy has not been altered. In effect, he claims that the Department of Defense is simply interested in results derived from normal therapy at the hospital.

The therapy involves exposing patients suffering from cancer that has progressed beyond surgery to total body radiation of between 100 and 300 rads, or occasionally to partial body doses of 50–100 rads. The Department of Defense is interested in monitoring the doses of radiation that produce nausea and vomiting and other effects that might impair the performance of troops on the battleground. Patients are told that the results of the treatment may be helpful in case people are exposed to large doses of radiation, but they are not told that the study is being funded by the Department of Defense.

So far, 81 people have been given the total radiation treatment at the University of Cincinnati, but 108 have been approached—27 were excluded either because they were found to be unsuitable for treatment or because they had indicated that they did not want to take part. They included patients suffering from cancer of the colon and lung and Ewing's tumour, and all had already been given treatment at the University of Cincinnati hospital. They are told that the therapy may relieve some of their pain but that it will not necessarily lengthen their lives. But they are not told that they may suffer nausea from the treatment, since that may bias the results.

The treatment is approved each year by the Committee on Human Research at the university. Some other cancer therapists have expressed doubts about both the ethics of the research and the efficacy of the therapy, however. In particular, it has been suggested that total body radiation is not used as a therapy elsewhere, and the Department of Defense says that it is not funding any similar research. The Cincinnati team, although unwilling to claim substantive evidence of success from its therapy, points out that out of four children treated for Ewing's tumour, three have survived for three and a half years.

DDT

Borlaug's Warning

by our Washington Correspondent

An impassioned plea on behalf of DDT was delivered in Washington last week by Norman E. Borlaug, recipient of the 1970 Nobel Peace Prize for his work on high yield wheat strains. Borlaug, who testified in a public hearing on the cancellation order imposed on the pesticide by the Environmental Protection Agency, said at a press conference later:

"Environmentalists today seek a simple solution to very complex problems. The pollution of the environment is the result of every human activity as well as the whims of nature. It is a tragic error to believe that agricultural chemicals are a prime factor in the deterioration of our environment.

"The indiscriminate cancellation, suspension, or outright banning of such pesticides as DDT is a game of dominoes we will live to regret.

"DDT, because it is a name popularly known to most segments of the public, has been the first target. Once that is accomplished, the so-called ecologists will work on hydrocarbons, then organophosphates, carbamates, weed killers, and, perhaps, even fertilizers will come under the assault of their barrage of misinformation.

"If this happens—and I predict it will if most DDT uses are cancelled—I have wasted my life's work. I have dedicated myself to finding better methods of feeding the world's starving populations. Without DDT and other important agricultural chemicals, our goals are simply unattainable.

"Perhaps more than any other single factor in the world today DDT has a unique contribution to the relief of human suffering. I need not reiterate its vital importance in malaria control.

"DDT critics will say, of course, that only domestic uses of the chemical are being reviewed in the hearings at which I appeared today. But I have spent my life working in the nations of the world to help them feed themselves. I know how they will react if we terminate uses of DDT in this country and, in effect, label it 'poison'. If it is not good enough for your purposes, they will reason, then it shouldn't be used in our countries. The impact will be catastrophic.".

DRUG ADDICTION

Fort Worth Deleted

by our Washington Correspondent

Plans to transfer a drug addiction research and rehabilitation centre from the National Institute of Mental Health to the Bureau of Prisons seems to be going ahead in spite of stiff opposition from powerful members of Congress. The centre, situated at Fort Worth in Texas, was due to be transferred to the Bureau of Prisons this month (see *Nature*, **232**, 597; 1971), but in July the House of Representatives blocked the move with a resolution designed to keep open all Public Health Service hospitals and outpatient clinics pending the findings of a thorough review of their functions. The resolution has, however, reportedly been emasculated in a conference committee appointed to iron out differences in the versions of the resolution passed by the House and by the Senate, and all references to the two research centres at Fort Worth and at Lexington in Kentucky have been deleted.

Opponents of the plan, led by Paul G. Rogers, chairman of the House Subcommittee on Public Health and Environment, argued that transfer of the Fort Worth centre to the Bureau of Prisons would in effect close down badly needed facilities for the treatment of drug addicts. NIMH officials have, however, countered that the loss of facilities will be offset by a sharp increase in the number of community health centres offering rehabilitation treatment and that, in any case, an addict is more likely to be rehabilitated if he is treated in his own community and not an isolated hospital.

While discussions about the future of the Fort Worth centre were taking place in Washington, however, many of the hospital's staff left, giving rise to serious doubts about the ability of the hospital to carry on operating in its present role, even if Congress did grant a stay of execution.

If, as now seems likely, the Fort Worth centre is transferred to the Bureau of Prisons for use as a medium security geriatric, alcohol and drug addiction hospital for prisoners, the move would underline the failure of the Narcotic Addict Rehabilitation Act of 1966. That act gave the courts the power to commit drug addicts for treatment instead of for prosecution for certain non-violent crimes such as mail theft, stealing cars and forging cheques.

Addicts committed under the terms of the Narcotic Addict Rehabilitation Act are sent to the Lexington and Fort Worth clinical research centres for detoxification and are then usually sent to aftercare centres in their local communities. In the first three full years of operation of the act, however, between July 1967 and June 1970, only 179 addicts were committed for treatment, and only 509 were committed after trial.

Congress had expected that about 900 addicts a year would be committed before trial alone, and that Lexington and Fort Worth would be bursting at the seams. Instead, the hospitals are running at half strength and seem destined for other things.

NEWS AND VIEWS

Seafloor Spreading and Mountain Building

CAN tectonic events on the continents be related to seafloor spreading several thousand kilometres away? The article by P. J. Coney on page 462 of this issue of *Nature* and that of R. W. R. Rutland in an earlier issue (*Nature*, **233**, 252; 1971) relate mountain building episodes in North and South America to changing seafloor spreading in the Atlantic. The bordering areas, the Caribbean and Scotia Arc, have also been studied recently in the light of seafloor movements by G. L. Freeland and R. S. Dietz (*Nature*, **232**, 20; 1971) and by I. W. D. Dalziel and D. H. Elliot (*Nature*, **233**, 246; 1971). This sudden flurry of articles reflects how details of seafloor spreading are now becoming available so that it is possible to relate such distant events with each other.

Perhaps the chief reason why seafloor spreading was not considered seriously until the 1960s was the apparent discrepancy between the essentially simple, localized pattern of spreading compared with the extensive, complicated patterns of mountain formation. The creation of new ocean floor takes place in an extremely narrow zone, possibly less than 30 m wide, at the centre of the ridge system, and this new oceanic lithosphere eventually descends back into the Earth's mantle at the oceanic trenches—again narrow zones compared with mountain systems of up to 1,000 km width. Between these two narrow active zones, the tectonic picture is quiescent; oceanic lithosphere is moving towards the trenches at different rates in different areas, although it is consistent over vast lithospheric plates on which only sedimentation is taking place, and the odd, persistent hot spot in the Earth's mantle is giving rise to a trail of volcanic islands. In contrast, mountain building occurs over wide regions of the continental crust with many phases of folding, faulting, igneous activity and metamorphism.

The discovery of major transform faults in the oceanic basins provided the first indisputable evidence of crustal movements of the magnitude required by the proponents of continental drift. On the whole, however, these faults could not be traced inland so that, for several years, it still seemed that there was little relation between the tectonics of the ocean floors and those of the continents. The establishment of plate tectonics indicated that there must be at least some broad correlation between these tectonically active areas although it still seemed improbable that detailed correlations would be made between changes in spreading rate and direction of the plates and the precise sequence of events in an orogenic system, even on the same plate.

It is, however, the simplicity and narrowness of the mechanism by which new ocean floor is added that are allowing determinations of detailed movements of the plates. If the creation ofseafloortook place over a wide region, many of these details would be lost in the "noise level" of the changing polarity of the Earth's magnetic field. These details, as they become available, are, however, becoming increasingly linked to specific changes in the degree of deformation, changes in the motion along faults, and so forth, within the orogenic systems of the continents. The current series of articles, such as that by Coney, mark only the start of this detailed correlation as they are based, so far, on magnetic records from those parts of the oceans which seem to reflect the simplest patterns, that is, where the locus of spreading has remained fixed, although the rate and direction of motion have changed. Coney is therefore able to distinguish three principal changes in spreading of the Atlantic plate from its initiation 150 to 180 million years ago, at 80, 40 and 9 million years ago. This means that he is able to correlate, for example, the evolution of the Azores with the Laramide orogeny and so forth, with only slight reference to movements of the Pacific plate.

Areas, such as the Atlantic north of Ireland, will take longer to interpret as changes have also taken place in the position of the axis of spreading. Similarly, the discovery that not all plate motions are symmetric with respect to the oceanic ridges (J. K. Weissel and D. E. Hayes, *Nature*, **231**, 518; 1971) must complicate the interpretation in some regions. Clearly, however, a synthesis of oceanic and orogenic motions on the Atlantic plates is becoming more feasible and, before long, the interaction between different plates will begin to emerge so that the tectonic activity will be seen in its true global perspective.

Putting the Cerebellum to Sleep?

ON page 481 of this issue of *Nature*, Siggins, Hoffer, Oliver and Bloom add another link to the growing chain of evidence which indicates that brain stem neurones rich in catecholamines are important regulators of activity levels in the cortices of the brain. They have demonstrated in rats that electrical stimulation in the neighbourhood of one such group of neurones leads to a prolonged inhibition of the impulse discharge from Purkinje cells—the output neurones of the cerebellar cortex. The amine-rich neurones concerned are those of the locus coeruleus (LC) in the rostral part of the pons. That axons of LC neurones terminate in the cerebellar cortex was first shown using histochemical techniques but the work of Siggins *et al.* represents the first attempt to use neurophysiological techniques to investigate the functioning of the pathway at the single neurone level. The potential importance of the path is emphasized by the finding that a single LC stimulus can produce a detectable decrease in the probability that individual Purkinje cells will discharge impulses; repetitive stimulation often leads to complete suppression of discharge.

It is difficult to speculate on the role of the projection in the normal operation of the cerebellar machinery. As Siggins *et al.* recognize, it has yet to be shown that the inhibition observed was directly mediated by synapses between the LC axons and the Purkinje cells. Such synapses have not been described but electron microscopical studies may yet help. At any event the long latent period of the effect suggests that the path is unlikely to be important in that aspect of cerebellar function which currently attracts most attention—the control of evolving movements. A role as a long-term regulator of cerebellar cortical responsiveness seems more plausible.

Interest in the result is enhanced when it is viewed against a more general background. Histochemical studies by Olson and Fuxe (*Brain Res.*, **28**, 165 ; 1971) have shown that the axons of LC neurones terminate at destinations as diverse as the cerebral cortex, the hypothalamus and the lateral and medial geniculate bodies, not to mention the pathway terminating in the cerebellar cortex. The pathway to the cerebral cortex has been investigated in a general way by observing the behavioural changes caused by pharmacological agents believed to stimulate or to depress it. These experiments suggest that high LC activity leads to behavioural arousal and an alerting of the cerebral cortex and this activity is signalled by desynchronization of the electroencephalograph. Reduced activity in the nucleus, or outright destruction of it, leads to the synchronous electroencephalograph which is characteristic of light sleep or waking repose. The new results apparently show that the LC axons to the cerebellum are inhibitory and the odds are therefore high that the other LC projections are also inhibitory, in which case the path to the cerebral cortex clearly does not cause arousal directly by exciting cortical neurones. It may prove necessary to regard the projection as functioning by turning down some cerebral cortical element the unchecked activity of which leads to electroencephalographic synchronization and behavioural repose.

HAEMOGLOBIN

Hammering the Sickle

from our Molecular Biology Correspondent

THIS week, dear reader, a tale with a moral, about molecular remedies for molecular disease. Sickle-cell haemoglobin is in all respects a normal functional molecule, except for the property, which the single substitution in the β-chain confers on it, of associating in the deoxygenated state to large tactoidal aggregates. When this happens in the cell, the consequences are exceedingly disagreeable. A simple problem, one might suppose, and an open challenge to protein chemists, to prove that theirs is a useful art, such as the hard-faced men by the National Institutes of Health should appreciate in tangible form. Better not to dwell on the more risible details, but to say only that until now they have failed prodigiously.

A new approach by a group of clinicians to the treatment of the condition was recently given wide publicity: having apparently encountered allusions to hydrophobic interactions between proteins, and the effect of urea on such forces, they proceeded to inject their patients with urea solutions. Protein chemists, knowing about the urea concentrations that might be effective in such situations, and guessing at the difficulties which might be involved in filling a patient with 8M urea, might have been permitted a pitying sneer. Astonishingly, however, this primitive ruse seemed to work. It is now apparent that this was in fact a consequence of the gradual formation in urea solutions —as every protein chemist also knows —of cyanate ions, which react with protein amino-groups.

The urea (which would not work anyway if too freshly made up) should therefore clearly be replaced by suitable doses of sodium cyanate. Kraus and Kraus (*Biochem. Biophys. Res. Commun.*, **44**, 1381 ; 1971) have now improved on this strategy by introducing carbamyl phosphate, a physiological substance, which is evidently capable of entering the red cell, with beneficial effects on the sickling tendency. They suggest that because phosphates in general compete with the natural cofactor, 2,3-diphosphoglycerate, for its site on the haemoglobin molecule, the carbamyl phosphate functions as an affinity reagent, entering the putative diphosphoglycerate site at the N-termini of the β-chain, and carbamylating the α-amino-groups (which indeed would be the most reactive at physiological *p*H). Indeed Caldwell, Nagel and Jaffé (*ibid.*, 1504) have shown that not only cyanate ions, but also carbon dioxide, which is known to bind noncovalently at the same site, will chase off diphosphoglycerate from normal human haemoglobin. One may also reflect that the loss by carbamylated haemoglobin of the capacity to bind the cofactor will lead to a relative increase in oxygen affinity (because the diphosphoglycerate binds only to the deoxygenated form). Thus the red cells will be less readily deoxygenated, and if they contain sickle-cell haemoglobin, the sickling will be inhibited. It is interesting to note that this provides a new retrospective rationale for the attempts once made to mitigate sickle-cell anaemia by administering carbonic anhydrase inhibitors. In addition to the decrease in *p*H that the accretion of carbon dioxide will engender, thereby bringing into play the Bohr effect to increase the oxygen affinity of the haemoglobin, inhibition by carbon dioxide of diphosphoglycerate binding would work in the same sense.

The ramifications of diphosphoglycerate binding are evidently endless. Lian, Roth and Harkness (*ibid.*, 151) now report that in intact red cells, the shift in the oxygen uptake curve on addition of the cofactor is substantially increased when the cells contain sickle-cell haemoglobin. It has long been known that the oxygen affinity of such cells is lower than those containing only normal haemoglobin, and Lian *et al.* also now find that this difference is still present, though much diminished, when the cofactor is absent. These findings must be set against the well-established identity of both the oxygen and diphosphoglycerate affinity of normal and sickle-cell haemoglobins in dilute solution, out of the cell. The situation is complex, and Lian *et al.* indulge in some contorted efforts at explanation. The formation, however, of aggregates in sickle-cell haemoglobin only at high concentrations must stabilize the deoxygenated state, and it is therefore reasonable to suppose that this will decrease the oxygen affinity, and increase the disphosphoglycerate binding in the cell which in turn will enhance the change in oxygen affinity.

The aggregation reaction in sickle cells is complicated by possibilities of participation of other forms of haemoglobin that are commonly present, such as normal adult and foetal haemoglobin. The true complexity of the situation has now been revealed by Bookchin and Nagel (*J. Mol. Biol.*, **60**, 263 ; 1971), who use the device of introducing cyanmethaemoglobins, which have the oxyhaemoglobin conformation, but do not bind oxygen, into mixtures. Sickle-cell haemoglobin forms gels only in the deoxygenated site, but the normal adult pigment will co-aggregate with it both in the deoxy- and the cyanmet- (that is, oxy-) conformations. Foetal haemoglobin by contrast will enter the aggregates in the cyanmet- but not in the deoxy- state. In another abnormal variant (Harlem C), capable of aggregating when deoxygenated, the interactions with other forms and other conformations are quite different. The results are by no means easy to explain in detail, but the authors are able, starting from the notion, originally advanced by Pauling, that the aggregates are helical structures, to make some inferences about the existence of separate secondary interaction sites across successive turns of a tight helix of low pitch; these are envisaged as distinct from the primary association in which the sickle-cell haemoglobin molecule is regarded as bivalent.

MITOCHONDRIAL DNA

Arrested Replication?

from our Cell Biology Correspondent

IF the exquisite experiments—for once that is no exaggeration—reported by Kasamatsu, Robberson and Vinograd in the current issue of the *Proceedings of the US National Academy of Sciences* (**68**, 2252 ; 1971) are anything to go by, it seems likely that the enigma of the mechanism of DNA replication will be solved by studies of mitochondrial DNA. And on reflexion that claim is not perhaps as surprising as it may seem at first sight, for the mitochondrial chromosome is after all probably nothing more than a heavily pruned bacterial chromosome.

Vinograd's group, in their peerless manner, have found that in two strains of mouse L cells growing exponentially about half the mitochondrial DNA molecules (closed circular DNA duplexes with a molecular weight of about 10×10^6) contain a short segment of triple stranded DNA called a displacement or D loop. This curious structure, revealed in their electron micrographs with a clarity that denies dispute, consists of a short chain of DNA which is apparently base paired to one of the closed circular DNA strands, such that a corresponding segment of the complementary closed circular DNA strand is displaced and loops out from the rest of the molecule. By heating mitochondrial chromosomes with these D loops to 90 C in 0.03 M NaCl, the short chain of DNA is released—it sediments at 7S in sucrose gradients—and concomitantly the two closed circular strands snap back together and the D loop is lost.

As Kasamatsu and her colleagues comment, the sedimentation properties of mitochondrial DNA with D loops suggest that the short displacing strand is about 3 per cent of the total length of the mitochondrial DNA molecule. On electron micrographs it measures 3.2 to 3.5 per cent the length of the complete molecule and assuming that the lower estimates of its length are the most accurate the displacing chain must be about 450±80 nucleotides long. This chain hybridizes with the light but not the heavy strand of L cell mitochondrial DNA and it is therefore probably a short segment of the heavy strand. Furthermore, the position of the D loop, and therefore the sequence of the 450 base segment of the heavy strand, seems to be unique.

Vinograd and his colleagues reach this latter conclusion from ingenious argument and experiment. As Clayton, Davis and Vinograd have shown, circular dimeric mitochondrial DNA molecules in human cells consist of two monomeric molecules joined head to tail. If this is also true of dimeric mouse L cell mitochondrial DNAs there should be two D loops per dimer and they should be diametrically opposed and separated by one genome length. Measurements of the position of D loops in electron micrographs of such dimeric molecules fulfil these expectations.

In short the properties of D loops eliminate the idea that they are intermediates in some DNA repair mechanisms, for repair would surely not be restricted to a unique site in the genome. But because they are present in up to 50 per cent of the mitochondrial DNA molecules isolated from exponentially growing cells, D loops may be an early stage in the synthesis of progeny mitochondrial heavy strands during a replication process initiated at a unique origin. Vinograd and his colleagues suggest that the initiation and synthesis of the progeny heavy strand can occur without a nick being introduced into either of the closed circular parental strands but growth of this chain is arrested when it is about 450±80 bases long. Further extension is then dependent upon nicks being introduced into the parental strands to allow their free rotation and unwinding. The occurrence of molecules which have nicked parental strands and displacement loops which range in size up to that of the complete genome supports this interpretation. In brief, closed circular molecules with D loops may well be mitochondrial genomes which have begun their replication but are hung up awaiting the attention of a nuclease without which they cannot continue. No doubt we shall not have long to wait before Vinograd's group can tell us more about this process and also about the synthesis of progeny light mitochondrial DNA strands complementary to the new heavy displacing strand.

Geometric Refinement of Crystal Structures

ALTHOUGH known interatomic distances are often used to find a chemically acceptable crystal structure concordant with an observed diffraction pattern, they are hardly ever used in the subsequent refinement stages of the analysis. In next Monday's *Nature Physical Science* W. H. Baur shows how, in some contexts, they can be incorporated most profitably and even used to overcome shortcomings in the observed crystallographic data.

Least-squares refinement, the most widely used refinement procedure at present, attempts by adjustments of the atomic coordinates and thermal parameters to improve the agreement between the observed and calculated structure amplitudes. It relies on the fact that the number of observed structure amplitudes greatly exceeds the number of adjustable atom parameters, but the ultimate accuracy achieved for any structure depends on the accuracy and extent of the intensity data. In the past, geometrical constraints have only been introduced into the process to treat certain atoms as a rigid group.

Inorganic crystallographers, especially those concerned with silicates, enjoy a situation not shared by many of their colleagues, and this has been exploited by Baur. Inorganic crystals are based on dense packing of anions interspersed by smaller cations and, although the cation angles may be distorted, the wealth of interatomic data now available (much of it derived from accurate single-crystal studies) has shown that contact distances are remarkably uniform. In 1969 Meier and Villiger (*Z. Krist.*, **129**, 411 ; 1969) realized that the number of interatomic distances in these structures exceeds the number of adjustable atom coordinates, described a least-squares procedure for adjusting the coordinates to minimize the spread of distances of a given type and demonstrated the usefulness and scope of the procedure. Baur, who has elsewhere been able to show that changes of interatomic distance are linearly related to changes of bond strength (using an extended electrostatic valence theory) and to derive empirical constants for a number of atom pairs, has now combined these linear relations with Meier and Villiger's least-squares refinement of distances. In particular he has shown how a rather poorly defined structure for the medium-pressure β-polymorph of $MgSiO_4$, originally deduced from powder data, could be refined to produce a far more homogeneous set of distances corresponding closely ($\langle \Delta d \rangle = 0.027$ Å) with those recently derived from a careful single-crystal study of the isomorphous β-$CoSiO_4$. Experience gained with earlier trials had shown that even more preferential weight should be given to the cation-oxygen distances than had been given by Meier and Villiger, and that this weighting is critical. When applied to accurately known structures the approach has proved capable of predicting Si–O distances to 0.01 Å or less.

It is a pity that the method is not more widely applicable: those people who study molecular crystals will surely be forgiven if they seem a little envious of a technique that allows their colleagues to transcend the limitations of their data.

ELECTRON MICROSCOPY

High Voltage at NPL

from a Correspondent

THE six AEI 1 MeV electron microscopes (installed at UKAEA, Harwell, the National Physical Laboratory, Imperial College, the Universities of Oxford and Birmingham and the British Iron and Steel Research Association, Sheffield) plus the 1 MeV JEOL machine at the Central Electricity Generating Board, Berkeley, and the original 750 keV instrument in Cambridge represent a UK investment in high voltage electron microscopy far exceeding that of any other country. About eighty people—mostly materials scientists using these various instruments and a few biologists—attended a conference organized by the Institute of Physics and the Royal Microscopical Society in conjunction with the National Physical Laboratory at Teddington from September 22 to 23.

Dr A. Howie (University of Cambridge) reviewed recent progress in understanding the penetration of electron beams in crystals and the remarkable channelling effects occurring above the "critical voltage". This voltage and related interference fringe spacings can be accurately measured to give information about lattice potentials as was shown for gold by Dr J. Steeds (University of Bristol) and for uranium by Drs B. Hudson and A. J. E. Foreman (UKAEA, Harwell). Local temperatures or alloy composition and order can also be determined in this way. Dr E. Butler (Imperial College, London) showed that in nickel-gold alloys 1 per cent compositional change could be detected over distances of about 1μ. Use of a mini lens to reduce the size of the illuminating spot to perhaps 0.1μ could make this a very powerful microanalysis technique. The discussion indicated that an improved theory which takes account of non-systematic reflexions would be useful for this work.

Above the threshold energy at which the fast electrons can knock atoms out of their lattice sites, the high voltage microscope is unrivalled for radiation damage studies *in situ*. Void growth, which causes serious swelling problems in fast reactor materials, can be simulated in the microscope at a rate 10^4 faster than in the reactor (Dr K. R. Williams *et al.*, CEGB). Accurate knowledge of flux density in the beam and temperature rise in the specimen are essential for quantitative interpretation of these experiments, as was pointed out by Dr M. J. Makin (UKAEA, Harwell).

The transparency of crystals several microns thick to fast electrons allows *in situ* experiments which are reasonably typical of bulk materials. Results presented included recrystallization processes in copper alloys (Dr R. Ray *et al.*, University of Birmingham), growth of strain-induced martensite in a titanium-zirconium alloy (Drs H. Flower and P. Swann, Imperial College, London) and an interesting film of dislocation motion during creep of polycrystalline aluminium (Drs K. F. Hale and M. Henderson Brown, NPL). The possible role of point defects in these experiments when the damage threshold energy is exceeded has not yet been assessed.

High voltage microscopes usually have more available space near the specimen which, in addition to the greater penetration of the electron beam, makes them very suitable for *in situ* experiments involving special stages. Some of these experiments were surveyed by Professor U. Valdrè (University of Bologna) and several new straining stages and environmental cells were described. The latter have been used in studies of cement hydration (Dr D. Allinson, NPL), the reduction of haematite (Drs N. Tighe and P. Swann, Imperial College, London) and the observation of 70 Å fringes in wet, partially stained catalase (Drs P. R. Ward and R. F. Mitchell, University of Cambridge).

Dr A. Glauert (University of Cambridge) listed the chief advantages of the high voltage microscope in biology as the ability to observe wet (but not living) specimens and to obtain both better resolution and, with stereo methods, a better three-dimensional picture of the structure in thick specimens. The ionization damage in organic materials is reduced at higher voltages but, as the polymer work of Drs M. J. Richardson and K. Thomas (NPL) demonstrated, this is often cancelled by the increased exposure time required with the photographic plates currently used.

Professor J. M. Cowley (Arizona State University) described a 1 MeV transmission scanning microscope now under construction and emphasized the advantages (chiefly reduced chromatic aberration and improved signal processing) which this technique has over conventional microscopy. This scanning instrument, which operates below the damage threshold, may well (at the cost of some resolution) offer significant advantages for the transmission studies of thick crystals. Its role at higher voltages, whether or not the probable damage produced is of interest, is rather more uncertain and Professor Cowley's results will be awaited with interest. In the meantime, the bold initiative which placed the high voltage conventional microscopes at the disposal of workers in Britain is being followed up with imagination and vigour.

HEPATITIS

Among Drug Addicts

from our Medical Virology Correspondent

TRANSMISSION of viral hepatitis through the communal use of syringes and needles by addicts for the injection of

---

Messenger RNA and the Endoplasmic Reticulum

THE elucidation of the way in which messenger RNAs, transcribed in the nucleus of eukaryotic cells, are matured, packaged and transported to the cytoplasmic translation machinery is one of the great challenges of cell biology. Over the past several years evidence has been accumulated which suggests that messenger RNAs occur in cell nuclei in the form of ribonucleoprotein particles—so-called informosomes—and in *Nature New Biology* next week Faiferman, Cornudella and Pogo report experiments with Krebs ascites cells which, they believe, indicate that these ribonucleoprotein particles migrate to the cytoplasm and become associated with the endoplasmic reticulum.

Faiferman and his colleagues gently lysed Krebs cells which had been labelled with RNA precursors and, by differential centrifugation, isolated the cell nuclei to which remained attached most of the cell's endoplasmic reticulum. After disrupting the nuclei in a French press, the nuclear chromatin was pelleted and the supernatant was found to contain 40–50S ribonucleoprotein particles as well as fragmented reticulum. The 40–50S particles are made in the presence of low doses of actinomycin D which block ribosomal RNA synthesis but cordycepin, which blocks messenger RNA synthesis in HeLa cells, blocks the synthesis of these particles in Krebs cells. To eliminate the possibility that the 40–50S particles are of mitochondrial origin, Faiferman and his colleagues have shown that their synthesis is not affected by ethidium bromide, which specifically inhibits the transcription of mitochondrial DNA.

Pulse chase experiments reveal that in the presence of amino-acids the 40–50S particles are rapidly chased into the endoplasmic reticulum. By contrast, when the cells are starved of amino-acids not only are the 40–50S particles broken down in the cytoplasm but also large numbers of ribosomes, which are normally attached to the reticulum, are found free in the cytoplasm. Faiferman *et al.* suggest therefore that the attachment of ribosomes and the 40–50S particles containing messenger are concurrent events. In short, they believe that messenger RNA is delivered to the reticulum in a 40–50S particulate riboprotein complex.

heroin, methylamphetamine and other drugs is a common and an increasingly serious problem in major urban centres throughout the world. The infection is spread by needles and syringes contaminated with blood. In a report from the Centre for Disease Control, Atlanta (*Hepatitis Surveillance Report*, No. 32; 1970), males between the ages of 15 and 29 years were identified as a high risk population for abusing parenteral drugs and the incidence of hepatitis among this age group is high. Once hepatitis is introduced into a group of drug addicts who use communal intravenous equipment, the disease spreads rapidly.

Cherubin and his associates (*Amer. J. Epidemiol.*, **95**, 510; 1970) detected the Australia antigen, which is specifically associated with serum hepatitis, in 54 per cent of patients who were known addicts and who were admitted with viral hepatitis to two hospitals in New York. In another group of young adults with clinical hepatitis who denied the use of drugs, Australia antigen was found in 50 per cent. On the other hand, the frequency of detectable antigen among healthy volunteer blood donors in New York is only 0.1 per cent. It was suggested that the high incidence of serum hepatitis in New York City might be attributable to a greater drug addiction problem than had hitherto been considered. It is, of course, true that the problem of hepatitis among drug addicts may well have been underestimated because there is some evidence that the virus of serum hepatitis may be transmitted not only by the parenteral route but also by the oral route and by sexual intercourse.

Another disturbing report has recently been published by Collins and Wells (*Milit. Med.*, **136**, 572; 1971) who noted a dramatic increase in the number of patients admitted with acute viral hepatitis to Brooke General Army Hospital between July 1969 and June 1970. The greatest increase in admissions was related to hepatitis associated with illicit drug abuse.

Drug abuse may be regarded in practice as a contagious disease because the habit is transmitted from one person to another. This approach makes it possible to apply to the study of drug addiction the methods used in the epidemiology of infectious disease and notification of hepatitis in young adults may well be used as one marker of drug abuse. Knowledge of the epidemiology of viral hepatitis among drug addicts is so far incomplete, but there can be no doubt that illicit drug users constitute an important reservoir of serum hepatitis in the community and the spread of infection to close family contacts, medical and nursing personnel and indeed to the population at large presents a real hazard.

ACETYLCHOLINE

Regulation of Binding

from our Neurochemistry Correspondent

A FURTHER addition to recent rapid advances in the application of techniques of molecular biology to the study of drug-receptor interactions (see, for instance, *Nature*, **229**, 523; 1971; and **231**, 91; 1971) is provided by evidence reported by M. E. Eldefrawi and R. D. O'Brien of Cornell University (*Proc. US Nat. Acad. Sci.*, **68**, 2006; 1971) that the binding of acetylcholine (ACh) to a preparation from the *Torpedo* electric organ containing cholinergic receptors is reduced at high concentrations of ACh.

Binding was measured by equilibrium dialysis using ^{3}H-ACh, and a particulate fraction in which all the acetylcholinesterase (AChE) was inhibited irreversibly. The binding sites studied were probably acetylcholine receptors, because O'Brien and his colleagues have shown previously that the binding of muscarone (a stable cholinergic agonist) to the same preparation was reduced after treatment with a variety of drugs known to act at cholinergic receptors, but was unaffected by other drugs. In the present experiments, binding of acetylcholine was found to reach saturation at a concentration just below 1 μM, but was markedly reduced at higher concentrations.

As pointed out by Eldefrawi and O'Brien in their discussion, this "autoinhibition" of acetylcholine binding to receptors is analogous to the reduction in activity of many enzymes, including acetylcholinesterase, at high concentrations of substrate. The latter effect,

Anti-θ Antisera and T Cells

IN recent years cellular immunologists have come to rely heavily on means of identifying the cell populations involved in immune responses. It is no longer good enough to say that changes have occurred in lymphoid organs after an antigenic stimulus, though the classic approach of the histopathologist is still obviously a prerequisite in any comprehensive study. The means adopted for cell identification have been many and various and are in most instances still only suitable for experimentation with small rodents of which the genetic constitution in relation particularly to tissue strain and species specific antigenicity can more readily be controlled.

One of the most useful devices has been the use of the theta (θ) alloantigen as an indicator for (T) cells of thymic origin. A recent review by Raff (*Transplant Rev.*, **6**, 52; 1971) summarizes such work. The problems with θ, however, are numerous. It is not absolutely specific for cells of thymic origin because it has been known for some time that some brain cells are θ positive. This is usually a minor problem for the experimentalist, but more serious are the possibilities, first, that not all T cells have easily detectable amounts of θ, as has been suggested by Miller and Sprent (*Nature New Biology*, **230**, 267; 1971), and, second, that the anti-θ antisera in use are not completely specific for T cells, which is the burden of a paper by Greaves and Raff which is to be published in next Wednesday's *Nature New Biology*.

Greaves and Raff prepared two batches of anti-θ antisera by injection of CBA thymocytes into AKR mice. Both sera had the same cytotoxic titre in the presence of guinea-pig complement as adjudged by either dye exclusion or release of ^{51}Cr from labelled target cells. It was found, however, that when the first serum (anti-θ_1) was used against lymphoid target cells in the presence of rabbit complement many more cells were killed than in the presence of guinea-pig complement. The conclusion was that a supposedly specific anti-theta serum was killing B cells. It is argued that the θ_1 antiserum contained an antibody directed against a previously unknown surface alloantigen which is present on the surface of B cells. The new antibody, however, required rabbit complement for expression of its cytotoxic potential.

Greaves and Raff present further studies in which anti-θ antisera were used to inhibit splenic rosette forming cells in the presence of guinea-pig complement. Anti-θ_1 had the capacity to inhibit both background and "immune" rosettes, but if it was absorbed with (autologous) AKR thymocytes its capacity to inhibit immune rosettes in the early phase of the response was limited. The authors conclude that anti-θ_1 contains an autoantibody which can act synergistically with anti-θ. Anti-θ_2, their second batch of antiserum, was deficient in the autoantibody. A corollary is that reacting T cells are deficient in θ antigen, a conclusion in line with that of Miller and Sprent.

Thus it seems that care must be taken in the use of anti-θ antisera as absolute criteria of T cells. This may restrict the use of fluorescent anti-θ antisera on tissue sections—because of staining of non-T lymphocytes. It should not, however, be thought that the analyses of the composition of various lymphoid cell populations in terms of T and B cells by the use of anti-θ sera in the presence of guinea-pig complement are invalid. Such a conclusion would be premature and almost certainly wrong.

however, is usually caused by an alteration in the effectiveness of the catalytic process rather than of the binding.

Of greater interest is the possible relation between this autoinhibitory effect and the blockade observed physiologically when neuromuscular preparations are exposed to high concentrations of acetylcholine—the phenomenon of "desensitization". Classical drug-receptor theory supposes that drug effects are a consequence of a change in the receptor to an "active" form, induced by binding of the agonist. On this basis, desensitization has been interpreted as being caused by the formation of "inactive" acetylcholine-receptor complexes, with a reduced ability to generate a physiological response. Receptors can thus exist in two forms, effective—R, at low ACh concentrations of acetylcholine—and desensitized—R″, at high concentrations of acetylcholine. The model also assumes that the affinity of R″ for acetylcholine is lower than that of R, but suggests no explanation of how these changes are brought about.

Eldefrawi and O'Brien propose a scheme whereby receptors may possess more than one type of binding site, occupation of low affinity sites leading to a reduction in binding to the high affinity or active sites and ultimately to a conformational change in the receptor, such that the affinity of the active sites for acetylcholine is decreased greatly. This would provide an attractive explanation for the molecular mechanism underlying desensitization, essentially analogous to the model for allosteric properties of enzymes. But so far there is no direct proof of multiple binding sites on receptors, and the authors should also perhaps first demonstrate a direct coincidence between physiologically observed desensitization and inhibition of binding *in vitro*.

BEHAVIOUR

Ethology in Edinburgh

from our Animal Behaviour Correspondent

THE wide range of topics discussed at the twelfth international ethological conference held in Edinburgh from September 7 to 15 reflects the diversity of interests of ethologists. The first morning of the conference was devoted to the neurophysiological mechanisms underlying sequences of invertebrate behaviour, and several speakers, including Dr A. O. D. Willows (Washington University), showed that patterns of overt behaviour may in some cases arise from patterns of impulses originating in particular neurones. Other plenary sessions covered imprinting, behaviour of social insects and orientation. Dr W. T. Keeton (Cornell University) reviewed the most recent evidence on the homing ability of pigeons and concluded that biologists simply do not understand how these birds are able to find their way home. Displacement activities (behaviours appearing "out of context" or at least where ethologists don't expect to find them) were discussed by Dr P. Sevenster (Leiden University) and Dr D. J. MacFarland (University of Oxford).

Professor N. Tinbergen (University of Oxford) stressed the importance of investigating not only the causation of behaviour but also its adaptedness—how it helps the animal to survive—and Professor K. D. Roeder (Tufts University) pointed out that this applies as much to physiology ("behaviour below the skin") as to ethology. As an illustration of the interaction between these two disciplines, he described the violent escape response of a sphingid moth to ultrasound, a response which he has recently found to depend on a novel type of sound receptor near the moth's mouth.

Dr R. P. Michael (Institute of Psychiatry) described work carried out at the Primate Research Centre at Beckenham which has revealed that the smell of certain substances in the vaginal secretions of female rhesus monkeys has the effect of dramatically increasing sexual activity in the males. Dr Michael is continuing this work on other species including humans.

For the first time, the conference included a representative from the Soviet Union. Dr D. K. Belyaev (Novosibirsk University) described the unexpected morphological and chromosomal changes that took place in a population of silver foxes selected primarily for behavioural traits.

During the afternoons three parallel sessions were held in different halls so that it was impossible for everybody to hear all of the hundred or so contributions. Judicious flitting from one room to another, however, did enable one to learn of the ability of electric fish to recognize the sex of other individuals on the basis of the frequency of their electrical discharge (Dr C. D. Hopkins, Rockefeller University), of the precision with which caddis fly larvae build their houses (Dr M. H. Hansell, University of Glasgow) and of the function of the "dance" that blowflies perform when they come across a drop of food (Dr M. C. Nelson, Tufts University), to mention just three from several noteworthy contributions.

Reactivity of Portland Cement Minerals

OF the two calcium silicates, which form the bulk of Portland cement, $3CaO.SiO_2$ is much more reactive than $\beta 2CaO.SiO_2$. Both these phases, however, develop similar ultimate strengths on hydration and, from the manufacturing point of view, cement richer in $\beta 2CaO.SiO_2$ is easier and cheaper to make than a cement richer in $3CaO.SiO_2$. Various empirical attempts have thus been made to increase the reactivity of $\beta 2CaO.SiO_2$ without gaining any fundamental understanding of the factors controlling the intrinsic reactivity of the minerals. In a communication to be published next Monday in *Nature Physical Science*, Ramachandran and Sereda have applied the Hedvall effect to gain some understanding of this difference in reactivities of $\beta 2CaO.SiO_2$ and $3CaO.SiO_2$.

$AgNO_3$ has a phase transition point at about 190° C. When a mixture of $AgNO_3$ and a reactive material, for example CaO, is cycled through this transition temperature a reciprocal pair forms very quickly. The authors have taken advantage of this fact in determining the reactivities of different minerals that may be present in Portland cement. The authors heated mixtures of $AgNO_3$ and an individual cement mineral (or fully hydrated $3CaO.SiO_2$) in a DTA (differential thermal analysis) apparatus and estimated the amounts of $AgNO_3$ used up to form reciprocal pairs in each case. From these data they calculated the quantities of Ca ions that took part in each case. For CaO and $Ca(OH)_2$, all the Ca ions took part in the reaction, but in the case of $3CaO.SiO_2$ and of $\beta 2CaO.SiO_2$ only 27 per cent and 6 per cent respectively of the Ca ions were involved. The presence of a higher proportion of more reactive Ca ions in $3CaO.SiO_2$ than in $\beta 2CaO.SiO_2$ seems to account for the higher reactivity of $3CaO.SiO_2$. The authors then infer that "in tricalcium silicate all Ca ions are not equivalent, a part of it being more reactive".

The origin of this high reactivity of some of the Ca ions in tricalcium silicate is not so obvious. In the structure of $3CaO.SiO_2$ (Jeffery, *Acta Cryst.*, **5**, 26; 1952), all the Ca ions are surrounded by six oxygen ions. In $\beta CaO.SiO_2$ (Midgley, *Acta Cryst.*, **5**, 307; 1952) half of the Ca ions have six oxygen ions as first neighbour and the other half have eight. Thus origins of the higher reactivities of some of the Ca ions in different calcium silicates seem to be extra-structural, and they may not be directly related to the intrinsic reactivities of different calcium silicates. The origin of the difference in reactivities of the cement compounds thus still remains unexplained.

PALAEONTOLOGY

Mammalian Evolution

from our Vertebrate Palaeontology Correspondent

THE nineteenth symposium on vertebrate palaeontology and comparative anatomy was held at Bristol on September 28–29, followed by two days of field excursions. The evolution of mammals, especially those of Africa, was a principal theme, because there is a large and active group at Bristol concerned with this subject. New work on the earliest known mammals, *Eozostrodon/Morganucodon* and *Kuehneotherium*, of the Triassic was described by Professor A. W. Crompton (Harvard University). Some workers have suggested that each of these two mammals gave rise to some of the groups of Jurassic mammals, but that they are so different from one another that they must have arisen from different lines of advanced mammal-like reptile—so making the mammals diphyletic. Professor Crompton strongly dissented from this view, pointing out a number of similarities between the two Triassic genera. He also noted that the epipterygoid of such cynodonts as *Probainognathus* is not greatly enlarged, indicating that this group could be ancestral to all the mammals, including the triconodonts (whose epipterygoid, unlike that of most other mammals, is small). An interesting discovery of early mammals in Britain was that of Dr M. Waldman (Stowe School). Though difficult to detect, and also difficult to remove from the hard matrix, his find of Middle Jurassic mammals in the Isle of Skye is of great potential interest, because the only other mammalian fauna of this age comes from the Stonesfield Slate of Oxford, which is no longer quarried.

Professor P. M. Butler (Royal Holloway College, London) suggested that the directions of jaw movement, as indicated by wear striations on the teeth, had passed through three principal phases in mammalian evolution. In Jurassic forms, the movement is partly transverse and becomes progressively more so, but the teeth shear past one another. Not until the Cretaceous do the teeth collide at the end of the jaw movement, so giving a crushing action. This in turn provided the possibility of a subsequent movement in another direction, so that two different sets of facets can be distinguished in the Tertiary herbivores and omnivores.

The studies of African mammals ranged from the problems of the phylogeny of the older forms to those of evolutionary rates and the comparison of later forms with those still extant. Mr G. J. Heal (University of Bristol) described sirenians which showed that two families of this order were already distinguishable in the Eocene, and Mr A. W. R. Wight (University of Bristol) described new specimens of Proboscidea and discussed the Eocene stratigraphy of Libya. Dr V. Maglio (Princeton University) analysed the rates of appearance and replacement of species in the Elephantinae and suggested that the group was in the middle of a phase of adaptive radiation when, in the Upper Pleistocene, there was a dramatic crash from eleven lineages to the present two. Dr A. W. Gentry (British Museum (Natural History)) compared the African Pleistocene antelopes with those of the continent today and pointed out that, because horn shape is a species recognition feature, the whole antelope fauna of any one time, within which such distinctions were essential, should be considered in any analysis.

At the other end of the chordate evolutionary story, Dr R. P. S. Jefferies (British Museum (Natural History)) described a new mitrate echinoderm which he believed showed evidence of being ancestral to the cephalochordates. Professor D. L. Dineley (University of Bristol) gave an interesting account of the Late Devonian fish fauna of Escuminac Bay, Canada, and Mr D. S. Broad (University of Bristol) described new heterostracans from Arctic Canada which showed evidence of the growth of each plate by accretion around a small number of original tubercles. The problem of interpreting the patterns of tetrapod tooth replacement was discussed by Dr R. DeMar (Liberal Arts College, Chicago), who showed that Edmund's Zahnreihe is only one of a number of theoretically possible alternatives, and that computer studies can show why some of these are not acceptable, because they lead to lengthy gaps in the tooth row.

Dr A. C. Charig (British Museum (Natural History)) pointed out that reptiles colonized more new ways of life in the Triassic than ever before or since, and suggested that this might be more than mere coincidence—though other possible reasons were not obvious. A valuable approach to the study of pterosaurs was presented by Dr G. R.

Visualizing Agglutinin Sites on Cell Surfaces

DURING the past several months the suggestion that transformed cells are more readily agglutinated by plant agglutinins than their untransformed counterparts because transformation leads to an increase in the number of agglutinin-binding sites exposed on the cell surface has been abandoned. For experiments such as those reported by Cline and Livingston and Ozanne and Sambrook in *Nature New Biology* (**232**, 155, 156; 1971) have shown that there is apparently no significant difference between the numbers of these binding sites on the surfaces of transformed and untransformed cells. These workers have suggested therefore that it is the distribution of agglutinin-binding sites rather than their numbers which is changed by transformation and in next Wednesday's *Nature New Biology*, Nicholson reports observing in the electron microscope just such a change; after transformation by SV40 virus the concanavalin A binding sites on the surface of mouse 3T3 cells are clustered whereas before transformation they are dispersed.

Nicholson was able to make these most elegant observations by preparing ferritin conjugated concanavalin A and then devising a new technique for preparing the cell surface membrane for electron microscopy. His technique involves first "strengthening" the cell membrane by briefly incubating the cells in 0.1 per cent formaldehyde before lysing them on an air–water interface and then picking up the membrane on electron microscope grids. Once on the grids the membranes can then be stained with the ferritin tagged concanavalin A.

The micrographs of stained membranes from transformed and untransformed 3T3 cells reveal at a glance the striking difference in distribution of binding sites. Single ferritin tagged concanavalin A molecules are randomly distributed on the membranes of the untransformed cells. By contrast the tagged concanavalin A molecules are in clusters, which are randomly distributed, on the membranes of the cells transformed by SV40. Although there are some 3 to 3.5 times more molecules of bound concanavalin A per unit area of transformed than per unit area of untransformed cell membrane, Nicholson points out that the total surface area of the transformed cells is only about half that of their untransformed counterparts.

Nicholson has also shown that after exposure to weak trypsin solutions, the concanavalin A sites on the surface of untransformed cells tend to be clustered, but within a few hours of enzyme treatment the sites assume their normal distribution.

There could therefore scarcely be a clearer demonstration of the fact that transformation causes a change in the distribution of these agglutinin-binding sites, which presumably accounts for the increased susceptibility of these transformed cells to agglutination. Whether this particular surface change also alters the social behaviour of cells remains to be seen.

Whitfield (University of Reading), who outlined the way in which theoretical aerodynamic studies and conventional anatomical studies can combine to throw new light on the functional anatomy of *Pteranodon.*

In two studies on modern reptiles, Dr G. L. Underwood (City of London Polytechnic) explained a dendritic classification which provides a very satisfactory picture of the evolution of boid snakes, and Mrs L. Sheppard (St Mary's Hospital, London) showed that the joints between the neural arches in lizards and snakes contain menisci, and suggested that these provide a flexible and compressible element needed in joints which permit movement in more than one plane.

SCHIZOPHRENIA

Biochemical Aspects

from a Correspondent

THE first joint meeting of the schizophrenia associations of Canada, America and Great Britain was held in London on September 28 and 29. Work on the genetic basis of schizophrenia was reviewed by Mr J. Shields (MRC Psychiatric Research Unit, Maudsley Hospital, London) in relation to current hypotheses and he concluded that the evidence for the existence of genetic factors rests on general arguments. Dr L. R. Mosher (National Institute of Mental Health, Bethesda), referring to a study of identical twins discordant for schizophrenia, reported having found different patterns of interaction between one parent and one twin (who was later to become schizophrenic). The twin as a result was repeatedly less favoured and a relationship was established with a contradictory and incomplete role expectation and without cognitive and communicative clarity.

Schizophrenia is a changing concept, said Dr D. Richter (MRC Neuropsychiatry Unit, Carshalton); it has altered as various subgroups have been isolated from the chief group of schizophrenias. Dr A. P. Ridges (University of Liverpool) emphasized the need for a definition of the criteria used in selecting patients and conducting experiments under carefully controlled conditions. She supported Dr Richter and said that altered activity of several enzymes or more probably isoenzymes could finally affect common metabolic pathways producing the symptoms of schizophrenia. Pink spot excretion seems to be associated with an altered catecholamine metabolism in schizophrenia. She reviewed other biochemical considerations including the suggested association between coeliac disease and schizophrenia.

The possible central effects in schizophrenia were considered by Dr A. Randrup (St Hans Hospital, Roskilde), who presented evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. Professor J. Yaryura-Tobias (University of Buenos Aires) considered that the basal ganglia are of prime importance in the aetiology of schizophrenia. Dr K. Smith (Washington University) reported that she has found trans-3-methyl-2-hexenoic acid in schizophrenic sweat. This finding has not yet been confirmed independently by other workers nor has its presence in other psychiatric patients been investigated. The adrenochrome hypothesis has evolved into the aminochrome-melanin hypothesis. Dr M. Altschule (Harvard Medical School) said that these unstable compounds are rapidly converted to melanin by everybody and, regardless of whether schizophrenics make more aminochromes or not, the fact is that at least one of their tissues, blood, is abnormally sensitive to the toxic effects of these compounds.

The term "orthomolecular psychiatry" introduced by Professor L. Pauling in 1968 has taught American psychiatrists to appreciate a principle well known to scientists. Dr R. Maclean (Vancouver) defined orthomolecular psychiatry as the treatment of mental disease by the provision of the optimum molecular environment for the mind especially the optimum concentrations of substances usually present in the body. Reports which followed testified that large doses of vitamins together with other therapy can improve the functioning of the brain in a variety of conditions. Successes with both children and adult psychiatric patients were reported by Dr A. Cott (New York) and Dr D. Hawkins (Nassau Medical Health Research Center, Long Island). Dr A. Hoffer (Saskatoon, Saskatchewan) outlined the treatment which is based on administering vitamin B3 in combination with other vitamins. The over-regulating mechanism of the body in various forms of stress results in hypoglycaemia and although this is not specific for schizophrenia it is common in schizophrenics and ought to be treated, said Dr R. Meiers (Belmont, California).

REACTOR TECHNOLOGY

Structural Mechanics

from a Correspondent

REACTOR technology requires a synthesis of many skills. The ability to deal with the structural mechanical problems which arise is vital to achieving economic, reliable and safe exploitation of nuclear power. For the first time, largely due to the inspiration of Professor Dr Ing T. A. Jaeger (Bundesanstalt für Materialprufung (BAM)), more than nine hundred delegates from the many disciplines involved in reactor technology met in West Berlin from September 20 to 24 to discuss these problems.

Introductory lectures allowed comparisons to be made between the different strategies applied to power reactor development within the European Community, in the United States and in the United Kingdom. The development of the different types of reactor systems was traced with particular emphasis on the ensuing mechanical–structural requirements.

The rapid progress of reactor technology has given rise to many structural problems which are consequent upon the use of sophisticated materials for various reactor system components. These components are required to operate under severe loading and hostile environmental conditions, and the analyses that must be made range from fuel element and cladding performance under mechanical thermal and irradiation, containment requirements, involving prestressed concrete steel lined vessels or heavy section steel vessels, to dynamic response characteristics of complete power installations. This stimulus has resulted in tremendous strides being made in the area of computational methods. It was underlined by the work of Professor J. H. Argyris (Stuttgart University) and others who have found that with the present generation of large computers it is quite possible to analyse almost any component, however complex, under virtually any loading conditions.

The basis of this development is the "finite element" method which, as the name suggests, splits up the problem into a large number of simpler standard elements which are combined in a suitable way. It has frequently proved to be the only feasible method in complex situations. There are, nevertheless, obvous limitations on cost and there remains the implementation of these procedures (or others) to produce the information required for design. Unfortunately analyses seem to have progressed faster than information on material response and it is not yet agreed how the working life of a structure can be predicted on the basis of failure criteria particularly in complex situations where irradiation damage, fracture and creep may interact. Similarly, analyses can only be as good as the input data they receive for situations such as accident conditions, earthquake disturbances or blast loading. It was emphasized by Professor R. K. Penny (University of Liverpool) that material and component testing of the correct type is essential and should be an interactive process with analyses in the overall optimization of design problems.

Politics of Nuclear Power in the United States

RICHARD WILSON
Department of Physics, Harvard University

In this article the issues surrounding nuclear power generation in the United States are critically examined and some of the misunderstandings are clarified.

NUCLEAR power came of age in the United States in about 1967. At that time about fifty large, new nuclear power stations were started. This dramatic switch from fossil fuel power came because the development of the power reactors had reached a point at which the cost became competitive with fossil fuel power. Two commercial experiments had been constructed and had worked well since 1961—a pressurized water reactor (PWR) at Rowe, Massachusetts, and a boiling water reactor (BWR) at Dresden, Illinois. In spite of rising construction costs, nuclear power remains competitive; oil and gas are beginning to run out in the USA and their prices are rising rapidly.

The American public was, however, slow to react. Scientists who had been arguing in favour of nuclear power—because it is cleaner than fossil fuel power—were pleased but took no particular notice or action. But during 1970 the public became alarmed as the full extent of the US nuclear power programme was made apparent. Until a few years ago, few public hearings in the USA on any subject were well attended and construction permits were obtained for these power stations without too much difficulty. But in 1970 the public began to question the wisdom of these plants both in hearings dealing with construction permits and in those dealing with operating licences.

The reactors being installed in the USA at present are of two types, both using enriched uranium fuel (up to 7% ^{235}U) with water as a moderator. In the PWR, water heated by the reactor produces steam in a heat exchanger inside the containment vessel and there is little release of radioactive gases. But, to make them easy to control, boron is added to the primary water in quantities which diminish as the fuel is consumed; this produces tritium which is released and added to the water effluent. The heat exchanger is omitted for reasons of economy in the BWR and the steam condenser after the steam turbine must be evacuated to achieve high efficiency. Air enters the condenser through leaks in seals and must be released, carrying with it a little radioactivity. The thermal efficiency of these stations is about 30%, compared with 40% for the advanced gas cooled reactors.

The first attacks on the nuclear power industry can be considered as a continuation of the attacks on the Atomic Energy Commission (AEC) because of weapons testing; the hazard to the general population due to radioactivity from weapons had been dramatized by Linus Pauling, Ernest Sternglass and others. The dose commitment from past tests (to the year 2000) of the US population is about 60 mrem (about 2 mrem a year). This is small, and it could be argued that it is tolerable but most of the scientists who were willing to argue in this way wanted to stop weapons tests anyway. Now the situation is different—Dr Sternglass has been joined by Dr John W. Gofman of the University of California and applies the same arguments to nuclear power. Dr Gofman argues[1,2] that if the whole population were exposed to the maximum allowed radiation as given by the International Commission on Radiological Protection (ICRP) guidelines (whole body dose 170 mrem per year), then between 9,000 and 110,000 more cancer deaths would be caused each year (depending on his assumptions). It seems probable from the recent work of Dr Alice Stewart at Oxford[3] that the unborn foetus is much more sensitive to cancer than is the adult; the probability of cancer induction also seems to remain linear with dose even at low doses. And cancer cases keep appearing for years after the insulting radiation dose. These findings seem so reasonable that, once understood, they are quickly accepted, although some would quote numbers a factor of five smaller than those of Gofman[4]. Dr Gofman's attacks on the AEC were made in a polemical style which delayed recognition of the issue, and brought forth an inept response from the AEC. Dr Storer[5], a pathologist, argued that there is non-linear behaviour at low doses—a proposition unsupported by reliable experiment and contrary to sound public policy. Moreover, the AEC unnecessarily defended the ICRP guidelines, and failed to define their admonition "as low as practicable".

Restricted Releases

The real answer is only just becoming apparent. Nuclear power now produces much less than 170 mrem average exposure a year. After intervention by scientists in Minnesota (led by Dr Dean Abrahamson) the Monticello reactor is being operated with releases restricted to less than 10% of the AEC regulations (10 CFR/20) and a simple computation shows that the average dose to the public within twenty miles will be less than 10^{-4} of 170 mrem a year. The State of Minnesota supported this restriction and tried to enforce it legally; Congress had, however, specifically given the AEC the right to set radiation regulations for nuclear plants, and this has been upheld by the US Federal Court. But in practice Minnesota will have its way. In Vermont, the Vermont Yankee Power Company signed an agreement with several conservation groups to abide by the lower standards of the Vermont Bureau of Public Health. Even so, Vermont Yankee is now entering what promises to be a long operating licence hearing; operation of the power station was scheduled for December 1971 and will certainly be delayed at a cost of $80,000 a day.

At Plymouth, Massachusetts, the Advisory Committee of the AEC on Reactor Safeguards has insisted on the earliest possible installation of a holdup filter to stop gases with short half lives. All existing BWRs will now be modified in a similar way. In May the AEC introduced new design criteria for light water reactors; the design must limit the worst fence post dose to 10 mrem per year, and the designed equipment must be used. Only two old reactors fail to meet this criterion now. The newer ones give lower radiation by a factor of as much as 10. The reprocessing plants are planning to hold up even ^{85}Kr within a few years.

The AEC is not the only agency to hide behind the ICRP and is not the worst offender. In spite of excellent practices by some of the leading hospitals, the average radiation dose to Americans due to diagnostic X-rays was 95 mrem in 1970 (ref. 6), twice the 1964 dose and seven times the average British dose. The American Medical Association has been silent.

Thermal Problems

Environmental groups have forced through important changes in thermal pollution standards. This is an old story in England where the River Trent, for example, which is small and sluggish by US standards, has 12,000 MW of installed generating capacity, more than any comparable river in the USA; cooling towers abound. Not only do nuclear power stations give out twice as much heat as fossil fuel power stations, but nuclear power is at present less efficient and there is no loss of heat up the chimney.

The first nuclear power station where this problem was faced was Connecticut Yankee at Haddam's Neck near the mouth of the Connecticut River. Cooling is done with river water, but a study of the effects of the heating is under way[7]. Other power companies have not been so fortunate; further up the Connecticut River at the Vermont Yankee station mechanical draught cooling towers which can work on a closed cycle have had to be installed. In the region of the Great Lakes, the Lake Michigan Environmental Conference wishes to set and enforce a rule allowing a maximum water temperature rise of 1° F. This matter involves many states and is being considered by the director of the newly formed US Environmental Protection Agency (Mr W. Ruckelshaus). Some scientists think the rule is extreme and unnecessary[8]. Lake Michigan, for example, has an area of 10,000 miles^2, and the heating from each power station is equivalent to solar heat over 1 mile^2. If the water is spread over the whole surface of the lake, all power stations up to the year 2000 could be accommodated[9] with a temperature rise of only 0.1° F, so the problem becomes one of achieving this uniformity. Particularly at issue is the fate of eight power stations under construction—the power companies estimate a cost of $200 million to add cooling towers at this stage.

The AEC was sensitive to criticisms of excessive political power and had no expertise in this field; hence it tried to avoid considering thermal pollution. It tried merely to "certify" the state or other federal standards and to consider only radiation and reactor safety in its hearings. Many people have tried to persuade the AEC to "hand over" all the hearing and compliance procedures to the Environmental Protection Agency. The Environmental Protection Act, as interpreted in July by the US Court of Appeals in the consideration of a nuclear power plant at Calvert Cliffs in Chesapeake Bay, insists, however, that the AEC has the right and duty to control all environmental matters. This will involve more delays—especially for those power stations which are now involved in operating licence hearings. It is ironic that conservation groups have forced the AEC into taking on more political power at a time when others accuse it of having too much.

Accidents and Safety

The third issue, least emphasized but most important politically, is that of safety. Each reactor makes enough plutonium in a year for thirty atom bombs, and radioactive material in proportion. Could an accident occur which would spread this over the countryside? Clearly a direct hit from a hydrogen bomb would do so, but what else?

If there is a catastrophic pipe failure in a light water reactor (which in itself is unlikely ever to happen) the depressurization (blowdown) will lead to loss of coolant (and moderator). Emergency core cooling must immediately be applied to prevent fuel melting and release of radioactivity. In a typical PWR 17,000 gallons of borated water held under pressure would immediately be injected into the vessel. But small scale (9 inch instead of 18 foot) tests held at the National Reactor Testing Station at Idaho in November of 1970 showed that the water injected during the blowdown fails to stay. Although calculations suggest that the system behaves better in the full scale reactor, the AEC first stalled on all new operating licences, and then in June issued tighter standards. The utility company must prove that the reactor can cope with a double-ended guillotine break, even if all water injected during the blowdown is lost. Superficially this does not affect the BWRs or the two helium gas-cooled reactors, but a few scientists argue[10] that none of the old safety arguments can be trusted, and that no new reactors should be licensed until a new independent study has been made.

This aspect of nuclear power is probably the least well comprehended. To understand this, we must understand other serious accidents. One can talk about the 88,000 direct coal mining deaths since 1907 (in addition to black lung and various other diseases) or the 2,000 people killed when a hydroelectric dam gave way near Belluno, Italy, in 1963, but there is no public understanding of such comparisons and even less acceptance. The US public will probably not accept nuclear power finally until there is a complete understanding of all types of accident—and an understanding which can be simply expressed.

The power companies are now becoming reconciled to long hearings on any power station. The Long Island Lighting Company has been trying to obtain a construction permit for a BWR at Shoreham, but this has already taken 12 months of hearings in spite of the help of some local citizens who work at Brookhaven National Laboratory. The President's Office of Science and Technology[11] has recommended that site searches take place in public over several years. This is a good development, but the USA will be faced with power shortages in the next few years while the power companies adjust to the new situation. Westinghouse is now designing reactors on barges to be floated 20 miles offshore.

The AEC, for its part, must quickly undertake the vital task of restoring public credibility. As a result of many factors, and in particular the SST and the Vietnamese war, the American public have learned to distrust the experts in Washington. The AEC has been talking for twenty years, but few members of the public have been listening. Now people are listening but the AEC must learn how to talk clearly and simply.

One possible consequence of failure to reorganize promptly is apparent in New York. Faced with forthcoming power shortages, and a completed nuclear power station which is not allowed to operate, Mayor Lindsay has overruled many of his advisers, and has allowed the installation of 2,000 MW of gas turbine power on the East River in New York, which now operates every hot summer afternoon. These are little better than jet engines blowing against windmills. The disadvantages in noise, thermal pollution, and consumption of expensive scarce fuels are obvious.

In Midland, Michigan, where a nuclear power station is held up by intervention in public hearings, Dow Chemical Company announced the transfer of part of its operation to New Orleans where power is cheaper. Local citizens took out a full-page advertisement attacking the interveners. Conservation societies now have great political power in the US—particularly on nuclear questions; they must use this power wisely.

[1] Gofman, J. W., *Testimony in Hearings Before the Joint Committee on Atomic Energy, Ninety-first Congress of the United States, Second Session on Environmental Effects of Producing Electric Power*, Part 2 **(1)**, 1388 (1970).

[2] Gofman, J. W., *Proc. April Meeting of the American Physical Society, Washington DC* (1971).

[3] Stewart, A. M., and Kneale, G. W., *Lancet*, i, 1185 (1970).

[4] Hamilton, L. D., *Proc. April Meeting of the American Physical Society, Washington DC* (1971).

[5] Storer, J. W., *Testimony in Hearings Before the Joint Committee on Atomic Energy, Ninety-first Congress of the United States, Second Session on Environmental Effects of Producing Electric Power*, Part 2 **(1)**, 2186 (1970).

[6] *US Public Health Service Report* (in the press).

[7] Merriman, D., *Sci. Amer.*, **222** (5), 42 (1970).

[8] Gustafson, P. F., *Bulletin of the Atomic Scientists* (March 1970).

[9] Asbury, J. G., *Effects of Thermal Discharges on the Mass/Energy Balance of Lake Michigan* (Argonne National Laboratory Report ANL/ESI).

[10] Union of Concerned Scientists, Cambridge, Mass., press release (July 1971).

[11] *Electric Power and the Environment* (Office of Science and Technology, 1970).

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA.

The Oxford Botanic Gardens: 1621 to 1971

C. D. DARLINGTON
Botany School, University of Oxford

On July 24 this year, the 350th anniversary of the old Physic Garden in Oxford was celebrated by the university. A guide to the old and new gardens has been published by the Oxford University Press and an exhibition set up in the Divinity School to mark the event.

LORD DANBY, the founder of the Oxford Botanic Garden, was one of the great adventurers of the Elizabethan age. Sir Philip Sydney took him as a page on his fatal expedition to the Netherlands in 1586 and soon afterwards he was exiled by Elizabeth to France for a murder; his brother was later beheaded for rebellion. This was the violent phase when he served as the model for the Romeo of Shakespeare[1]. But after these youthful indiscretions, Henry IV of France befriended him, James I made use of him, and in good time he found himself an Earl and a Knight of the Garter. It is in this respectable phase that his bust appears adorning the Inigo Jones gateway of his garden (Fig. 1).

Danby was prompted to set up his garden in Oxford by Thomas Clayton, the professor of medicine. But no doubt it was his own experiences which made him demand a wall to defend the garden against friends and foes as well as against frost. The wall took twelve years to build and proved strong enough to meet all these dangers. Civil war in the seventeenth century, a bridge and road scheme in the eighteenth century, and an expansion plan in the nineteenth century were all withstood by Danby's wall.

Jacob Bobart had been given a 99-year contract as keeper of the garden for himself and his heirs in 1637, a contract which allowed a far-sighted policy. By 1641 he had produced the necessary heir and by 1648 he had issued a catalogue of the 1,600 plants that he had collected. Many had been grown from seed sent by other gardens and, in about 1666, he or his son raised the hybrid plane and recognized it as a hybrid. It is now known as the London plane but its finest specimens are still to be seen in Oxford.

Evidently the garden flourished for the 82 years that it was managed by the Bobarts, in spite of financial difficulties. Loggan in 1675 shows their orangery and the four gated enclosures which they had made for their beds. Here Pepys and Evelyn came with other inquisitive visitors; here the physicians learnt their drug plants, among them Radcliffe who built the Camera with the profits of his profession. Here, too, Robert Hooke must have sought out the plants for his *Micrographia*. And here the younger Bobart began his collaboration with Robert Morison[2].

Morison came from Aberdeen, fought for Charles II and followed him into exile, and then studied several sciences in Paris. He was uncouth in appearance but the King liked him and, after his restoration, made him his own physician as well as his own gardener. Sponsored by Charles in 1669 he undertook the additional duties of professor.

Morison brought with him from France several "striped" herbs and trees including the mottled sycamore. They still survive in the garden, embodied in the great classified collection of variegated plants. Next, in collaboration with the younger Bobart, he began a detailed account of the flora of Oxfordshire; the joint experience of the two men of raising plants from seed gave them a new point of view. Their recognition of the pro-

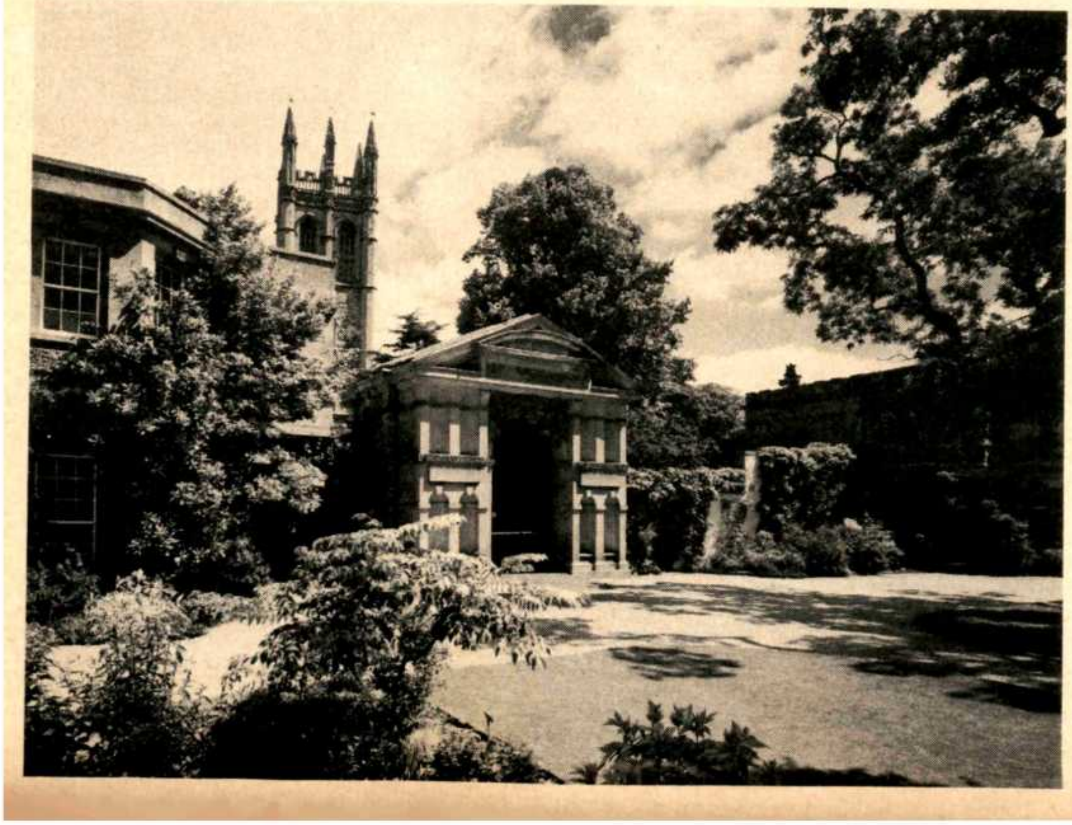


Fig. 1 The Inigo Jones gateway

cesses of sexual reproduction (which they shared with John Ray) led them to use the reproductive rather than the vegetative organs as the basis of classification and helped to make explicit the daring idea of genetic relationship, the slow advance of which was to cover the next three hundred years.

When Morison died prematurely in London in 1683, knocked down by a coach in the Strand, his salary, which was to have been paid by the King, had long been in arrears. The younger Bobart then became the unpaid professor and had to complete their joint work alone. It was this devoted work which must have attracted the attention of William Sherard, then an undergraduate in law at Oxford. Later Sherard in his capacity as British consul at Smyrna from 1703 to 1717 acquired a fortune and was able at last to indulge his botanical interest. He then began to make extensive journeys collecting plants, a library and, most important of all, a collaborator—Johann Dillen or Dillenius.

Dillenius was one of a succession of German botanists who excelled as draughtsmen and, by their comprehensive labours, made it possible to establish the systematic ideas of Ray, Morison and later Linnaeus. Sherard first installed Dillenius as Foreign Secretary of the Royal Society and then set to work to establish him as professor at Oxford, with an endowment both for the chair and for the garden. He was careful to exclude the danger, endemic in Oxford at that time, of any connexion with the Church.

The immediate fruit of Sherard's gift was the production of Dillenius's *Historia Muscorum*, the most thoroughly illustrated early account of the lower plants; in this way the practice of plant illustration took root in Oxford. The next professor, Humphry Sibthorp, did little himself. He may, have had Joseph Banks in the audience of his single lecture but we have to suppose that it was the garden and not the lecture that later inspired Banks to establish the Royal Gardens at Kew. Sibthorp's artistic interests are shown, however, by his acquisition not only of seed of the Chinese ginkgo but also of a Chinese painting of the tree, now on show at the Divinity School in Oxford. Accordingly it was a botanical artist, Georg Dionysius Ehret, that Sibthorp engaged as head gardener. Ehret stayed only a year for he would not take orders from Sibthorp. He made his way to London, but his descendants left their mark in Oxford. The fourth generation gave us Sir Arthur Evans, Keeper of the Ashmolean Museum, by whose enterprise the plant decorations at Knossos were to be discovered.

The urge to collaborate with artists and to seek them out abroad descended to Sibthorp's young son for whom he made way in 1784. For John Sibthorp arrived in Vienna to find that the fifth century illustrations of Dioscorides had recently been brought from Constantinople and, by order of Maria Theresa, had been engraved and some twenty-five copies printed. Equipped with one of these and with ample funds, he was accompanied by yet another German artist, Ferdinand Bauer, as he made his way to Greece with the object of having every plant in the classical catalogue drawn again from nature and printed and published in the modern style. He and Bauer later extended this original purpose, first to include the general flora of Greece and then the fauna as well.

Before this plan could be fulfilled Sibthorp died after only two journeys in Greece. But he had endowed the publication of his work. Bauer completed the illustrations, and after forty-four years all the 960 coloured plates had appeared in the ten volumes of his splendid *Flora Graeca*.

It was now 1840, however, and times had changed. The study of physic and practice of draughtsmanship had ceased to be the mainsprings of plant science. New possibilities had arisen and a new kind of teacher appeared in Oxford to seize the opportunity. This was Charles Daubeny, an amiable man who was willing to expound any science known at the time—except theology. He was elected in succession to the chairs of chemistry, botany and rural economy and he held them concurrently. Quick to learn, it was said he had picked up what botany he needed from the De Candolles during a few months in Geneva. He was neither profound nor original and he did not know enough to be pedantic. But he was willing to try other people's ideas and eager to teach them. The result was that he and his contemporary Acland proved to be the most effective of university innovators.

The young Daubeny realized the value of Liebig's experiments with mineral fertilizers as soon as he heard of them. He at once tried them out on plots in what he called the Botanic Garden. His pupil John Lawes took the idea home with him and founded Rothamsted. Later, an older Daubeny at once saw the merits of evolutionary theory and when the British Association came to Oxford in June 1860 it was Daubeny who opened the Darwinian debate. The title of his paper (*On sexuality in plants*) pointed back to Morison and forward to the present day[3]. And it was Daubeny who concluded the debate with a party that evening at his house in the Botanic Garden: in the heart, as we may say, of enemy country.

Daubeny and Acland saw the need for unifying the sciences and fitting them into a larger scheme of education. It was a development of this vision when, within a few years of his death in 1867, Lawson and Lankester were joining together to teach a course in biology in the Botanic Garden. But this development was not to prosper. The craving for specialization in teachers, the demand for independence of professors, but above all the great fear in the university of expanding science, turned the current of opinion in the opposite direction. In these circumstances the university appealed to Dr Hooker[2], the director of Kew, for guidance. It was relieved to learn from him that botany in Oxford could well stand by itself and could stand for ever on the four acres of the Botanic Garden.

With this guidance, teaching as well as research fell into decay. The attachment of botany to medicine, which could have carried both subjects into a creative experimental period (putting Mendel and Garrod together), remained static for a further eighty years in the forms of an ancient syllabus. In 1883 Lawson emigrated and the Sherardian Chair fell vacant; once again there was no paid teacher or manager in the garden. In 1901 Daubeny's now useless experimental field was sold. And appropriately, forty years later, the superintendent's seed list was still titled in Latin and his office was still lit by the light of a single candle.

Today these setbacks and holdbacks can be seen from a happier position. In 1944 the garden itself was doubled in size and on the new ground there has been arranged a collection of roses whose history of hybridization and selection is now defined by pedigree and also by chromosomes. In 1954 a Genetic Garden, now in the University Parks, was added to display, with materials both for teaching and research, not only the results but the processes of evolution. And in 1969 an anonymous benefactor put a third garden at the disposal of the university, the established and now rehabilitated arboretum of Nuneham Courtenay.

We can now look back at the contrasted succession of naturalists and experimenters who have worked and taught in the Botanic Garden. Through them runs the continuity of the garden itself with its collections of plants, its series of glass-houses (now rebuilt for the seventh time) and its long line of gardeners. Notable among these are the Bobarts and the Baxters—fathers and sons—whose skill has been passed on from generation to generation and who have maintained the garden, disseminating their knowledge and materials worldwide through catalogues, exchange lists and guides. In the future, even more than in the past, we may expect that the practical wisdom of the garden and of the gardeners will continue to hold together the branching strands not of one but of several sciences.

---

[1] Rowse, A. L., *The Times*, July 24 (1971).

[2] *Makers of British Botany* (edit. by Oliver, F. W.) (Cambridge University Press, 1913).

[3] Daubeny, C. G. B., *Dictionary of National Biography* (1900).

General Model for the Structure of all Myosin-containing Filaments

J. M. SQUIRE[*]

Biophysics Institute, Aarhus University, University Park, DK-8000 Aarhus

It is suggested that the way in which the molecules are packed is common to all types of myosin filament. This leads to quantitative predictions about the component molecules.

It has been known for some time that thin actin-containing filaments, all of basically the same structure, occur in all the types of muscle so far studied[1-6]. These muscle types include vertebrate striated muscle (VStM), vertebrate smooth muscle (VSmM), insect fibrillar flight muscle (IFM) and molluscan muscles (MM). On the other hand, it is generally accepted that the myosin-containing filaments in the muscles mentioned are structurally quite different[5-11] apart from the following points of similarity. (i) All the myosin filaments are known to have an axial repeat of about 144 Å. (ii) It is thought that the heavy meromyosin portions[12] of the myosin molecules which form the filaments project from the surfaces of the filaments to form cross-bridges which can interact with neighbouring actin filaments[8,13]. Evidence of such projections has been obtained directly by electron microscopy (refs. 10, 11 and 15–17 and J. V. Small, and J. M. Squire, in preparation) and has been deduced from X-ray diffraction studies of intact muscle[13,14,18,19].

The apparent differences between these myosin-containing filaments are considerable. (i) The filaments can either be cylindrical (VStM, IFM and MM) or ribbon-like in shape (VSmM)[9]. The cylindrical filaments vary greatly in diameter and length although they probably all taper at each end, there is probably end to end polarity of the cross-bridges and probably a bare zone half way along the filaments[20]. The VSmM myosin ribbons have opposite polarity on opposite faces (ref. 17 and J. V. Small, and J. M. Squire, in preparation). (ii) Those myosin-containing filaments which have been analysed structurally (VStM, IFM and VSmM) have projections arranged on nets which apparently have quite distinct symmetry and spacings (except for the common 144 Å axial period) in the different types of filaments. In VStM the myosin projections are thought to be arranged on a two-strand 6/1[†] helix of repeat 429 Å (ref. 8), in IFM[11] they are thought to be arranged on a two-strand 16/3 helix of true repeat about 1150 Å and in VSmM the projections are thought to be arranged on each face of the ribbon in a lattice of approximate dimensions $a=100$ Å (across the ribbon face), $b=150$ Å and $\gamma=74°$ which corresponds to an axial repeat of about 720 Å (ref. 17).

The relationship between projections in these models of the different filaments is shown in Fig. 1. In VSmM it is very probable (J. V. Small, and J. M. Squire, in preparation) that each lattice point in Fig. 1*a* is associated with the origin of the heavy meromyosin portion of one myosin molecule. In biochemical estimates of myosin content for VStM[20] it seems that there may be two myosin molecules associated with each lattice point (see ref. 8, page 418). In insect flight muscle the estimates of myosin content suggest that there are either two molecules per lattice point which seems to agree with Reedy's observations of transverse sections of insect muscle in the electron microscope[11] or there are three molecules per lattice point[21].

It is known that the myosin molecules extracted from different muscles, although different in some respects, have basically the same form[12] and approximately the same molecular weight[5,22]. They consist of a two-chain α-helical rope in the rod portion of the molecule which is linked at one end to a globular unit (the myosin head)[20,23,24] comprising two large globular subunits[12] which may be associated with several light chains[25,26]. One might ask why these similar molecules pack together in such different ways in different muscles. On the other hand, one might consider whether there are alternatives to the existing models[8,11,27,28,30] for the structures of these filaments which will satisfactorily account for the published experimental data and in which the packing of myosin molecules is basically the same in all types of myosin-containing filament. This communication represents the first step in the exploration of such an idea.

Clues to Basic Myosin Filament Structure

I have quoted results from the analysis of the structure of the myosin-containing ribbons in VSmM[9,17]. The ribbons and their cross-bridges have been visualized directly by electron microscopy. Optical diffraction analysis of micrographs of thin longitudinal sections in which the planes of the ribbon and the section were parallel has given direct information about the cross-bridge lattice on the faces of the ribbons (ref. 17 and J. V. Small, and J. M. Squire, in preparation). Fig. 1*a* shows the form of this lattice. We can therefore start with this evidence and use it to help to deduce alternative structures for the other myosin-containing filaments.

---

[*] Present address: Biophysics Department, King's College, 26–29 Drury Lane, London WC2.

[†] The notation m/n helix has been used throughout to denote a helix which repeats after n turns in which there are m residues.

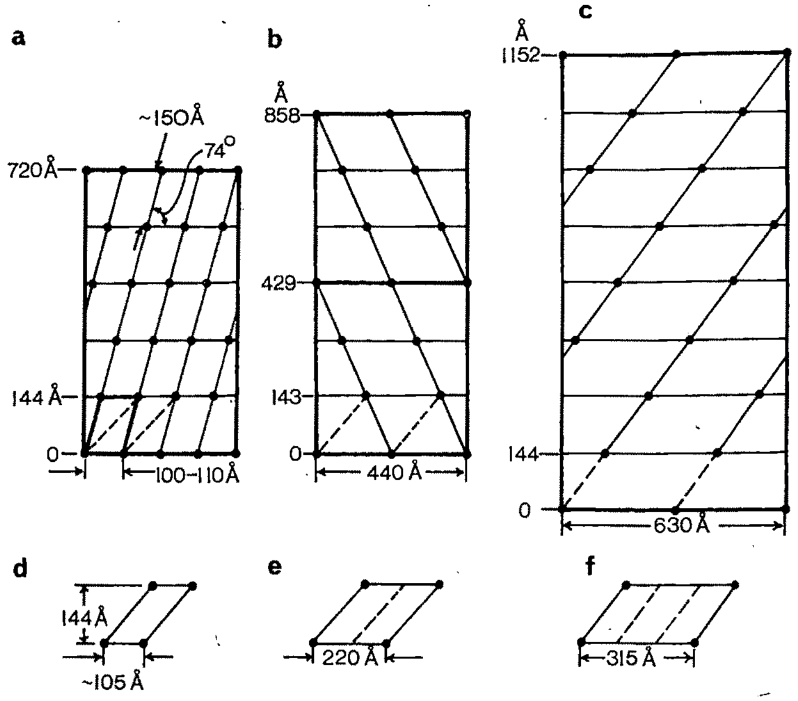


Fig. 1 *a*, The probable lattice of cross-bridges on each face of the myosin ribbons of vertebrate smooth muscle (ref. 17 and J. V. Small, and J. M. Squire, in preparation). *b*, Radial projection of the cross-bridge lattice in the model of the myosin filaments in vertebrate striated muscle proposed by Huxley and Brown[8]. *c*, Radial projection of the model for the cross-bridge lattice on the thick filaments of insect flight muscle proposed by Reedy[10]. *d*, *e* and *f* show repeating units in *a*, *b* and *c* respectively and demonstrate how these units are related.

First of all, if we take two of the repeating units (Fig. 1*d*) on the ribbon lattice shown as a dashed line in Fig. 1*a* we generate a repeating unit similar to that in the two-strand 6/1 helix of VStM (Fig. 1*b* and *e*). It is thought that there are probably two molecules per lattice point in the VStM filaments on the basis of two-strand 6/1 helical symmetry[8]. This suggests that a four-strand 6/1 helical structure (Fig. 2) with one molecule per lattice point (as for the ribbons) might be a possible alternative arrangement to the two-strand 6/1 helix. Such a four-strand 6/1 helix would generate the observed repeat of 429 Å, it would have a residue repeat of 143 Å giving the observed meridional X-ray reflexion and in projection at right angles to the filament axis (that is, the view obtained in longitudinal section of muscle[29]) it would appear almost identical to the same projection of a two-strand 6/1 helix. The only difference in the X-ray diffraction pattern would be that the intensity distribution on layer lines other than the third, sixth, and so on would relate to J_4 and higher order Bessel functions but not to J_2 Bessel functions as for the two-strand 6/1 helix. Using the intensity profile on the first layer line given by Huxley and Brown[8] it is possible to estimate (without attempting to fit this curve exactly) that a J_4 Bessel function in this region would relate to a helix of radius about 160–170 Å. These layer lines arise solely from the arrangement of cross-bridges on the myosin filament[8] and most of the mass of the cross-bridges is located in the heavy meromyosin regions which form the bridges. It is therefore not unreasonable that most of the diffracted intensity on first and second layer lines should relate to a radius of 160–170 Å which lies between the surface of the filament backbone (radius about 70 Å) and the surface of the actin filaments (approximate radius 200 Å)[8]. In this case it would seem that the myosin heads are quite close to the neighbouring actin filaments. It will be shown elsewhere that the proposed model can account for the observed exchange of mass from myosin to actin when a relaxed muscle goes into rigor[13]. It is therefore possible that the four-strand 6/1 helix is a satisfactory alternative model for the myosin filament in VStM.

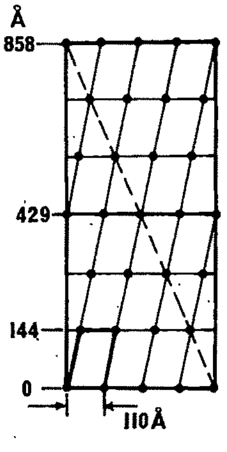


Fig. 2 Possible alternative model for the cross-bridge lattice on the thick filaments of vertebrate striated muscle in radial projection. The bold line outlines a repeating unit similar to that of the lattice in Fig. 1*a*. The dashed line represents one of the four 6/1 helical strands.

Next we can consider the structure of the myosin-containing filaments in insect flight muscle. This time Fig. 1 shows that three repeating units of the ribbon lattice (*d*) would give a repeating unit rather like that on the proposed two-strand 16/3 helical model for these filaments (*f*). If we take the estimate of myosin content[21] which gives three molecules per lattice point on this model (and not two), then an alternative model with the same helical symmetry but with six molecules equally spaced around the myosin filament on one level seems possible. This would generate a six-strand 16/3 helix (Fig. 3) with a long repeat of about 1150 Å. The experimental evidence from which the original model was deduced was from X-ray diffraction work[16] and electron microscopy[10,11,16]. The electron micrographs of longitudinal sections of insect flight muscle were analysed by optical diffraction and reflexions which were orders of a repeat of 1150 Å were observed. From the X-ray diffraction results it was deduced that the residue repeat of the cross-bridge lattice on the myosin filaments is about 144 Å. These results taken together led Reedy to propose the two-strand 16/3 helical model for the myosin filaments. The alternative six-strand 16/3 helix also has a repeat of 1150 Å and a residue of 144 Å and would equally well account for these results.

The other experimental evidence which needs to be explained is the appearance of "flared-X" configurations of the projections in Reedy's very elegant electron microscope work on serial

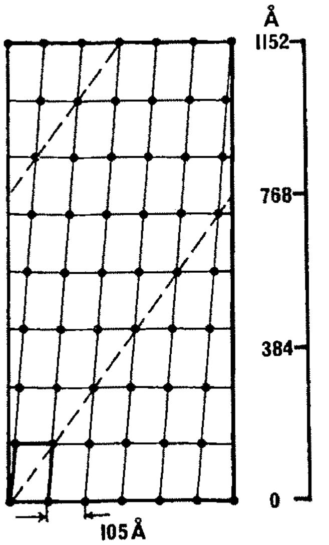


Fig. 3 A possible model for the thick filaments in insect flight muscle; radial projection of units arranged on a six-strand 16/3 helix of repeat 1152 Å. The dashed lines follow one and a half turns of the 16/3 helix.

thin transverse sections of glycerinated insect flight muscle[11]. These seem to show that there are at most only four cross-bridges with origins within one repeat of 146 Å along the myosin filaments. But Reedy states[10] that the "flared-X" configurations cannot be convincingly demonstrated in sections of relaxed insect flight muscle where presumably the myosin heads are not necessarily cross-linked to actin filaments. It is therefore possible that cross-bridges are only visible in Reedy's micrographs if they were cross-linked to actin before fixation. In this case the proposed six-stranded model would require that even in a rigor muscle (in which it is commonly assumed that all the cross-bridges are attached to actin) only four out of six of the cross-bridges on one level are cross-linked. These would then form the flared-X configurations seen in Reedy's micrograph and the remaining cross-bridges would not be preserved and visualized. Evidence for this will be given elsewhere (J. M. Squire, in preparation). It is therefore possible that a model for the myosin filaments in insect muscle in which there are six cross-bridge origins on one level may adequately account for the existing experimental data on the structure of these filaments.

Implications of Alternative Models

I have now arrived at the situation where the structure of the myosin-containing filaments in VSmM, VStM and IFM might be explained in terms of models with basically the same distribution of myosin heads on the surfaces of the filaments. If it is assumed that the myosin molecules pack together with about 1000 Å of the rod portion of the molecules associated with the backbone of the filaments (the remainder forming the cross-bridges) and also that the myosin rods are close packed in the backbone with a separation of about 20 Å, then the suggested arrangements of projections would require the rod portions to pack into a surface layer of myosin molecules of thickness about 30–40 Å. This may of course vary for cylindrical filaments of different total diameter and will be slightly larger for the smallest filaments. Taking reasonable values (which can only be approximate) for the dimensions of the backbones of the filaments being considered (about 140 Å diameter in VStM[8,30], about 200 Å diameter in IFM[10,11] and the thickness of myosin ribbons in VSmM about 145 Å (ref. 17 and J. V. Small, and J. M. Squire, in preparation)) then the filament profiles drawn in Fig. 4 are obtained. An approximation has been made here in that differences in filament structure in the bare zones and tapered ends of the cylindrical filaments have been neglected. The diameters given do not relate to the bare zone but to the regions on the filaments from which most of the cross-bridges originate. In these regions the filaments would all consist of a core of protein other than myosin surrounded by a layer of myosin of thickness 30–40 Å. The observations of "hollow" filaments (or at least filaments which have a central core with different staining properties from the periphery) in muscle cross-sections are quite numerous[16,31] and favour this sort of model. Szent-Györgyi *et al.*[32] have also suggested that there may be a constant surface area per cross-bridge (and therefore a constant thickness of myosin) on the surfaces of the paramyosin filaments of different molluscan muscles. This is discussed in the next section.

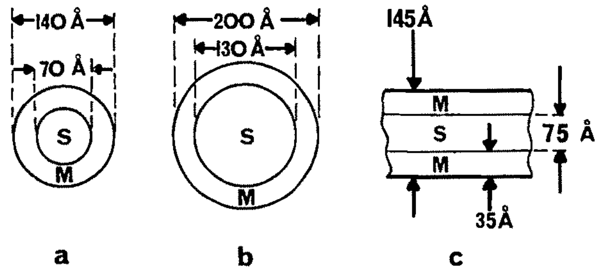


Fig. 4 Profiles of different myosin-containing filaments assuming the myosin rods are confined to a layer of thickness 35 Å on the surface of the filaments. *a*, Vertebrate striated muscle filaments; *b*, insect flight muscle filaments; *c*, ribbons in vertebrate smooth muscle. The myosin projections do not form part of this layer.

If we now consider just those filaments which are cylindrical in shape we can calculate approximately the percentage volume of the filament backbone which will be taken up by the core in filaments of different diameter if the myosin is all in a surface layer of thickness, say, 35 Å, and it is assumed that 60% of the mass of myosin molecules is in the projections and does not form part of the filament backbone. Fig. 5 shows this percentage volume (V_c) plotted as a function of the diameter of the backbones of the filaments. The two points which would correspond to the models illustrated in Fig. 4 for VStM and IFM are shown on this graph together with points related to other models for these filaments.

Paramyosin-containing Filaments

If the ideas mentioned above have any basis in reality, then the paramyosin-containing filaments in molluscan muscles should have the same sort of structure with a 35 Å layer of myosin muscles surrounding an inner core of paramyosin. In this case we are in the fortunate position of having experimentally determined values for the proportions of paramyosin (P) and myosin (M) in particular muscles. We can therefore plot several points on Fig. 5 which correspond to experimental results for filaments of widely varying diameter. Recent estimates of the ratio P : M by weight in different muscles are 25% : 75% in *Pecten* striated adductor muscle (private communication of preliminary results by P. M. D. Hardwicke, and J. Hanson); 50% : 50% in *Venus mercenaria* white adductor muscle[32], 33% : 67% in the translucent adductor muscle of *Crassostrea virginica*[32] and finally in *Crassostrea* opaque adductor muscles 57% : 43%[32] or 75% : 25% (private com-

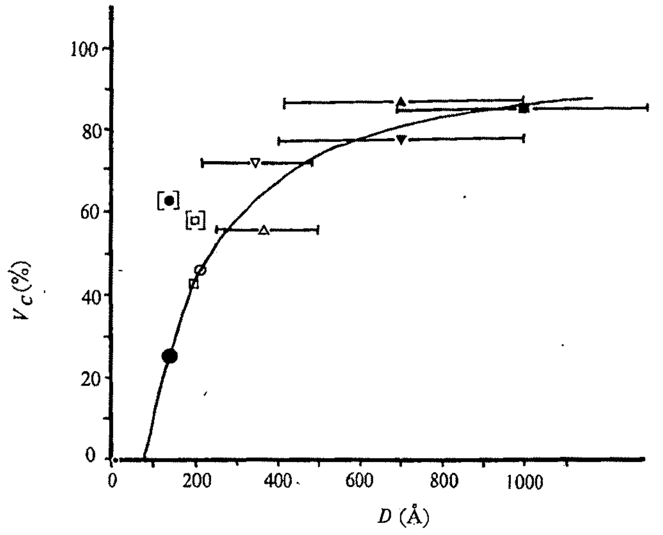


Fig. 5 The bold line represents a plot of the volume of the core (V_c) expressed as a percentage of the total volume of cylindrical myosin-containing filaments of different diameter (D), assuming that there is a layer of myosin of thickness 35 Å on the surfaces of these filaments, and that 60% of the mass of the myosin molecules is in the cross-bridges and does not form part of the backbone. ● and □ represent the appropriate points for filaments in VStM and IFM on the basis of the models shown in Figs. 2 and 3 respectively. The same symbols in brackets refer to existing models[8,10] for the myosin filaments in the muscles. The remaining points represent experimental results obtained from the relative amounts of paramyosin and myosin in different molluscan muscles. Error bars on these points represent the standard deviation of the filament diameters about the mean assuming the sort of distribution of filament diameters observed in *Crassostrea* opaque adductor muscle[34]. ○, *Pecten* striated (P. M. D. Hardwicke, and J. Hanson, personal communication); ▽, *Venus* red[32]; ■, *Venus* white[32]; △ *Crassostrea* translucent[32]; ▼, *Crassostrea* opaque[32]; ▲, *Crassostrea* opaque adductor muscles (P. M. D. Hardwicke, and J. Hanson, personal communication).

munication from P. M. D. Hardwicke, and J. Hanson). In *Pecten* the paramyosin filaments have a diameter of about 200 Å to 250 Å (except in the bare zone). In the other muscles the paramyosin filaments can have a variety of diameters. Published estimates of the range of diameters (which also include the effects of the tapering of the filaments) are as follows: 200 Å to 500 Å in *Venus* red muscle[33], 500 Å to 1500 Å in *Venus* white muscle[15], 150 Å to 600 Å in *Crassostrea* translucent muscle[15] and 200 Å to 1500 Å with a peak of the distribution at about 700 Å (standard deviation 300 Å) in *Crassostrea* opaque adductor muscles[34]. Although these sizes cannot be taken too literally it is probable that "average" values of the diameter will reflect the relative filament sizes in the different muscles. Fig. 5 shows how the experimental figures relate to the model and demonstrates that in each case there is sufficient paramyosin to fill the core of the different filaments.

Although there is obviously considerable uncertainty in the results, we can conclude from the general agreement between the theoretical curve and the plotted points that, at least, the model is consistent with the available data. This serves to emphasize one point made in Szent-Györgyi *et al.*[32] that the surface area per myosin head is probably a constant feature of all paramyosin-containing filaments. As in the other filaments this area would be about 144 Å × 100–110 Å = 15,000 Å^2.

On this basis one can suggest possible packing arrangements for the myosin heads on the surfaces of different paramyosin-containing filaments. The paramyosin in these filaments is known to pack to give a surface appearance[35] well known as the Bear–Selby net (Fig. 6*a*). The axial repeat of the net is always about 720 Å but the "a" spacing varies greatly in filaments from different muscle types and also with the diameter of the filaments within one muscle type[7]. Fig. 6 shows the Bear–Selby nets of filaments in four different molluscan muscles (for details see caption) and illustrates how these nets can be subdivided very simply (assuming all the lattice points in the Bear–Selby net are equivalent) to give repeating units of area about 15,000 Å^2. The mean "a" spacings are obtained mostly from X-ray diffraction results[36] and are assumed to be similar to the "a" spacings on the surface of the paramyosin core of the filaments[7]. It is therefore quite possible that the lattices drawn in Fig. 6 represent the packing distribution of myosin on these filaments. It should be noted, however, that in VSmM evidence has been obtained that the filament backbone and the cross-bridge lattice on the myosin ribbons do not have the same axial repeat (J. V. Small, and J. M. Squire, in preparation). In spite of this the present model would require that the myosin molecules pack with a side-spacing of about 100 Å. Evidence in support of this is shown in Fig. 7 where a micrograph of a paramyosin filament ("a" spacing, 630 Å) from the anterior-byssus retractor muscle (ABRM) of *Mytilus* is shown by self-convolution[37] to exhibit a lateral repeat of about one fifth of the "a" spacing (that is, 120–130 Å) which is generated by a rectangular net of the sort drawn in Fig. 6*e*.

Discussion and Predictions

So far I have tried to show that it may be possible to interpret the existing experimental data on the form of the myosin-containing filaments in different muscles in terms of one basic structure. The details of this interpretation will be given later

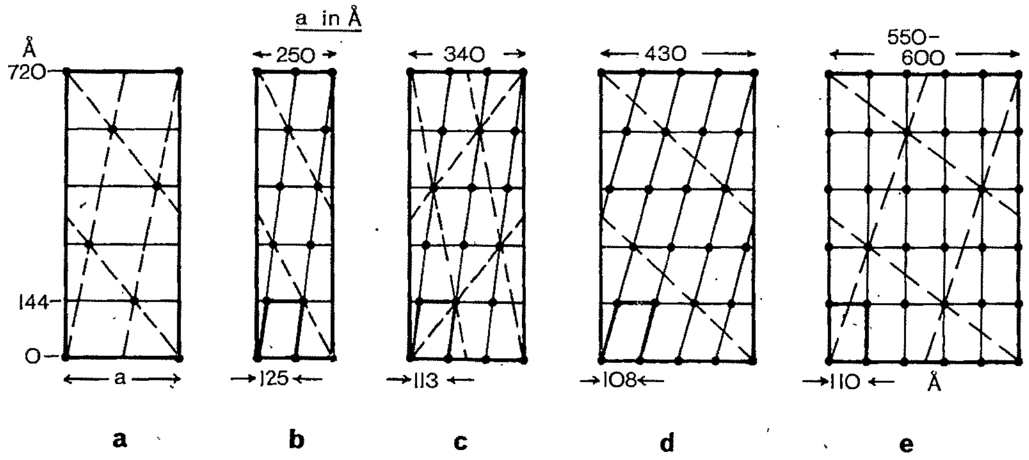


Fig. 6 *a*, The lattice (Bear–Selby net) on the surface of paramyosin filaments from which the myosin has been removed; *b–e* represent subdivisions of the Bear–Selby nets on the surfaces of paramyosin filaments in different molluscan muscles to give repeating units of area 15,000 Å^2. It has been assumed that lattice points in the Bear–Selby nets are all equivalent. *b*, *Venus* adductor muscle; *c*, *Crassostrea* adductor muscle; *d*, *Mytilus* adductor muscle; *e*, *Mytilus* ABRM.

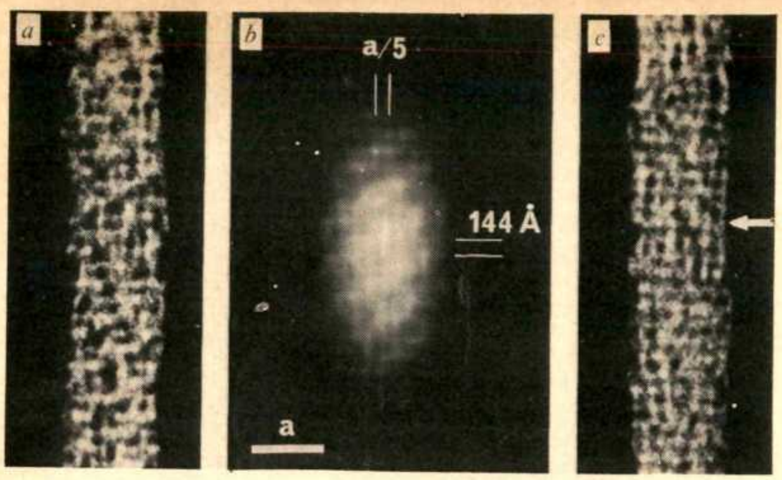


Fig. 7 *a*, Electron micrograph of part of a negatively stained paramyosin filament (courtesy of Professor J. Lowy) from *Mytilus* ABRM; *b*, self-convolution function of part of the filament in *a* which shows that "a" spacing of the Bear–Selby net on this filament is clearly divided into five sub-periods; *c*, another region of the same filament as *a* showing a regular near-rectangular arrangement of subunits (compare Fig. 6*e*).

(manuscript in preparation) but I would like to list some of the predictions from this idea which can be tested experimentally. The following predictions assume that all the myosin filaments contain a core protein and are not hollow. The numerical values given obviously depend critically on the exact values taken for the filament diameters (which are only known approximately) and may need to be modified when even more accurate values are available. (i) All myosin-containing filaments of the same backbone diameter will have approximately the same proportion of myosin to core protein by volume and also by weight if the core proteins have the same density. (ii) In general the cores of myosin-containing filaments will have a diameter (or thickness) equal to the total diameter (or thickness) of the filaments less about 70 Å. (iii) In particular the core of the myosin-containing filaments in VStM would have a diameter of about 70 Å and would contain about 10–15% by weight of the total mass of the filaments. This assumes that the density of the core protein is the same as that of the myosin rods and that the projections contain 60% of the mass of the myosin molecules. A model with two molecules per 144 Å repeat would have about 40% of the filament mass in proteins other than myosin. (iv) In insect flight muscle approximately 20–25% of the mass of the myosin filament would be in the core (using the same assumptions) and the diameter of the core would be about 130 Å. Reedy's model with four myosin heads per 146 Å repeat would have about 40–45% of the filament mass in the form of proteins other than myosin. (v) In VSmM the ribbons which have a core of thickness about 75 Å (J. V. Small, and J. M. Squire, in preparation) would have a total ribbon thickness of about 145 Å and the core would account for approximately 25–30% of the total mass of the ribbons. (vi) The structure of the myosin layer will be different in the regions of cylindrical filaments corresponding to the bare zone. On the basis of the type of molecular packing scheme proposed by Huxley[20] in which myosin molecules pack antiparallel in the bare zone, the type of packing scheme proposed recently[38] from studies of myosin assembly may well occur if modified to fit the filament lattice described here. The thickness (35 Å) of the layer of myosin rods in regions other than the bare zone is that required to close pack the rod portions of about two or three molecules, and it is possible to generate very simple packing arrangements for the myosin rods in these layers which will produce the required surface lattices. Details of these will be given elsewhere. The lattices which have been drawn in Figs. 1*a*, 2, 3 and 6 are not all identical, but the very small differences in packing which would change one lattice to another are attributable to the small differences in chemical composition of the rod portions of the different myosin molecules involved. It is also possible that the packing of the core protein(s) affects the precise ordering of the myosin molecules on the surfaces of the filaments. (vii) One further implication of the model is that the core protein(s) and not the myosin in a filament will determine the precise shape and size of that filament. On this basis the vernier length-determining mechanism proposed by Huxley and Brown[8] would require the presence of at least two core proteins in the thick filaments of VStM and IFM.

Many experiments come to mind which would test the ideas presented here. The extraction and identification of core proteins, together with an estimation of their quantity, is one obvious type of experiment. Electron microscopy could be used to analyse further the cross-bridge arrangements on the myosin-containing filaments of different striated muscles. If it turns out that there is a structure common to all myosin-containing filaments it will be an open question whether or not there is any relationship between that structure and the function of the different muscles in which the filaments occur. One might suggest that the overall shape and size of the filament affect the speed and function of the different muscles but that the precise cross-bridge lattice does not. It would still remain to be answered whether this form of lattice would be critical for an efficient acto-myosin interaction during contraction of a muscle and therefore for an efficient sliding filament mechanism.

I thank Professor J. Lowy for permission to use the micrograph shown in Fig. 7 and him, Professor J. Hanson, Dr J. Haselgrove, Dr J. V. Small and Dr P. J. Vibert for comments on the text.

Received June 10; revised September 6, 1971.

[1] Bear, R. S., *J. Amer. Chem. Soc.*, **67**, 1625 (1945).
[2] Carsten, M. E., and Katz, A. M., *Biochim. Biophys. Acta*, **90**, 534 (1964).
[3] Hanson, J., and Lowy, J., *J. Mol. Biol.*, **6**, 46 (1963).
[4] Hanson, J., and Lowy, J., *Proc. Roy. Soc.*, B., **160**, 449 (1964).
[5] Hanson, J., and Lowy, J., *Brit. Med. Bull.*, **21**, 264 (1965).
[6] Lowy, J., and Vibert, P. J., *Nature*, **215**, 1254 (1967).
[7] Elliott, A., and Lowy, J., *J. Mol. Biol.*, **53**, 181 (1970).
[8] Huxley, H. E., and Brown, W., *J. Mol. Biol.*, **30**, 383 (1967).
[9] Lowy, J., and Small, J. V., *Nature*, **227**, 46 (1970).
[10] Reedy, M. K., *Amer. Zoologist*, **7**, 465 (1967).
[11] Reedy, M. K., *J. Mol. Biol.*, **31**, 155 (1968).
[12] Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H., *J. Mol. Biol.*, **42**, 1 (1969).
[13] Huxley, H. E., *J. Mol. Biol.*, **37**, 507 (1968).
[14] Lowy, J., Poulsen, F. R., and Vibert, P. J., *Nature*, **225**, 1053 (1970).
[15] Hanson, J., and Lowy J., *Proc. Roy. Soc.*, B, **154**, 173 (1961).
[16] Reedy, M. K., Holmes, K. C., and Tregear, R. T., *Nature*, **207**, 1276 (1965).
[17] Small, J. V., Lowy, J., and Squire, J. M., *Proc. First European Biophysics Congress* (Vienna, 1971).
[18] Miller, A., and Tregear, R. T., *Nature*, **226**, 1060 (1970).
[19] Tregear, R. T., and Miller, A., *Nature*, **222**, 1184 (1969).
[20] Huxley, H. E., *J. Mol. Biol.*, **7**, 281 (1963).
[21] Chaplain, R. A., and Tregear, R. T., *J. Mol. Biol.*, **21**, 275 (1966).
[22] Perry, S. V., *Prog. Biophys.*, **17**, 325 (1967).

[23] Rice, R. V., *Biochim. Biophys. Acta*, **52**, 602 (1961).
[24] Rice, R. V., in *Biochemistry of Muscle Contraction* (edit. by Gergely, J.), 41 (Little, Brown, Boston, 1964).
[25] Stracher, A., *Biochem. Biophys. Res. Commun.*, **35**, 519 (1969).
[26] Weeds, A. G., *Nature*, **223**, 1352 (1969).
[27] Pepe, F. A., *J. Mol. Biol.*, **27**, 203 (1967).
[28] Pepe, F. A., *J. Mol. Biol.*, **27**, 227 (1967).
[29] Huxley, H. E., *J. Biophys. Biochem. Cytol.*, **3**, 631 (1957).
[30] Huxley, H. E., *Harvey Lectures, Series 60*, 85 (Academic Press, New York and London, 1966).
[31] Franzini-Armstrong, C., *J. Cell. Sci.*, **6**, 559 (1970).
[32] Szent-Györgyi, A. G., Cohen, C., and Kendrick-Jones, J. *J. Mol. Biol.*, **56**, 239 (1971).
[33] Philpott, D. E., Kahlbrock, M., and Szent-Györgyi, A. G., *J. Ultrastruct. Res.*, **3**, 254 (1960).
[34] Elliott, G. F., *Proc. Roy. Soc.*, B, **160**, 467 (1964).
[35] Selby, C. C., and Bear, R. S., *J. Biophys. Biochem. Cytol.*, **2**, 55 (1956).
[36] Elliott, G. F., thesis, Univ. London (1960).
[37] Elliott, A., Lowy, J., and Squire, J. M., *Nature*, **219**, 1124 (1968).
[38] Harrison, R. G., Lowey, S., and Cohen, C., *J. Mol. Biol.*, **59**, 531 (1971).

Cordilleran Tectonic Transitions and Motion of the North American Plate

PETER J. CONEY

Department of Geology, Middlebury College, Middlebury, Vermont

This article examines the relationship between tectonic events in western North America and in the North Atlantic Ocean.

I WISH to propose that there is a correlation between the timing of principal tectonic events in western North America since late Jurassic time and submarine data from the North Atlantic Ocean. The correlation is particularly striking if inferences about relative plate motions from Atlantic magnetics[1] are combined with inferences about absolute plate motions[2-4].

An analysis of the timing of tectonic events in western North America since late Jurassic suggests that important periods of tectonic reorganization (tectonic transitions) took place at about 80 m.y. BP and at about 40 m.y. BP. The data strongly indicate that the tectonic transitions were felt from Alaska to northwestern South America and that they represent fundamental turning points in the tectonic evolution of the Cordillera. The tectonic transitions are best described as boundaries of duration about 5 to 10 m.y. which separate longer periods of up to 60 m.y. The transition periods mark reorganizations of tectonic behaviour, whereas the longer periods separated by transitions are more internally consistent.

The 80 m.y. Transition

The principal significance of the 80 m.y. transition is that it marks the boundary in time and space between classic Laramide orogeny[5] and Nevadian–Sevier orogeny[6]. The transition extends, however, from Alaska to northern South America, far from type areas in the western United States.

Features associated with the transition include the end of major plutonism in the Sierra Nevada of California[7] and the termination of Franciscan deposition[8,9]. In southern California an important unconformity at the base of the Asuncion Formation at about 80 m.y. places a roof on major metamorphism and plutonism[10,11]. In southern Alaska, plutonism was initiated near 80 m.y. after a pause of more than 50 m.y. (ref. 12), and deformation of the slate–greywacke belt in the Kodiak–Kenai–Chugach mountains around the Gulf of Alaska began[13-15]. There is some evidence from northwestern Canada that right strike-slip on the Tintina fault diminished after 80 m.y. (ref. 16), but through going right strike-slip on the Chatham fault in southeastern Alaska may have started about this time[17]. In the Canadian Cordillera the transition marks the boundary in space and time between late Jurassic to early upper Cretaceous Columbian orogeny[18] and Laramide orogeny[18]. There is a low in the frequency of radiometric age determinations from western Canada at about 80 m.y. just before a compound peak extending to 40 m.y. (ref. 19), and there is a marked increase in amount of detritus shed from the Cordillera eastward over the plains from about 80 m.y. to late Palaeocene[20]. Most deformation to the west of the Sevier orogenic belt of eastern Nevada and western Utah, and within this belt, ceased at about this time[6] whereas Laramide orogeny[21,22], accompanied by a distinct increase in plutonism, broke across the Cordilleran foreland soon afterwards. From the southwestern United States to southern Mexico, widespread carbonate shelf conditions in the Mexican geosyncline terminated near 80 m.y. and were followed by detrital wedges, plutonism, and major compressive deformation[23,24]. In the Greater Antilles, arc volcanism, plutonism, emplacement of ultramafics and compressive deformation seem to be bracketed by Turonian (about 85 m.y.) and upper Eocene (about 40 m.y.)[25-28]. Deformation and metamorphism in the Venezuelan Coast Ranges ceased in late Cretaceous time and were replaced by a distinctly different tectonic behaviour involving considerable oblique right strike-slip and related thrusting[29].

Widespread deformation and plutonism initiated or intensified near 80 m.y. in the northwestern Andes of South America[30,31].

Transition at 40 m.y.

The principal significance of the 40 m.y. transition is that it marks the end of Laramide compressive deformation in the western United States[32]; the transition can, however, be tracked from northwestern Canada south to the Isthmus of Tehuantepec in southern Mexico, and in the Greater Antilles. In Western Canada, with the exception of the Cascade–southeastern British Columbia region, plutonism and volcanism also almost cease at 40 m.y. (ref. 19), and in the western United States there is a pause in igneous activity[33]. After this pause there was a widespread increase of ignimbrite eruption in the Rocky Mountains and Great Basin[33,34]. In the Greater Antilles, plutonism, volcanism, emplacement of ultramafics, and compressive deformation apparently finished near 40 m.y., but the Lesser Antilles appear to have initiated activity at about this time[25]. Even in California, compressive deformation involving thrusting of Great Valley rocks over Franciscan rocks appears to have ceased near late Eocene time[35].

Several regions are not noticeably affected by the 40 m.y. transition. Southern Alaska, west of the Fairweather fault system, seems to have experienced plutonism from about 80 m.y. to 15 m.y., and compressive deformation from about 80 m.y. to 35 m.y., then again from about 20 m.y. to the present[15]. The northwestern Andes are not strongly effected by the 40 m.y. transition, and tectonism and plutonism, which accelerated after 80 m.y., have continued intermittently to the present[30,31]. Tectonics from Alaska to South America after 40 m.y. has been very variable from region to region, but the timing of volcanism, plutonism, compressive deformation, rifting, and strike-slip faulting fits well with predictions from submarine magnetics in the northeastern Pacific Ocean[36]. A mid-Tertiary tectonic transition, reported to have taken place between about 30 and 9 m.y. from region to region around the Pacific margin[37], is characterized by renewal of strike-slip and related compressive deformation on the San Andreas fault of California, block faulting in the Great Basin, volcanism and plutonism in the Aleutians, southern Alaska, the Cascades, and in Mexico and Central America[36,38–41].

Transitions and Plate Motion

The fact that both the 80 and 40 m.y. tectonic transitions cut through the whole array of data for western North America strongly suggests that these important turning points in Cordilleran tectonic evolution are the result of fundamental reorganizations in plate motions.

Changes in relative plate motions in the northeastern Pacific are indicated at about 55 m.y. and 7 m.y. by departures from parallelism of adjacent magnetic anomalies at these times[42]. Atwater[36,43] has also suggested that changes in plate motions took place at about 80 m.y. and about 15 m.y. as well. There is apparently nothing in the geometry of magnetic stripes in the northeastern Pacific Ocean which would suggest that anything unusual, such as the end of the Laramide orogeny and related deformation, occurred between, say, 50 and 40 m.y. on the adjacent North American continent. This suggests that we are looking in the wrong ocean.

Turning to the North Atlantic for relative motions of the North America plate, Pitman and Talwani[1] suggest that changes in relative plate motion occurred at about 80, 63, 53, 38, and 9 m.y. (their work was based on the analysis of magnetic anomalies). Although spreading between North America and Africa may have started after 180 m.y. and continued to the present, it is important to realize that spreading between North America and Eurasia apparently commenced at about 80 m.y. This is suggested by the fact that north of the Azores, the oldest anomalies and extrapolated time lines adjacent to present continental shelves are 80 m.y. old or less. This would suggest that spreading broke into the North Atlantic north of the Azores at about 80 m.y. and that spreading subsequently moved into the Arctic, thus separating the two continents. A major reorganization of plates at about 80 m.y. is indicated. Also, Pitman and Talwani[1] suggest that spreading between North America and Europe almost ceased between 38 and 9 m.y. The evidence for this is the close proximity of anomalies 15 (38 m.y.) and 5 (9 m.y.). From the analysis of sea-floor spreading in the North Atlantic, the 80 and 40 m.y. time lines seem important because the start of spreading between North America and Europe at 80 m.y. and its almost complete termination near 40 m.y. bracket the Laramide orogeny and related deformation in western North America. This suggests that both the 80 m.y. and 40 m.y. tectonic transitions, and the tectonic responses in the western Cordillera before, between, and after these transitions, were strongly influenced by motions of the North America plate; these were reflected in the relative spreading history recorded on the floor of the North Atlantic.

Absolute Plate Motion

If the proposal mentioned previously is valid, there is a strong incentive to determine absolute motions of the North America plate during the past 180 m.y. The only hypothesis which would permit analysis of absolute motion is that of Morgan[2–4] who has resurrected a long dormant notion of Wilson[44] that aseismic ridges and seamount chains on the ocean floors are generated by plate motion over mantle "hot spots" and mark dead reckoning tracks of absolute plate motion. A similar idea has been used by Dietz and Holden[45]. Seamount chains in the North Atlantic are of particular concern because they might provide a fix on the trajectory of the North America plate. The principal seamount chain west of the Mid-Atlantic ridge is the New England (or Kelvin) chain which extends from off Cape Cod in a southeasterly direction towards the Corner Rise near 34° N, 51° W. The only "hot spot", or convection plume[2–4], apparently capable of generating the New England–Corner Rise chain is the Azores[3]. Dating of the time of formation of the New England–Corner Rise chain would perhaps give the direction of absolute motion of North America during that time.

The White Mountain magma series of New England is a complex of granitic intrusions, rink dikes, calderas and volcanics which apparently initiated activity about 180 m.y. ago[46]. The complex cannot be related to subduction at a continental margin and seems to have more to do with the opening of the Atlantic Ocean[47]. Morgan[3] has suggested that the New England seamount chain might anchor on the White Mountain magma series and that both may have been produced by the Azores "hot spot". This suggests that the seamount chain was generated by a northwesterly motion of the North America plate over the Azores "hot spot" as the plate moved away from a proto-Atlantic ridge after 180 m.y. BP. The age of the southeast end of the chain near the Corner Rise is apparently 80 m.y. old or less, because anomaly 32 is close by[1] and drilling at Joides Site 10 (ref. 48) was into Campanian sediments just south of the southeast end of the chain. Because the Azores lie northeast of the Corner Rise, motion of the North America plate must have abruptly changed from northwesterly to southwesterly after the "hot spot" constructed the Corner Rise at or after 80 m.y. The change in plate motions suggested by initiation of spreading between North America and Europe at about 80 m.y. may correspond to the "elbow" at the Corner Rise. No clear seamount chain extends from the Corner Rise to the Azores, but the Corner Rise heads in that direction. Continuity may have been broken by proximity of the "hot spot" to the spreading ridge axis and associated transform faults[44]. Relative motion data[1] suggest that North America presumably drove southwesterly to south-southwesterly after 80 m.y., then slowed down between 40 m.y. and 9 m.y. Thus the northwesterly rotation between 180 and 80 m.y. brackets Nevadian–Sevier–Columbian orogeny, and the southwesterly rotation between 80 and 400 m.y. brackets the Laramide orogeny.

Conclusions

I propose that after the North America plate broke away from the Africa plate between 180 and 150 m.y. it migrated northwesterly over the Azores "hot spot" until about 80 m.y. (Fig. 1*a*). During this time the leading edge of North America failed over an overridden and entrained Benioff zone producing the Nevadian–Sevier–Columbian orogeny. A mid to late Jurassic age for initiation of actual separation[49] would fit well with the initiation of detrital wedges shed eastward from the Cordillera starting in upper Jurassic time[6,20]. It seems likely that this northwesterly rotation drove the Alaska–Kolyma[50] arm of the North America plate into the trailing edge of Eurasia in upper Jurassic to lower Cretaceous time[51] to produce the Verhoyansk Mountains and several intraplate adjustments in Alaska. Some transform rifting between North America and Eurasia in the Arctic is indicated. Once North America and Eurasia had locked and the Alaska–Kolyma peninsula between them had consolidated, North America began separating from Eurasia north of the Azores at about 80 m.y. and rotated southwesterly about a pole near northeastern Alaska. As spreading later broke into the Arctic, the rotation became more complex and eventually reached its present position at the head of rifting in Siberia. The change

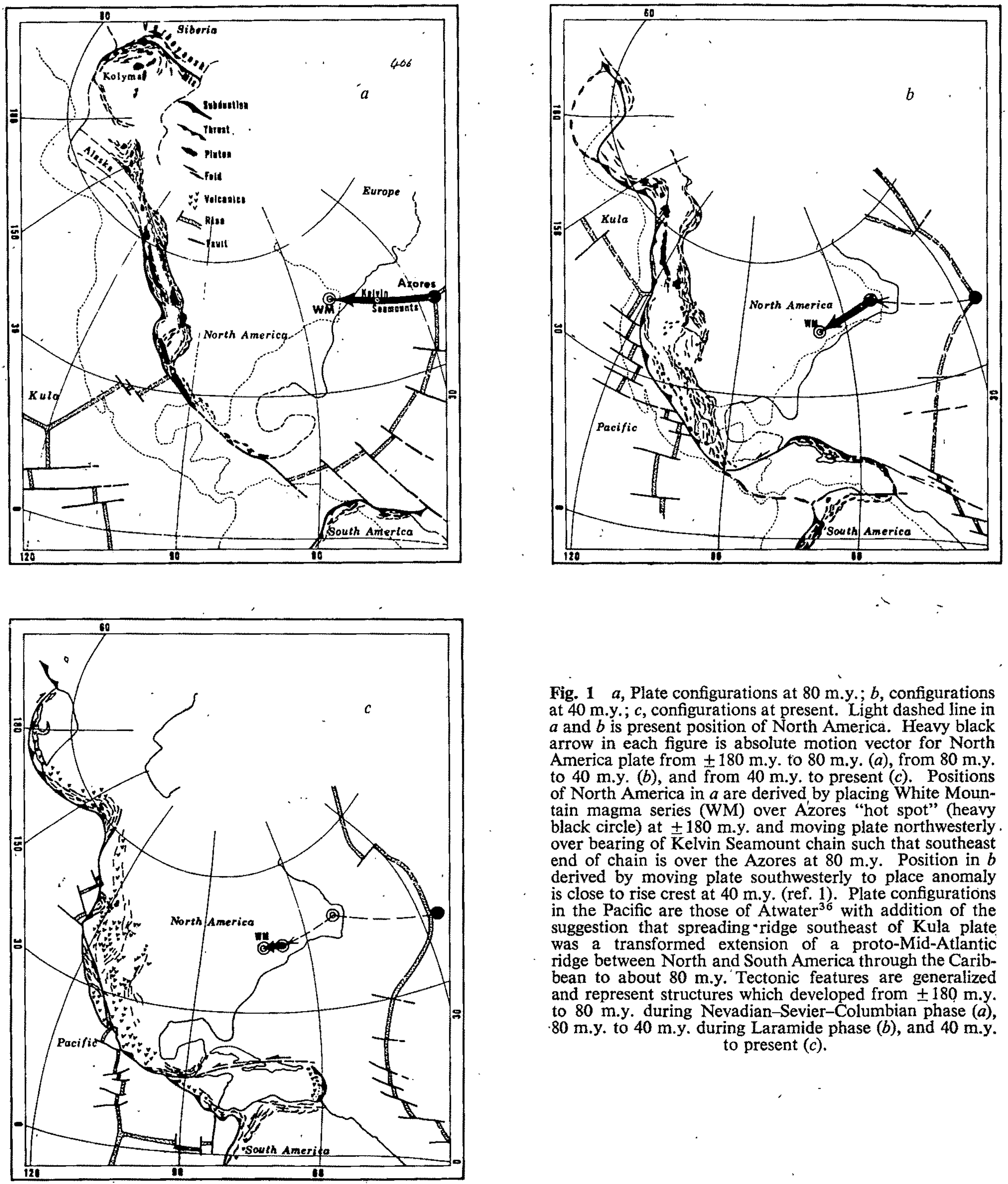


Fig. 1 *a*, Plate configurations at 80 m.y.; *b*, configurations at 40 m.y.; *c*, configurations at present. Light dashed line in *a* and *b* is present position of North America. Heavy black arrow in each figure is absolute motion vector for North America plate from ±180 m.y. to 80 m.y. (*a*), from 80 m.y. to 40 m.y. (*b*), and from 40 m.y. to present (*c*). Positions of North America in *a* are derived by placing White Mountain magma series (WM) over Azores "hot spot" (heavy black circle) at ±180 m.y. and moving plate northwesterly over bearing of Kelvin Seamount chain such that southeast end of chain is over the Azores at 80 m.y. Position in *b* derived by moving plate southwesterly to place anomaly is close to rise crest at 40 m.y. (ref. 1). Plate configurations in the Pacific are those of Atwater[36] with addition of the suggestion that spreading ridge southeast of Kula plate was a transformed extension of a proto-Mid-Atlantic ridge between North and South America through the Caribbean to about 80 m.y. Tectonic features are generalized and represent structures which developed from ±180 m.y. to 80 m.y. during Nevadian–Sevier–Columbian phase (*a*), 80 m.y. to 40 m.y. during Laramide phase (*b*), and 40 m.y. to present (*c*).

in motion at about 80 m.y. apparently caused the 80 m.y. transition, and the new southwesterly motion from 80 to 40 m.y. initiated widespread failure of the Cordilleran foreland to produce the Laramide orogeny (Fig. 1*b*). A slowing of that motion after 40 m.y. ended that orogeny. I suggest that the 30° bend in the strike-slip faults of Alaska[52] can almost be eliminated by a rotation of the Corner Rise to the Azores about a pole in northeastern Alaska, thus explaining the Alaska orocline[15,52–54]. Other features, such as subduction of Atlantic lithosphere by the Greater Antilles between 80 and 40 m.y., are perhaps also explained by North America plate motion.

Since 40 m.y. (Fig. 1*c*) North America has moved in more of a southwest by west direction towards the East Pacific Rise; eventual contact with the rise off southern California in mid-Tertiary time[36] and possible changes in Pacific plate motions near 25 m.y. (ref. 41) explain much of the tectonic history after 40 m.y. Motion of North America and South America past an almost stationary Caribbean plate was probably slightly convergent and explains many features on the margins of the Caribbean such as oblique right strike-slip in northern Venezuela[29], compressive and left strike-slip deformation in Chiapas, Mexico and Guatemala[55,56], disruption of the Greater Antilles and Central America along the Bartlet–Motagua fault zones[57], volcanism in the Lesser Antilles and present seismicity[58].

The close correspondence of phases of major compressive deformation during Nevadian–Sevier–Columbian andLaramide Cordilleran tectogenesis with timing of North America plate motions suggests to me that compressive tectonics in western North America are the result of abortive west-directed efforts at subduction of continental lithosphere on the leading edge of an actively driving continental plate. This deformation was perhaps facilitated by softening of the plate margin (up to 1,000 km wide) by complex plutonic patterns associated with overridden, entrained and sometimes double[59] subduction zones. This suggests that active plate motion somehow transmits compressive stress through the plate and that Cordilleran tectonic styles such as those in the Rocky Mountain fold and thrust belt are due to massive westward cratonic underthrusting as visualized by Misch[60,61]. By contrast, it would appear that active driving and underthrusting of oceanic lithosphere are easily accomplished and little stress is transmitted across subduction zones from the subducting oceanic lithosphere to the upper plate much beyond the arc. If North America had remained fixed during Mesozoic and early Tertiary time the Cordilleran orogen would more likely have resembled present day western Pacific arcs and margins where active subduction-related tectonics occurs in relatively narrow belts compared with Cordilleran styles. Thus if an Andean–Cordilleran type orogen exists[62] it is confined to leading edges of actively driving continental plates, and one can conclude that several other combinations of absolute motions are capable of producing as many other orogenic types.

I am grateful to William R. Dickinson and Tanya Atwater for discussions. Norman Silberling, Ben Page, David Jones, George Plafker, Eli Silver, Warren Hamilton, Peter Lipman, Greg Davis, Trobe Grose, Peter Misch, and J. O. Wheller were also most helpful. Manik Talwani kindly provided a preprint of work in the North Atlantic, and Eli Silver a preprint of work on the Hawaii–Emperor seamount chain. I would finally thank Jason Morgan for a lecture and discussion at Stanford University and a preprint which provided the framework for consideration of possible absolute motions.

Received July 15, 1971.

[1] Pitman, W. C. III, and Talwani, Manik, *Bull. Geol. Soc. Amer.* (in the press).
[2] Morgan, W. J., *EOS*, **51**, 822 (1970).
[3] Morgan, W. J., *Nature*, **230**, 42 (1971).
[4] Morgan, W. J., *Geol. Soc. Amer. Mem.* (in the press).
[5] Eardley, A. J., *Structural Geology of North America*, second ed., 295 (Harper and Row, 1962).
[6] Armstrong, R. L., *Bull. Geol. Soc. Amer.*, **79**, 429 (1968).
[7] Evernden, J. F., and Kistler, R. W., *US Geol. Sur. Prof. Paper*, 623 (1970).
[8] Bailey, E. H., Irwin, W. P., and Jones, D. L., *Calif. Div. Mines and Geol. Bull.*, 183 (1964).
[9] Ernst, W. G., *J. Geophys. Res.*, **78**, 886 (1970).
[10] Compton, R. R., *Calif. Div. Mines and Geol. Bull.*, **190**, 277 (1966).
[11] Page, B. M., *Calif. Div. Mines and Geol. Bull.*, **190**, 255 (1966).
[12] Reed, B. L., and Lanphere, M. A., *Geol. Soc. Amer. Bull.*, **80**, 23 (1969).
[13] Burk, C. A., *Geol. Soc. Amer. Mem.*, 99 (1965).
[14] Stoneley, R., *Quart. J. Geol. Soc. London*, **123**, Pt. 1, 25 (1967).
[15] Plafker, George, *US Geol. Sur. Prof. Paper* 543–I (1969).
[16] Roddick, J. A., *J. Geol.*, **75**, 23 (1967).
[17] Loney, R. A., Brew, D. A., and Lanphere, M. A., *Geol. Soc. Amer. Bull.*, **78**, 511 (1967).
[18] Gabrielse, H., and Wheeler, J. O., in *Geology of Western Canada, Geol Sur. Canada Econ. Geol. Report*, No. 1 (edit. by Douglas, R. W.), 450 (1969).
[19] Gabrielse, H., and Reesor, J. E., in *Royal Soc. Canada Spec. Publ.*, No. 8, 96 (1964).
[20] Price, R. A., and Mountjoy, E. W., in *Geol. Assoc. Canada Spec. Publ.*, No. 6, 7 (1970).
[21] Grose, L. T. (in the press).
[22] Weimer, R. J., *Soc. Econ. Paleo. and Mineral Spec. Publ.*, No. **15**, 270 (1970).
[23] Cserna, Zoltan de, *Geol. Rundschau*, **50**, 595 (1960).
[24] Fries, jun., Carl, *Mexico Univ. Nac. Inst. Geol. Bull.*, 60 (1960).
[25] Butterlin, J., *Centre Nat. de la Res. Scientifique* (1956).
[26] Khudoley, K. M., *Amer. Assoc. Petrol. Geol. Bull.*, **51**, 668 (1967).
[27] Dengo, G., *Gulf Coast Assoc. Geol. Trans.*, **19**, 311 (1969).
[28] Meyerhoff, A. A., Khudoley, K. M., and Hatton, C. W., *Amer. Assoc. Petrol. Geol. Bull.*, **53**, 2494 (1969).
[29] Bell, J. S. (in the press).
[30] Burgl, H., *Tectonophysics*, **4**, 429 (1967).
[31] Hosmer, H. L., thesis, Univ. Michigan (1959).
[32] Gilluly, James, *Quart. J. Geol. Soc. London*, **119**, 133 (1963).
[33] Damon, P. E., and Mauger, R. L., *Soc. Mining Eng. Trans.*, March, 99 (1966).
[34] McKee, E. H., Noble, D. C., and Silberman, M. L., *Earth Planet. Sci. Lett.*, **8**, 93 (1970).
[35] Swe, W., and Dickinson, W. R., *Geol. Soc. Amer. Bull.*, **81**, 165 (1970).
[36] Atwater, Tanya, *Geol. Soc. Amer. Bull.*, **81**, 3513 (1970).
[37] Dott, R. H., *Science*, **166**, 874 (1969).
[38] Grow, J. A., and Atwater, Tanya, *Geol. Soc. Amer. Bull.*, **81** (1970).
[39] Lipman, P. W., Prostka, H. J., and Christiansen, R. L. (in the press).
[40] Christiansen, R. L., and Lipman, P. W. (in the press).
[41] Jackson, E. D., Silver, Eli, and Dalrymple, B. G. (in the press).
[42] Menard, H. W., and Atwater, Tanya, *Nature*, **219**, 463 (1968).
[43] Atwater, Tanya, *Geol. Soc. Amer. Prog. with Abstracts*, **3**, 75 (1971).
[44] Wilson, J. T., *Nature*, **207**, 907 (1965).
[45] Dietz, R., and Holden, J. C., *J. Geophys. Res.*, **75**, 4939 (1970).
[46] Foland, K. A., Quinn, A. W., and Giletti, B. J., *Amer. J. Sci.*, **270**, 321 (1971).
[47] Coney, P. J., *Geol. Soc. Amer. Bull.*, **81**, 739 (1970).
[48] Peterson, M. N. A., *et al.*, in *Initial Reports of the Deep Sea Drilling Project*, **2**, 217 (US Govt. Printing Office, Washington DC, 1970).
[49] Hallam, A., *J. Geol.*, **79**, 129 (1971).
[50] Churkin, jun., Michael, in *Proc. Geol. Seminar on North Slope Alaska*, Amer. Assoc. Petrol. Geol., Pac. Sec. (edit. by Adkinson, W. L., and Brosge, M. M.) (1970).
[51] Hamilton, Warren, *Amer. Assoc. Petrol. Geol. Prog. with Abstracts*, 2nd Int. Symposium Arctic Geol., 24 (1971).
[52] Grantz, A., thesis, Univ. Stanford (1966).
[53] Carey, S. W., in *Continental Drift, a Symposium* (Univ. Tasmania, 1958).
[54] Hamilton, Warren, *Geol. Soc. Amer. Bull.*, **81**, 2553 (1970).
[55] Viniegra O., Francisco, *Amer. Assoc. Petrol. Geol. Bull.*, **55**, 478 (1971).
[56] King, P. B., *US Geol. Sur. Prof. Paper 628* (1969).
[57] Hess, H. H., and Maxwell, J. C., *Geol. Soc. Amer. Bull.*, 64 (1953).
[58] Molnar, Peter, and Sykes, L. R., *Geol. Soc. Amer. Bull.*, **80**, 1639 (1969).
[59] Lipman, P. W., Prostka, H. J., and Christiansen, R. L., *Geol. Soc. Amer. Prog. with Abstracts*, **3**, 148 (1971).
[60] Misch, Peter, in *Inter. Assoc. Petrol. Geol. 11th Ann. Field Conf. Guidebook*, 17 (1960).
[61] Misch, Peter, *Geol. Soc. Amer. Prog. with Abstracts*, **3**, 164 (1971).
[62] Dewey, J. F., and Horsfield, B., *Nature*, **225**, 521 (1970).
[63] King, P. B. (compiler), *Tectonic Map of North America* (US Geol. Survey, 1968).

Pure Chemistry Library,
SCIENCE COLLEGE.
92, Upper Circular Road,
CALCUTTA.

LETTERS TO NATURE

PHYSICAL SCIENCES

New Point γ-ray Source Lib γ-1: Evidence for Time Variation and Possible Identification with PKS 1514-24

DURING the past 2 yr evidence has been presented for the existence of four point sources of high energy γ-radiation ($E_\gamma > 50$ MeV). Three were found by the Case-Melbourne (C-M) collaboration using high altitude balloon flights from Australia (Fig. 1) and two of these sources, Gγ 2+3 and Gγ 341+1, coincided closely with known high energy X-ray sources[1,2]. A group from the Moscow Physical Engineering Institute detected a source with a counter telescope on Cosmos 251[3]. Three aspects of the Russian source are particularly noteworthy: (1) the source position, $54° < \alpha < 75°$, $+4° < \delta < +9°$, contains the unusual Seyfert galaxy 3C 120[4] which varies at both radio[5,6] and optical[7] frequencies; (2) the γ-ray flux is $F_\gamma = 5 \times 10^{-4}$ γs cm^{-2} s^{-1} ($E_\gamma > 100$ MeV), a factor of 30 greater than any of the C-M sources; and (3) most of the detected γ-ray flux must have been due to photons above 500 MeV, because inserted between the counters of the Cosmos counter telescope were 3.7 radiation lengths of lead in which multiple scattering and energy loss greatly reduce the efficiency between 100 and 500 MeV. The Russian group's claim for time variability of 3C 120 as a γ-ray source, because it was not observed on earlier surveys, is not valid. The surveys in this energy range which were cited (refs. 8 and 9) did not cover this region of the sky. The OSO-3 detector (ref. 10), which did cover this area, has a sensitivity to point sources which is about the same as the reported flux from 3C 120.

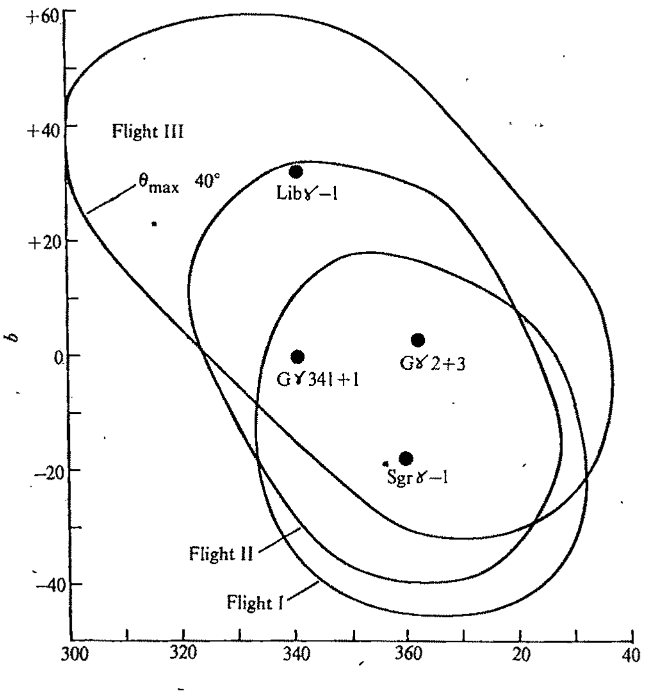


Fig. 1 Sky regions covered by the three flights. Boundaries are shown where the exposure is 50% and events are restricted to zenith angles $0 < 40°$. The black dots represent the positions of the four sources so far observed including the new source Lib γ-1 at $l = 340°$; $b = 30°$.

In this article we report a further point γ-ray source Libra γ-1 detected on the balloon flight of November 26, 1969, which was described earlier (flight III of ref. 2). The signal-to-noise ratio is 6σ for $E_\gamma > 100$ MeV, better than for any of the previous sources and furnishing further confirmation for the existence of this type of object. No excess was seen from this direction on an earlier flight, so for the first time we have direct experimental evidence for the time variation of a γ-ray source. The angular resolution circle in the direction of Lib γ-1 includes PKS 1514-24[11] which is also an optical variable[12,13].

The method of analysis is similar to that used previously[1,2] except that, after the approximate source position was ascertained from the search programme which had rectangular bins of $\Delta\alpha = 4°$, $\Delta \sin\delta = 0.08$, a series of concentric annular rings of equal area was drawn about the preliminary location of the source: $\alpha = 227°$, $\delta = -22°$. The radius of the first circle was 2.12° with an outer radius of 6° for the eighth annulus. The higher flux from Lib γ-1, combined with the reduced atmospheric background found at the 8.8 GeV geomagnetic cutoff for flight III, made this more detailed analysis possible. In Fig. 2 is shown the number of events for succeeding rings proceeding out from the source position. After the fourth ring, which has a radius of 4.2°, the intensity has decreased to the background level. The number of events for the energy groups—*a*, all energies (the effective threshold is 50 MeV); *b*, $E_\gamma > 100$ MeV; *c*, $E_\gamma > 500$ MeV—are listed in Table 1. For $E_\gamma > 100$ MeV there is an excess of 40 events above the 45 predicted from the background level, a 6.0σ result. The flux for $E_\gamma > 100$ MeV is $F_\gamma = (2.4 \pm 0.6) \times 10^{-5}$ γs cm^{-2} s^{-1}, greater than for Sgr γ-1, Gγ 2+3, or Gγ 341+1, but still a factor 20 less than for the Cosmos 251 source.

Our flight II (ref. 2) also had a good exposure to this region but, as can be seen in Fig. 2*d*, no evidence for Lib γ-1 was found on this flight, which was made on February 26, 1969. Thus only an upper limit can be set. With 95% confidence the flux is $F_{95} < 1.4 \times 10^{-5}$ γs cm^{-2} s^{-1}. The γ-ray flux increased by at least a factor of two over this nine month interval.

It is not surprising to find a time variation in the γ-ray flux from a localized object since a great variety of time variations is seen at lower frequencies. In particular high energy X-ray sources have appeared and disappeared between exposures and have also exhibited flare type of emission with a time scale of a few minutes[14]. We have examined our data to see if any variation occurred during the flight itself, but as is shown in Fig. 3, the number of events per half hour interval follows the expected response within the statistical accuracy.

Table 1 Number of Events detected in Different Energy Groups

Flight date and geomagnetic cutoff	Source	Energy groups	No. x in central area*	Background in equal area N*	D_x	P_x	Flux γs cm^{-2} s^{-1}
III Nov. 26–27, 1969 2224–0636 UT 8.8 GeV	Lib γ-1	All energy $E>$ 100 MeV $E>$ 500 MeV	121 85 25	75 45 14	6.4×10^{-7} 4×10^{-8} 5×10^{-3}	6.4×10^{-5} 4×10^{-6} 0.39	$2.8\pm0.8\times10^{-5}$ $2.4\pm0.6\times10^{-5}$ $0.7\pm0.4\times10^{-5}$
II Feb. 26–27, 1969 1815–0205 UT 4.5 GeV	Lib γ-1	$E>$ 100 MeV	60	66	0.82	1.0	95% upper limit 1.5×10^{-5}

D_x is probability of one distribution having number x or greater with mean N.

P_x is probability of one "central" area with number x occurring in an area 100 times the central area—that is, the area observed (assuming Poisson distribution).

* To nearest whole number.

Recently Bond[15] and Biraud[13] have pointed out that the radio source PKS 1514-24, identified as an E or N galaxy[11,16], coincides with the remarkable optically varying object AP Lib, which has been observed since 1935 to vary in magnitude between 14.5 and 16.4. A variation of 0.7 magnitude was observed in this source over one 5 h interval. In a systematic search for coincidences between irregular variable stars and radio sources Biraud added AP Lib and W Com to BL Lac and BW Tau which were already known. Our preliminary position for Lib γ-1 is $\alpha=227°\pm2°$, $\delta=-22°\pm2°$; 2.7° of arc from the optical and radio position of PKS 1514-24, $\alpha=228.7°$, $\delta=-24.2°$ and within our angular resolution circle.

It is remarkable that two of the three γ-ray sources which are not correlated with X-ray sources should coincide with two of the four objects listed by Biraud. An earlier γ-ray survey[9] covered BL Lac and W Com but they should be observed again for as long an exposure as possible in view of the time varying nature of Lib γ-1.

Shklovsky[17] has proposed a theory which explains the radio variations of 3C 120 and predicts surges of γ-rays from it and similar objects such as QSOs and Seyfert galaxies which show time variations at lower frequencies. The γ-rays are generated by bremsstrahlung from the fast electrons which produce the varying radiation at lower frequencies by synchrotron radiation. Shklovsky predicts the surges of γ-rays should last the order of several days or weeks and be separated by intervals the order of a year. Our observation is consistent with this time scale since we could detect no large variation over 6 h for Lib γ-1 but a definite variation over 9 months. If Shklovsky's theory is correct, γ-rays should be observed

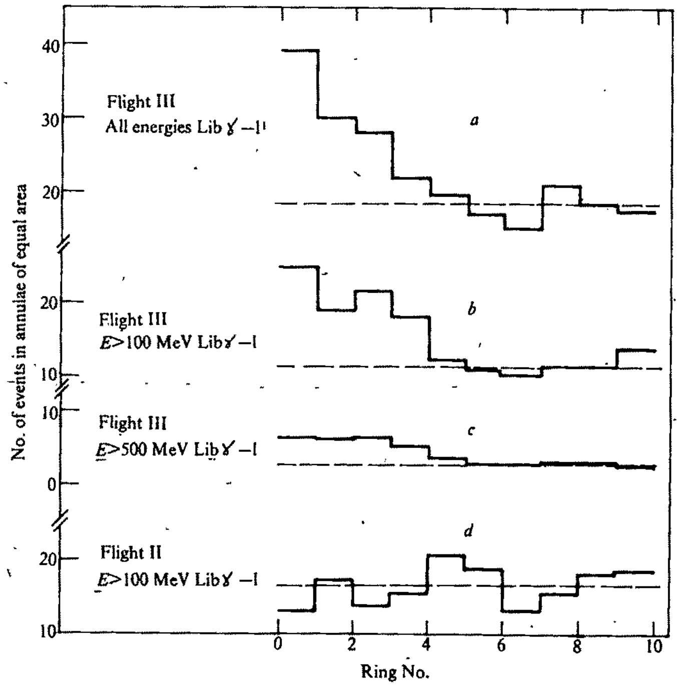


Fig. 2 Number of events plotted for circular annulae about the central position of source Lib γ-1 for flight III and flight II. The radius of ring number 1 is 2.1° and of ring number 8 is 6.0°. The dashed lines correspond to the means for the rings from number 4 to number 8.

Table 2 Comparison of γ-source Positions with Objects listed by Biraud[13]

Possible γ-source	RA	Dec.	Radio and/or optical source	Radio and optical position RA	Radio and optical position Dec.	Type
Lib γ-1 (ref. 1)	$227°\pm2°$	$-22°\pm2°$	AP Lib PKS 1514–24	228.7°	−24°	E or N, variable
(ref. 3)	$54°<\alpha<75°$ $+4<\delta<+9$		BW Tau 3C 120 PKS 0430+5	67.5°	+5°	Seyfert, strongly variable
			W Com ON 231	184.5°	+28°	Variable
			BL Lac VRO 42 22 01	330.0°	+42.0°	Similar to AP Lib

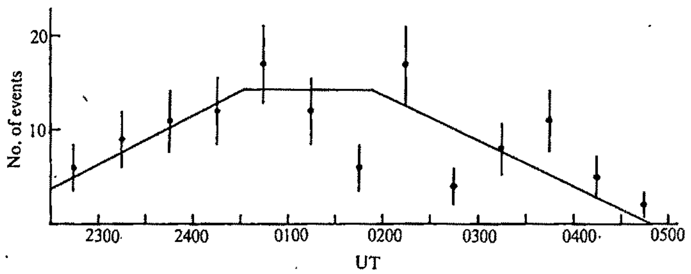


Fig. 3 Number of events per half hour interval received from the source direction during flight III. The full line shows the expected response curve for a constant source.

from a given object only 1–10% of the time and thus supposedly our second period of observation coincided with an outburst. An extended period of observation would then be required to delineate the time history of Lib γ-1. The γ-ray energy spectrum will furnish an additional test of the theory. If the spectrum follows a power law in the 10–100 MeV region, the corresponding electron energy spectrum can be deduced. If, however, it is a pion spectrum with the characteristic peak at 70 MeV (which may be red-shifted if PKS 1514–24 is far away), nucleon–nucleon collisions or nucleon–antinucleon annihilation will be responsible for the γ-radiation and Shklovsky's mechanism will be ruled out.

We thank J. Maynard and J. Panettieri for help. We also acknowledge the careful film analysis of Mesdames C. Conoboy, M. Samuels, L. Satz, Y. Strauss, N. Varma, D. Laba, M. Nash and M. Reardon, and thank E. Curwood, D. Scott, J. Hillier and the launch crew at the Mildura Base for the flights, and T. Gross, US Atomic Energy Commission, D. Sumner and W. Wilson, Office of Naval Research, R. Leslie, Australian Department of Supply, the Royal Australian Air Force, and the Australian Bureau of Meteorology for their assistance. This work was supported in part by grants from the National Science Foundation and the Australian Research Grants Committee.

G. M. FRYE, JUN.
P. A. ALBATS
A. D. ZYCH

Department of Physics,
Case Western Reserve University,
Cleveland, Ohio 44106

J. A. STAIB

Department of Physics,
Madison College,
Harrisonburg, Virginia 22802

V. D. HOPPER
W. R. RAWLINSON
J. A. THOMAS

Physics (RAAF) Department,
University of Melbourne,
Parkville N-2, Victoria

Received September 13, 1971.

[1] Frye, G., Staib, J., Zych, A., Hopper, V., Rawlinson, W., and Thomas, J., *Nature*, **223**, 1320 (1969).
[2] Frye, G., Albats, P., Zych, A., Staib, J., Hopper, V., Rawlinson, W., and Thomas, J., *Nature*, **231**, 372 (1971).
[3] Volobuev, S., Gal'per, A., Kirillov-Ugryumov, V., Luchkov, B., and Ozerov, Y., *Sh ETF Pis. Red.*, **13**, 28 (1971).
[4] Penston, M., *Inf. Bull. Var. Stars*, 255 (1968).
[5] Pauliny-Toth, I., and Kellerman, K., *Astrophys. J. Lett.*, **152**, L169 (1968).
[6] Hobbs, R., Hollinger, J., and Marandino, G., *Astrophys. J. Lett.*, **154**, L49 (1968).
[7] Penston, M., and Cannon, R., *IAU Colloq. on Non-Periodic Phenomena in Var. Stars*, Budapest (1968).
[8] Fichtel, C., Kniffen, D., and Ogelman, H., *Astrophys. J.*, **158**, 193 (1969).
[9] Frye, G., and Wang, C., *Astrophys. J.*, **158**, 925 (1969).
[10] Clark, G., Garmire, G., and Kraushaar, W., *Astrophys. J. Lett.*, **153**, L203 (1968).
[11] The Parkes Survey of Radio Sources, *Austral. J. Phys., Astrophys. Suppl.*, 8 (1969).
[12] Ashbrook, M., *Harvard Annals*, **109**, No. 7 (1942).
[13] Biraud, F., *Nature*, **232**, 178 (1971).
[14] McClintock, J., Ricker, G., and Lewin, W., *Astrophys. J. Lett.*, **166**, L73 (1971).
[15] Bond, H., *Astrophys. J. Lett.*, **167**, L79 (1971).
[16] Westerlund, B., and Wall, J., *Astron. J.*, **74**, 335 (1969).
[17] Shklovsky, I., *Astronomicheskii Zhurnal*, **47**, 742 (1970).

Short Term Variability of Pulsations in the X-ray Flux from Cygnus X1

IN a recent sounding rocket flight in the southern hemisphere (launched from Woomera, South Australia, on November 11, 1970) the X-ray source Cygnus X1 was observed by a 1,000 cm^2 collimated proportional counter sensitive to X-rays in the range 2–14 keV. Each X-ray event was indicated through the telemetry link by a 300 μs analogue pulse whose height was proportional to the incoming photon energy. This method of data transmission allows good timing accuracy together with a reasonable spectral resolution.

The data were folded using a technique similar to that used by Boldt *et al.*[1] at a number of trial periods in the range 50 ms to 1.2 s, and a chi-squared statistical test carried out on the resulting light curve to search for the presence of significant periodic variations in the X-ray flux.

The detector observed the source twice during the flight, the two scans being separated by 55 s. During the first scan significant fluctuations were observed at periods of 57.50, 115.24 and 229.74 ms. Table 1 shows the significance of the departure from the expected count rate. *P* is the probability that such a value of chi-squared would arise from random data with no pulsation present. The smaller the value of *P* the more significant the fluctuation. Analysis of the second scan revealed no significant pulsation at the $P=0.01$ level at any period in the range tested. The maximum values of chi-squared

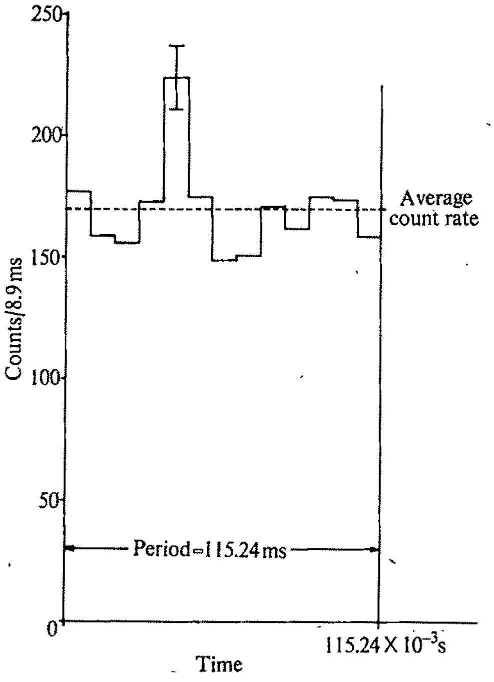


Fig. 1 "Light curve" at 115.24 ms period.

and the periods at which they were observed are also shown in Table 1. We interpret these results as indication of a drop in the fluctuating fraction of the X-ray output of the source in the 55 s separating the two scans. If similar behaviour is present at higher energies it may explain the negative result of Manchada *et al.*[2].

Table 1 Significance of Departure from Expected Count Rate

Period		Chi-squared (for $\nu = 10$)	P
Scan 1	57.50 ms	29.4	0.001
	115.24 ms	24.0	0.01
	229.74 ms	23.3	0.01
Scan 2	55.66 ms	19.5	0.04
	113.55 ms	15.5	0.10
	231.93 ms	14.4	0.11

The steady flux from Cygnus X1 was the same as measured by previous observers and showed no change between the two scans to within the experimental accuracy of 3%.

Fig. 1 shows the light curve at 115.24 ms, the only light curve in these data that is easily interpreted at the present time. By altering the number of phase bins within the light curve we have estimated the width of the pulse and also the maximum count rate during the pulse. These parameters and estimates of the pulsed energy output of the source are listed in Table 2. As the light curves at the other periods reported here are more complex we defer discussion of their interrelationship to a later communication.

Table 2 Measured Parameters of the 115.24 ms Light Curve

Period	115.24 ms
Pulse width	9.2 ± 0.4 ms
Count rate during pulse	0.64 ± 0.18 photons cm^{-2} s^{-1} in range 2–14 keV
Average flux	1.31 ± 0.05 photons cm^{-2} s^{-1} in range 2–14 keV
Energy in pulse as a fraction of total energy	0.04 ± 0.01
Pulsed energy (with period 115.24 ms) radiated by source, in energy range 2–14 keV (assuming a distance of 1.0 kpc (ref. 5)	1.5×10^{34} erg s^{-1}

The only period detected in this flight that has been previously reported is that of 230 ms. Other observers have detected periods of 290 ms and 1.1 s (ref. 3) and 73 ms (ref. 4), although these were not significant in our data. We note, however, that there are certain harmonic relationships between some of these periodicities.

We thank the staff at MSSL, BAC, BAC(A) and SRC (SRMU) for their assistance in this project. A. M. C. acknowledges an SRC studentship.

A. M. CRUISE
A. C. NEWTON
C. E. CHAPMAN

Mullard Space Science Laboratory,
University College London,
Holmbury St Mary,
Near Dorking, Surrey

Received September 13, 1971.

[1] Boldt, E. A., Holt, S. F., and Serlemitsos, P. J., *Astrophys. J. Lett.*, **164**, L9 (1971).
[2] Manchada, R. K., Iyengar, V. S., Agrawal, P. C., Gokhale, G. S., Kunte, P. K., and Sreekantan, B. V., *Nature Physical Science*, **232**, 190 (1971).
[3] Holt, S. F., Boldt, E. A., Schwartz, D. A., Serlemitsos, P. J., and Bleach, R. D., *Astrophys. J. Lett.*, **166**, L65 (1971).
[4] Oda, M., Gorenstein, P., Gursky, H., Kellogg, E., Schreier, E., Tananbaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **166**, L1 (1971).
[5] Gursky, H., Gorenstein, P., Kerr, S. J., and Grayzeck, E. J., *Astrophys. J. Lett.*, **167**, L15 (1971).

Soft X-ray Emission from Galactic Radio Spurs

Shklovsky and Sheffer[1] have recently raised the interesting possibility that the galactic radio spurs could be soft X-ray sources. As radio spurs have often been considered to be very old supernova remnants[2,3], and since three old remnants have been found to be soft X-ray sources[4,5], this idea has particular merit. Shklovsky and Sheffer point out that the soft X-ray (0.21 to 0.28 keV) map of Bowyer *et al.*[6] shows enhanced emission in the northern galactic hemisphere in a region coincident with secondary radio ridges of the North Polar Spur[7] and that this correlation may provide experimental verification of the hypothesis concerning the possible supernova remnant origin of the spurs.

The purpose of this letter is to question the proposed association between radio spurs and soft X-ray emission in general and to take issue with the particular identification of soft X-ray emission observed by Bowyer *et al.* with the North Polar Spur. The main points of our argument are that an independent interpretation of the radio spurs exists[8] which shows most of these features are not related to SNR. A possible exception is the Cetus Arc[9], a configuration in the southern galactic hemisphere, which we discuss in depth. We conclude on theoretical grounds that this object is not likely to be an X-ray source. From the observational viewpoint the soft X-ray enhancement observed by Bowyer *et al.* can be interpreted in terms of solar X-rays resonantly scattered in the Earth's atmosphere. Other investigators who have scanned the North Polar Spur and portions of other galactic spurs (in particular the Cetus Arc) at large solar zenith angles have not observed any enhancement at soft X-ray energies.

With regard to possible interpretations of the galactic radio spurs, we call attention to the fact that Mathewson[8] has been able to explain most of the observed radio features in terms of synchrotron radiation from electrons spiralling along a helical component of the local magnetic field. With this interpretation, he also accounts for the polarization orientation of the nonthermal galactic radio background and the large-scale orientation of the optical polarization vectors of nearby stars.

One feature Mathewson's model is not able to explain is the Cetus Arc. The spectral index of the polarized radiation between 408 and 620 MHz is steeper in this region than in others in the Galaxy[10], and H_α filaments[11,12], H_β radiation[13], and neutral hydrogen (M. P. Hughes, A. R. Thompson and R. S. Colvin, unpublished results) have been detected in the region. It seems reasonable to interpret that this feature is an old and unusually large supernova remnant. If the Cetus Arc is indeed such a remnant, it is useful to estimate whether it is likely to be a soft X-ray emitter. The X-ray emission spectrum of a supernova remnant varies with time as it is strongly influenced by the dynamics of the expansion. Three phases can be distinguished in the expansion of a typical shell[14,15].

(1) The free expansion phase, which proceeds up to a time when deceleration effects become important. The diameter at such a time is $D = 4.2\ (M/M_\odot)^{1/3}\ n_H^{-1/3}$ pc, where M is the ejected mass and n_H the unperturbed interstellar number density.

(2) A deceleration phase which is energy-conserving (or adiabatic). During this phase the shell diameter varies as

$$D \approx 25.8 \left(\frac{E_0}{n_H}\right)^{1/5} \left(\frac{t}{10^4 \text{ yr}}\right)^{2/5} \text{pc} \qquad (1)$$

where E_0 is the energy released in the explosion in units of 7.5×10^{50} erg and t is the age in years. In this case the shock velocity is given by

$$v_s \approx 505 \left(\frac{E_0}{n_H}\right)^{1/5} \left(\frac{t}{10^4 \text{ yr}}\right)^{-3/5} \text{km s}^{-1} \qquad (2)$$

Heiles[16] demonstrated that this phase continues only up to a time when radiation losses become important and thereafter the assumption of energy conservation is no longer valid. Cox[15] has shown that when line emission by heavy elements is included in the radiative losses, the thermal energy content of the whole remanant varies with time as

$$\frac{E}{E_0} = 1 - \left(\frac{t}{t_c}\right)^{17/5} \qquad (3)$$

where $t_c = 5.6 \times 10^4 \ E_0{}^{4/17} \ n_H{}^{-9/17}$ yr. The shell diameter at this time is

$$D_c = 50.8 \ E_0{}^{5/17} \ n_H{}^{-7/17} \text{ pc} \qquad (4)$$

Shortly after the remnant has cooled to a dense shell, it enters the next and final phase.

(3) The momentum-conserving phase in which the radius varies as

$$D \sim \left(M_0 v_0\right)^{1/4} \ n_H{}^{-1/4} \ t^{1/4} \qquad (5)$$

where $M_0 v_0$ is the shell momentum at the time equation (5) becomes first applicable. Assuming the final shell momentum is 75% of the momentum at the time the temperature of a mass element drops faster from radiation than from expansion, the shock velocity in this phase is

$$v_s \approx 165 \ E_0{}^{16/17} \ n_H{}^{-1} \left(\frac{D\text{pc}}{40}\right)^{-3} \text{ km s}^{-1} \qquad (6)$$

During phase 1 and early in phase 2 the X-ray emission is most likely nonthermal in nature and due to the high-energy tail of the same electron distribution responsible for the nonthermal radio emission[17]. This is probably the case for Cas A, Tycho's supernova and the Crab Nebula. The spectra have already steepened in the X-ray range due to radiation and expansion losses. Continuous injection of high-energy particles seems indicated over a time scale of hundreds of years. For remnants late in phase 2, the X-ray emission is probably thermal radiation from the shock-heated plasma present in the shell. The temperature behind the shock is, for a monoatomic gas,

$$T = \frac{3\mu}{16k} v_s^2 \qquad (7)$$

where μ is the mean mass per particle, k is Boltzmann's constant, and v_s is the shock velocity. Using $\mu = 0.61 \ m_H$, corresponding to a 10% helium abundance by number, for $E_0 = n_H = 1$ and $t = 10^4$ yr, we see from equations (2) and (7) that $T \sim 3 \times 10^6$ K.

As a tentative test of these ideas, we list in Table 1 the diameters, ages and shock speeds for two well studied remnants, Vela X and the Cygnus Loop, with a comparison between the values obtained from equations (1), (2) and (7) and the observations[4,5]. For Vela X, it is assumed the age of the pulsar PSR 0833–45[18] to be that of the remnant. For the Cygnus Loop, the expansion velocity of the filamentary system may not be representative of the shock velocity[22] and a new estimate for the parameters has been obtained by scaling the Vela X values to the diameter of the Loop using equation (2).

Although our understanding of supernova remnants is far from complete, the reasonable agreement between theory and experiment for these two remnants lends confidence to attempts to use these models to predict the emission spectrum of the Cetus Arc. To do this, we need an estimate of the linear diameter and expansion velocity of this feature.

Hughes *et al.* (unpublished work) have compared the neutral hydrogen absorption velocities observed in the spectra of extragalactic radio sources in the direction of the Cetus Arc with velocities obtained from optical absorption lines in the spectra of nearby stars with known distances in this region. From this they obtain a distance to the centre of the Arc, assuming spherical symmetry, of $d \leq 170$ pc. The linear diameter, as implied from the radio angular diameter, is then $D \leq 240$ pc. The upper limit for the proper motion of the H_α filaments is 0.5 arc s yr^{-1} (ref. 11) and this provides an upper limit for the expansion velocity of $v_s < 90 \text{ km s}^{-1}$.

From a comparison of these values with equations (4) and (6), we see that the Cetus Arc ought to be well into the momentum-conserving phase. Cox has computed a number of detailed models for supernova remnants in this phase[15]. The model which is most appropriate for comparison with the Cetus Arc is characterized by $v_s = 70 \text{ km s}^{-1}$. For this model, Cox derives a shock temperature of $T \sim 6 \times 10^4$ K. Alternately, using equation (7), we find that for $v_s < 90 \text{ km s}^{-1}$, the corresponding temperature is $T < 9.5 \times 10^4$ K. In either case, the upper limit for the temperature of the Cetus Arc is at least a factor of 40 below that needed in order for this object to be a source of soft X-rays, and almost all the energy is radiated in the form of ultraviolet, visible, and infrared lines.

Direct estimates of conditions in the Cetus Arc can be made by scaling values relevant for Vela X to the 240 pc diameter of the Arc using either equations (1) and (2), relevant for a remnant in phase 2, or equations (5) and (6), relevant for a remnant in phase 3. This approach assumes that the parameters E_0 and n_H are the same in the two objects. In equations (1) and (2), the ratio E_0/n_H enters as the one-fifth power; hence, the effects of any possible differences in these parameters tend to be minimized while the remnant is in phase 2. In equation (6), however, this ratio enters directly, so estimates for v_s while the remnant is in phase 3 are less reliable. In Table 1 we list the values for the age, shock velocity, and shock temperature for the Cetus Arc derived in this manner. With both models, the predicted shock speeds are consistent with the observational upper limit; and in both cases, the temperature is far below that necessary for the object to be a source of soft X-rays.

Considerable soft X-ray data that relate directly to this subject are available. The soft X-ray enhancement detected by Bowyer *et al.*[6] near the North Polar Spur is best interpreted

Table 1 Observed and Computed Parameters for Old Supernova Remnants

Source	Diameter (pc)	Age (yr)	v_s (km s^{-1})	kT (keV) Observed	kT (keV) Predicted	References
Vela X	35	1.13×10^4	600	0.23	0.4	5, 18, 19
Cygnus Loop	41	6.7×10^4	*110*	0.37	0.014	4, 20, 21
		1.8×10^4 *	440		0.23	
Cetus Arc	240	1.4×10^5 *	30	—	1.3×10^{-3}	11, and Hughes *et al.* unpublished
		2.7×10^7 †	2		5×10^{-6}	

Note: Velocity in italics is an observational value. For the Cetus Arc the observational upper limit to the velocity is $v_s < 90 \text{ km s}^{-1}$. The corresponding temperature is $kT < 8.0 \times 10^{-3}$ keV.

* Computed using energy-conserving theory, scaled from Vela X.

† Computed using momentum-conserving theory, scaled from Vela X.

as the result of atmospheric scattering of solar X-rays, because the Sun was close to the horizon at this position in the sky at the time of their flight[23]. This effect has been examined in some detail by Tomblin[24] and appears to be well understood. Other investigators have scanned portions of galactic radio spurs at soft X-ray energies with more favourable solar zenith angles: Henry *et al.*[25] and Palmieri *et al.* (unpublished work) have scanned the North Polar Spur; Bunner *et al.*[26], Hayakawa *et al.* (unpublished work) and Baxter *et al.*[27] have scanned Loop III[28]; and Bunner *et al.*[26,29], Hayakawa *et al.* (unpublished work) and Baxter *et al.*[27] have scanned the Cetus Arc. No soft X-ray enhancements are present in the published data of these investigators near the positions of these features.

We conclude both on theoretical and observational grounds that galactic spurs are not, in general, sources of soft X-ray emission. The Cetus Arc may well be a supernova remnant; if it is, however, it has long ago ceased to be a source of X-ray emission.

This work was supported in part by an NASA grant.

SERGIO A. ILOVAISKY *
STUART BOWYER

*Department of Astronomy
and Space Sciences Laboratory,
University of California,
Berkeley,
California* 94720

Received August 31, 1971.

* Permanent address: Centre d'Etudes Nucléaires de Saclay, 91 Gif sur Yvette, France.

[1] Shklovsky, I. S., and Sheffer, E. K., *Nature*, **231**, 173 (1971).
[2] Hanbury Brown, R., Davies, R. D., and Hazard, C., *Observatory*, **80**, 191 (1960).
[3] Elliot, K. H., *Nature*, **226**, 1236 (1970).
[4] Gorenstein, P., Harris, B., Gursky, H., Giacconi, R., Novick, R., and Vanden Bout, P., *Science*, **172**, 369 (1971).
[5] Palmieri, T. L., Burginyon, G., Grader, R. J., Hill, R. W., Seward, F. D., and Stoering, J. P., *Astrophys. J.*, **164**, 61 (1971).
[6] Bowyer, C. S., Field, G. B., and Mack, J. E., *Nature*, **217**, 32 (1968).
[7] Large, M. I., Quigley, M. J. S., and Halsam, C. G. T., *Mon. Not. Roy. Astron. Soc.*, **131**, 335 (1966).
[8] Mathewson, D. S., *Astrophys. J. Lett.*, **153**, L47 (1968).
[9] Large, M. I., Quigley, M. J. S., and Halsam, C. G. T., *Mon. Not. Roy. Astron. Soc.*, **124**, 405 (1962).
[10] Mathewson, D. S., Broten, N. W., and Cole, D. J., *Austral. J. Phys.*, **19**, 93 (1966).
[11] Elliot, K. H., and Meaburn, J., *Astrophys. Space Sci.*, **7**, 252 (1970).
[12] Meaburn, J., *Z. Astrophys.*, **65**, 93 (1967).
[13] Reay, N. K., *Mon. Not. Roy. Astron. Soc.*, **151**, 299 (1971).
[14] Spitzer, L., *Diffuse Matter in Space* (Interscience, New York, 1968).
[15] Cox, D. P., thesis, University of California, San Diego (1970).
[16] Heiles, C., *Astrophys. J.*, **140**, 470 (1964).
[17] Tucker, W. H., *Astrophys. Space Sci.*, **9**, 315 (1970).
[18] Gunn, J. E., and Ostriker, J. P., *Astrophys. J.*, **160**, 979 (1970).
[19] Milne, D. K., *Austral. J. Phys.*, **21**, 201 (1968).
[20] Minkowski, R., in *Nebulae and Interstellar Matter* (edit. by Middlehurst and Aller) (University of Chicago Press, 1968).
[21] Doroschenko, V. T., *Sov. Astron. A-J*, **14**, 237 (1970).
[22] Tucker, W. H., *Science*, **172**, 372 (1971).
[23] Bowyer, C. S., and Field, G. B., *Nature*, **223**, 573 (1969).
[24] Tomblin, F. F., *J. Geophys. Res.*, **76**, 163 (1971).
[25] Henry, R. C., Fritz, G., Meekins, J. F., and Friedman, H., *Astrophys. J.*, **153**, 11 (1968).
[26] Bunner, A. N., Coleman, P. L., Kraushaar, W. L., McCammon, D., Palmieri, T. L., Shilepsky, A., and Ulmer, M., *Nature*, **223**, 1222 (1969).
[27] Baxter, A. J., Wilson, B. G., and Green, D. W., *Canad. J. Phys.*, **47**, 2651 (1969).
[28] Quigley, M. J. S., and Halsam, C. G. T., *Nature*, **208**, 741 (1965).
[29] Bunner, A. N., Coleman, P. L., Kraushaar, W. L., and McCammon, D., *Astrophys. J. Lett.*, **167**, L3 (1971).

Is Mercury from Hawaiian Volcanoes a Natural Source of Pollution?

THE development of more sensitive techniques[1] for the analysis of mercury has resulted in reports of previously unsuspected amounts of mercury in the environment. There are now several well documented cases of mercury pollution deriving from industrial sources, but sources of other forms of mercury pollution, as in ocean-going fish[2], for example, are not so well defined[3]. The widespread distribution of mercury in the environment may, however, be in part a consequence of the physical properties of the element, the manufacture of the element by the smelting of cinnabar, and the fact that certain microorganisms can convert mercury to the gas dimethyl mercury.

Another possible source of atmospheric mercury is vulcanism. A recent review[5] states that "data on mercury contents of fumaroles are lacking because of the rarity of volcanic eruptions and high-temperature fumaroles and, until recently, the lack of adequate methods of analysis. Hawaiian and Alaskan fumaroles should be studied". We now report that the active vulcanism on the island of Hawaii is producing significant amounts of mercury.

Atmospheric mercury was obtained by drawing 0.2–0.4 m^3 of air through 15×150 mm U-tubes containing traps of 10 ml. of concentrated H_2SO_4, 1 ml. of concentrated nitric acid and about 500 mg of powdered copper (reagent grade). Variations on this procedure included the use of different glassware and the replacement of copper by silver, gold and other heavy metals.

Field air samples were collected using a portable battery-powered pump (Mine Safety Appliances No. 92–814, Bureau of Mines Approval No. 2F-2004). After sample runs of 30 to 90 min duration, trapping vessels were sealed for analysis at the laboratory in Honolulu. Further samples were collected using Mine Safety Appliances No. 93-494 cellulose filters, which retain particles down to 0.3 μm diameter.

Table 1 Atmospheric Mercury Levels in Selected Hawaiian Sites

Sample site and time	Trap	Volume of air sample (m^3)	Hg μg/m^3
A, Manoa Campus, University of Hawaii, Honolulu (Oahu)			
1700–1900 h, April 18, 1971, temp. 25° C, clear, no wind	(1) Cu/acid	0.50	0.034
	(2) Ag/acid	0.47	0.019
	(3) Cu/acid	0.40	0.065
		Average	0.04
B, Ala Moana Beach, Mamala Bay, Honolulu (Oahu)			
1200–1400 h, May 13, 1971, temp. 30° C, clear, mild breeze from north	(1) Cu/acid	0.36	0.60
	(2) Cu/acid	0.36	0.71
	(3) Cu/acid	0.36	1.41
		Average	0.91
C, Sulfur Banks, Hawaii Volcanoes, National Park (Hawaii)			
1000–1200 h, April 10, 1971, temp. 20°–22° C, intermittent light rain	(1) Cu/acid	0.36	26.6
	(2) Charcoal	0.34	26.3
	(3) Cu/acid	0.36	17.1
		Average	23.3
1100–1300 h, May 8, 1971, temp. 22° C, clear to light rain	(1) Cu/acid	0.15	15.4
	(2) Cu/acid	0.34	27.4
		Average	21.4
	(3) Cellulose filter	0.34	0.38
	(4) Cellulose filter	0.24	0.50
		Average	0.44

Trap contents were transferred quantitatively to acid-washed flasks containing 100 ml. of concentrated nitric acid and diluted to 200 ml. with distilled water. Portions of 20 ml. were analysed for mercury content by flameless atomic absorption at the 253.65 nm resonance line. We used a Utopia Instruments flameless mercury analysis kit[6] and a 10 cm absorption cell mounted on the burner of a Beckman 1301 atomic absorption spectrophotometer. Samples were compared with appropriate blanks and standards. The results are summarized in Table 1. The Sulfur Banks within Hawaii Volcanoes National Park is a site where steam and gas are emitted more or less continuously through crevices and cracks which vent into the open air. Control samples were taken in the Manoa Valley which has light traffic and no industry. Tradewinds usually carry clean air masses over the head wall of the valley. A second control site was urban, non-residential, and in proximity to heavy traffic, but also was without industrial development.

Table 2 Boiling Points of Selected Mercury Compounds

Compound	Boiling, sublimation (S) or decomposition (D) point (°C)
Hg	356.58
Hg_2Br_2	S 345
$HgBr_2$	322
Hg_2Cl_2	S 400
$HgCl_2$	302
Hg_2I_2	S 140, D 290
HgI_2 (β-form)	354
Hg_2O	D 100
HgO	D 500
HgS	S 583.5

The results show that 98% of the mercury issuing from the Hawaian fumaroles is either in the form of a gas or of particles less than 0.3 µm in diameter. Several mercury compounds as well as the pure metal may become gaseous under the conditions of temperature and pressure found in volcanic fumaroles (Table 2)[7]. The magnitudes of mercury content in these fumaroles' discharges agree quite well with reports from thermal areas in Kamchatka and the Kuriles[5]. The background figures for Honolulu seem high when compared with other reports, but this may simply be because this site is close to a volcano.

The Hawaiian Islands possess industries using mercury. The sugar industry (the state's third largest behind the military and tourism), for example, has used mercury-containing fungicides. Mercury batteries, lamps and switches are used by many individuals and businesses. Military consumption of mercury is not known. In spite of the industrial consumption of mercury in Hawaii, however, it is still reasonable to suspect that the volcanoes supply large quantities of mercury to the environment. During a period of heavy volcanic activity it is not uncommon to have a high atmospheric turbidity extending along the island chain. This haze may be very noticeable in downtown Honolulu on the island of Oahu, more than 200 miles NNW of the source. Since our laboratory has turned its attentions toward mercury studies this phenomenon has not recurred so that we have been unable to verify the efficacy of atmospheric dispersal of mercury from volcanic activity. We are now planning a programme of continued air monitoring for mercury.

In conclusion, we do not wish to discount the mercury contribution of industrial pollution, but only to suggest that in Hawaii, at least, natural as well as human factors may be at work.

We thank Park Superintendent Balaz and Chief Ranger Hewitt of Hawaii Volcanoes National Park for their cooperation and advice in the course of this study; Adele Shimabuku and James Barres for field collection and sampling; and also Dr Irving Breger, United States Geological Survey, for reviewing this manuscript. This work was supported in part by a grant from the National Aeronautics and Space Administration.

ALAN ESHLEMAN
SANFORD M. SIEGEL

Department of Botany,
Environmental Center,
University of Hawaii

BARBARA Z. SIEGEL

Department of Microbiology,
University of Hawaii,
Honolulu, Hawaii 96822

Received June 28; revised August 27, 1971.

[1] Manning, D. C., *Atomic Absorp. Newsl.*, **9**, 97 (1970).
[2] *Environment*, **13**, 4 (1971).
[3] Siegel, S. M., Eshleman, A., Umeno, I., Puerner, N., and Smith, C. W., *Proc. Mercury in the Western Environment Workshop* (Oregon State University, in the press).
[4] Challenger, F., *Quart. Rev.*, **9**, 255 (1955).
[5] White, D. E., Hinkle, M. E., and Barnes, I., *Prof. Pap. US Geol. Surv.*, No. 713, 25 (1970).
[6] Miltelhauser, H. M., *Atomic Absorp. Newsl.*, **9**, 6 (1970).
[7] *Handbook of Chemistry and Physics*, forty-eighth ed. (Chemical Rubber Co., Cleveland, 1968).

Proposed New Method for Separating Isotopes

RECENTLY[1] a double mercury discharge vessel was used (Fig. 1), in which a large current running in the outer vessel irradiated the small current discharge in the inner vessel with 2537 Å resonance radiation. This irradiation caused the running voltage and electron temperature in the inner discharge to be reduced appreciably over the non-irradiated mode of operation. The explanation offered was that the neutral Hg atoms in the inner vessel absorbed the resonance radiation and entered the 6^3P_1 state (see Fig. 2), and from this level the atom was ionized by electron impact. In addition the 6^3P_0 and 6^3P_2 metastable levels are populated from the 6^3P_1 level by electron collisions and by cumulative absorption and radiative decay of higher levels. Because the radiation field is doing approximately half the ionization work, the electron temperature drops correspondingly.

The present communication suggests the possibility that such a double discharge could form the basis of an isotope separator, if the outer, irradiating discharge were run in a pure isotope and the hyperfine components of the resonance line are separate. We show in Fig. 3 the hyperfine components of the Hg 2537 Å line and it is seen that ^{200}Hg and ^{202}Hg could be separated by this method. In this situation only the isotope in the inner discharge which corresponds to that in the outer will be excited, and thus the Hg ions which are accelerated to the discharge wall by the radial electric field will be predominantly of one isotope. The details of the collection of ions at the wall are not considered here.

It is interesting to note that this method differs from other methods in current use in that it does not depend directly on

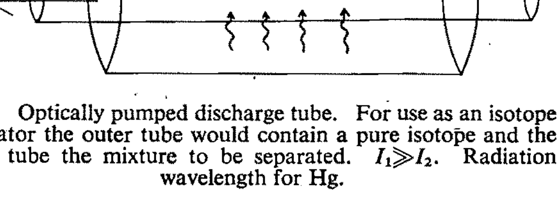


Fig. 1 Optically pumped discharge tube. For use as an isotope separator the outer tube would contain a pure isotope and the inner tube the mixture to be separated. $I_1 \gg I_2$. Radiation wavelength for Hg.

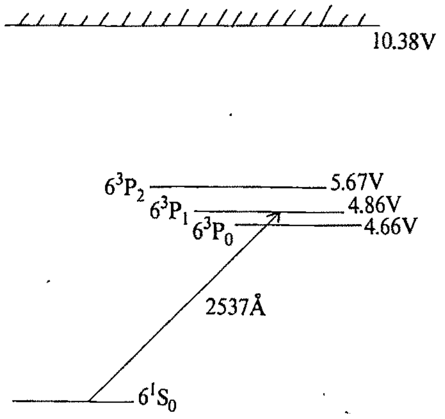


Fig. 2 Simplified Hg energy level diagram.

the isotope mass ratios, which for heavy elements are almost unity.

In the case of Hg isotope separation the inner discharge changes to the radiation-assisted mode only for Hg gas pressures greater than 10 mtorr[1]. At these higher pressures the ion on its way to the wall will suffer charge exchange collisions with Hg atoms of all isotopes and so the ion wall current will not be of the pure isotope. In the case of Hg, further experiments in our laboratory have shown that the addition of a few mtorr[1] of N_2 to the Hg facilitates the transition to the radiation-assisted mode, at only 1 mtorr of Hg, where the charge exchange mean free path for Hg^+–Hg collisions is fairly long. It is believed that this improvement is caused by collisions between Hg 6^3P_1 atoms and N_2 in the $v=0$ vibration state, giving Hg 6^3P_0 and N_2 ($v=1$), (ref. 2), and because the Hg 6^3P_0 metastable state has a much longer lifetime than that of Hg 6^3P_1, the electron impact ionization makes more efficient use of the radiation assistance. If this is the explanation, then CO should be even more efficient an additive than N_2 (ref. 2), because the quenching cross-section of CO for $6^3P_1 \rightarrow 6^3P_0$ is much greater than that of N_2. Such high ionization potential additives as N_2 and CO result in Hg being the predominant ion even up to molecular gas pressures considerably greater than the Hg pressure.

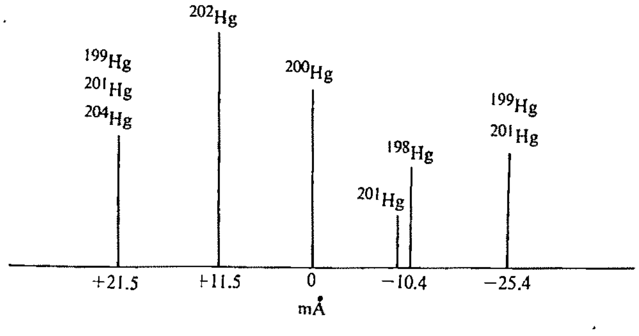


Fig. 3 Hyperfine structure of the 2537 Å resonance line.

Extension of this method to other elements is dependent on the fulfilment of several criteria, among which are that, first, the hyperfine components must not overlap and, second, that the mean free path for radiation absorption be equal to or less than the mean free path for charge exchange.

This latter criterion is probably not satisfied by uranium, although its spectroscopic properties are poorly known[3].

We thank Professor D. W. Holder for support in this work which was carried out under contract from the Culham Laboratory (UKAEA). P. C. S. is indebted to NRC (Canada) for support.

P. C. STANGEBY
J. E. ALLEN

Department of Engineering Science
and University College,
Oxford

Received July 19, 1971.

[1] Stangeby, P. C., Allen, J. E., and Fraser, D. A., *Proc. Tenth Gas Ionization Conf.*, 30, Oxford (1971).
[2] Mitchell, A. C. G., and Zemansky, M. W., *Resonance Radiation and Excited Atoms*, second ed. (Cambridge University Press, 1961).
[3] Mann, J. B., *J. Chem. Phys.*, **40**, 1632 (1964).

On the Formation of the Solar System

THE object of this article is to point out the significance of some properties of Jupiter (the most massive planet in the solar system) for the problem of the origin of the solar system. The mass of Jupiter is 0.001 solar masses, and its orbital eccentricity is 0.05. The following discussion shows that these properties of Jupiter are primaeval, and that they have not been affected by the evolution of the solar system.

I shall start by considering what would happen to the planetary system if the mass of Jupiter and/or the eccentricity of its orbit around the Sun increased Shelus and I[1] and Shelus (unpublished) have already tried to answer this in a study of the elliptical restricted problem of three bodies. Periodic orbits for a body of infinitesimal mass in the presence of two massive objects, moving around each other in eccentric orbits, were computed. The mass ratios for the two primaries used in this study were 1,000, 100, 10, 5, and 1. The periodic orbits were tested for stability under the framework of a linear stability analysis. From these computations, it is clear that increasing the mass of Jupiter and/or increasing its orbital eccentricity makes the orbits of the "small" planets, such as the Earth, unstable. A small planet, when put in one of the unstable orbits, will tend to collide with one of the primaries, or it will be thrown out of the system. Some stable periodic orbits exist, but they are located either very close to one of the two primaries or very far away from both of them.

If Jupiter had originally possessed 40 or 50 times its present mass, it would have made the planetary system unstable over a period much shorter than 4.5×10^9 yr. The planetary system would also have become unstable if Jupiter's orbital eccentricity were as small as 0.5. It should be mentioned that the increase of the eccentricity tends to destroy the stability of the orbits of the small planets more effectively than does the increase of the mass of Jupiter. Thus the fact that the planetary system has survived for a period of 4.5×10^9 yr leads us to the conclusion that the above mentioned properties of the most massive planet (Jupiter) are original properties of the system. If they are original, then any satisfactory theory of the formation of the solar system must take them into account.

The above discussion is obviously relevant to the problem of the existence of other planetary systems in the Galaxy. It is clear that double stars, which normally have mass ratios in the range 1 to 10 and eccentricities as high as 0.9, cannot possess planetary systems such as our own. Further, double stars, such as Lalande 21185, in which one of the components is a low mass "dark" star, may not possess planetary systems similar to our own, for their mass ratios are in the range 10 to 50, and the eccentricities in the range 0.3 to 0.9. The number of multiple stars in the Galaxy is very high, and at least 60% of all stars in the solar neighbourhood are found in double systems[2]. As the formation of double stars seems to be favoured, the occurrence of planetary systems cannot be a universal phenomenon. We may conclude that the total number of planetary systems in the Galaxy is probably much smaller than that estimated by some workers[3].

I thank Professor H. Bondi and Dr P. Shelus for discussions.

SHIV S. KUMAR

Leander McCormick Observatory,
University of Virginia,
Charlottesville,
Virginia

Received November 23, 1970; revised July 19, 1971.

[1] Shelus, P. J., and Kumar, S. S., *Astron. J.*, **75**, 315 (1970).
[2] Worley, C., in *Low Luminosity Stars* (edit. by S. S. Kumar) (Gordon and Breach, New York, 1969).
[3] Huang, S. S., *Astrophys. J.*, **150**, 229 (1967).

Dust transported in the North-east and South-east Trade Winds in the Atlantic Ocean

THE dust in the atmosphere over the oceans can yield an insight into the processes of deep-sea sedimentation and into certain aspects of oceanic pollution[1-4]. The importance of wind transport for the introduction of land-derived material to the oceans varies from one area to another, and the highest reported content of dust is found in the north-east trade wind system over the North Atlantic Ocean[5], but there seem to be no data on the dust loadings in the south-east trades of the Atlantic. To establish the concentrations and kind of dust in this important wind system, collections were made during July and August 1970 in both the SE and NE trades of the Atlantic Ocean between about 16° S and 23° N.

The dusts were collected on nylon meshes, and the volume of wind passing through the meshes was calculated using a calibrated anemometer. Two meshes, each 1 m^2, were exposed for each collection and the weights of dust collected were corrected for the collection efficiency of the meshes, which was assumed to be 50% (ref. 1). Samples with a prefix SH were collected in the SE trades off the coast of St Helena from a small fishing boat; those with a prefix GH were collected on the RMMV Good Hope Castle. The ship's track, collection areas and dust loadings are shown in Fig. 1, and collection details are given in Table 1.

Dusts collected in the SE trades between about 16° S and the equator have an average concentration of 0.55 $\mu g\ m^{-3}$ of air, and are predominantly brown or grey in colour. With the exception of dust GH3, which contained some shipboard contaminants, the dust loadings are quite similar and have only a relatively small range of concentration (0.33–0.70 $\mu g\ m^{-3}$ of air). Sample GH4 was collected in variable winds of the intertropical convergence zone, and has a similar dust loading to the SE trades. This sample, however, has a distinct red–brown colour, suggesting an origin for much of the dust in the deserts of West Africa. There was a dramatic change in dust loadings when the ship entered the NE trades; the red–brown dusts have an average concentration of 24.5 $\mu g\ m^{-3}$ of air.

Table 2 Particle Size Analyses of the Dusts*

Sample	<2 μm	2 to 4 μm	4 to 8 μm	8 to 16 μm	16 to 32 μm	>32 μm
Average of Dusts SH1 to SH5	74	14	9.0	2.0	1.0	Trace
GH1	60	15	13	6.7	3.2	2.1
GH2	50	20	18	7.0	3.0	2.0
GH3	60	20	13	3.0	2.5	1.5
GH4	91	4.6	2.5	1.7	0.2	Trace
GH5	78	12	6.7	2.2	0.8	0.3
GH6	84	6.8	4.8	2.6	1.2	0.6
M1†	79	13.5	6.5	1.0	Trace	Trace

* Particle size determinations were made by a microscopic counting technique, and the results are given as the percentage number of particles in each size class. The analyst was L. R. Johnson.

† M1 dust collected off West African coast between 17° 39′ N, 16° 36′ W and 18° 55′ N, 17° 22′ W—see ref. 5 for collection details. The particle size analysis given in ref. 5 was on a weight percentage basis and differs from that given here which is based on percentage number of particles.

The particle size analyses of the dusts are given in Table 2. Dusts collected to the south of St Helena have only a trace amount of material in the size class >32 μm. By contrast, dusts GH1, GH2 and GH3 contain more material both in the >32 μm and the 8 to 16 μm size range; this is probably the

Table 1 Dust Collection Details

Sample	Location*	Wind system	Colour of dust	Dust loading ($\mu g\ m^{-3}$ of air)	Remarks
SH1	Off the coast of St Helena Island, position 15° 54′ S to 16° 01′ S and 05° 37′ W to 05° 46′ W	SE trades	Light brown	0.78	Winds SE to SSE
SH2		SE trades	Brown–grey	0.33	
SH3		SE trades	Brown–grey	0.51	
SH4		SE trades	Light brown	0.54	
SH5		SE trades	Brown	0.37	
GH1	13° 19′ S, 08° 41′ W 08° 16′ S, 14° 36′ W	SE trades	Light grey	0.44	Wind SE
GH2	08° 16′ S, 14° 36′ W 06° 08′ S, 14° 42′ W	SE trades	Grey	0.51	Wind SE. Some contamination from ship smoke; dust loadings therefore represent upper limits.
GH3	06° 08′ S, 14° 42′ W 00° 00′ S, 16° 00′ W	SE trades	Grey–black	0.93	
GH4	00° 00′ S, 16° 00′ W 12° 27′ N, 17° 28′ W	Doldrums	Red–brown	0.51	Winds variable
GH5	12° 27′ N, 17° 28′ W 20° 20′ N, 17° 43′ W	NE trades	Red–brown	23	Winds NNE
GH6	20° 20′ N, 17° 43′ W 23° 09′ N, 17° 05′ W	NE trades	Red–brown	26	Winds NNE

* For samples GH1 to GH6 the location given is the ship's position at the start and finish of each collection.

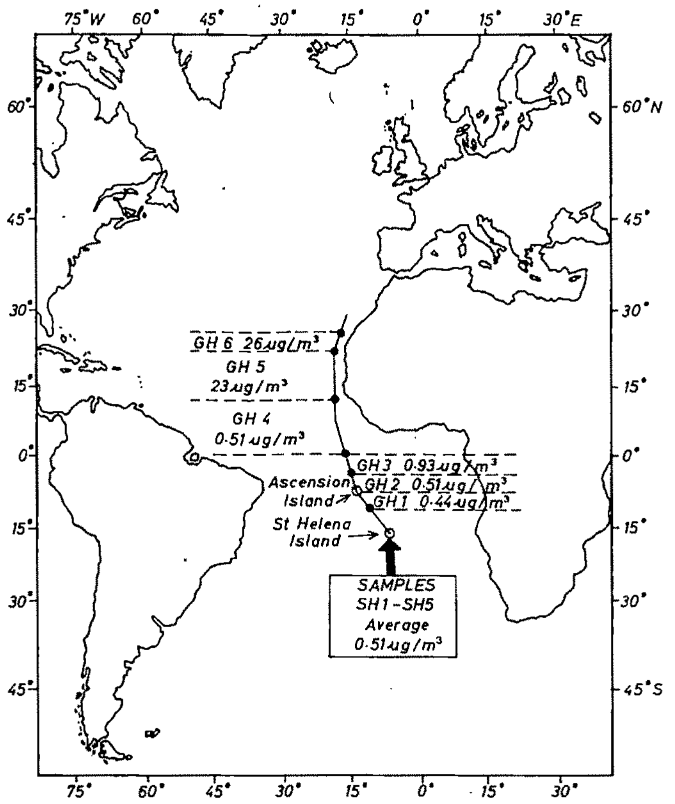


Fig. 1 Collections of atmospheric dusts in the south-east and north-east trade winds. The solid line indicates the partial track of the RMMV Good Hope Castle. The sample reference numbers are given together with the atmospheric dust loadings (calculated assuming a 50% mass collection efficiency).

result of an injection of material into the local atmosphere from the islands of St Helena and Ascension. Dusts GH5 and GH6 have a particle size distribution very similar to that of other dusts collected in the same area (Table 2). Sample GH4 has a larger proportion of small sized particles than either GH5 or GH6. The colour and mineralogy of GH4 indicate that a large proportion of the dust has the same origin as GH5 and GH6, that is, West African deserts, but it will have lost some larger sized material because it has been transported for a greater distance.

The mineralogy of some of the dusts is given in Table 3. The mineralogy of aeolian dusts has been widely reported in the literature[1,2,3,5] and only those features which highlight the differences between dusts of the NE and SE trades will be mentioned here. (1) It has been suggested[1] that dolomite probably from the Sahara Desert region characterizes NE trade wind dusts. Our data further indicate the usefulness of this mineral as an indicator of a Sahara Desert origin as it was not identified in any of the dusts from the SE trades; (2) the principal difference in the <2 µm clay mineralogies of the NE and SE trade wind dusts is that montmorillonite is far less abundant in the last of these. In South Atlantic deep-sea sediments montmorillonite has its highest concentration in mid-ocean areas where it constitutes between 30% and 50% of the total <2 µm clay minerals[7]. This has been interpreted in terms of either an aeolian input of montmorillonite or the *in situ* formation of this mineral from volcanic material of the Mid-Atlantic Ridge[7,8]. Our results indicate a relatively low montmorillonite content for South Atlantic dusts and support the hypotheses of the *in situ* formation of this mineral from local volcanic debris.

Our data and those given in the literature allow a tentative comparison between the dust-carrying capacities of the NE and SE trades of the Atlantic Ocean. The NE trades have a large reservoir of loose surface deposits in the desert areas of West and North Africa. Such an origin is reflected in the reddish colour usually shown by these NE trade dusts. Both the Kalahari (an inland desert) and the Namib (a coastal desert), both in southern Africa, could act as a source of dust for the SE trade winds. It has been shown[1,9] that dust originating in the Sahara Desert can be transported in relatively large quantities about 6,000 km westwards across the Atlantic. For example, dust-loadings at Bermuda in summer averaged about 6 µg m^{-3} of air. The influence which the Sahara reservoir exerts on the dust loadings of the NE trades is apparently much greater than the combined effects of the Kalahari and Namib deserts on the SE trades. In the atmosphere off St Helena, which lies about 2,500 km from the coast of South West Africa, the average dust loading is approximately 0.51 µg m^{-3} of air. This dust loading was obtained from collections made in the southern hemisphere winter; dust loadings will vary considerably from day to day and season to season depending on local meteorological conditions. From the haze-frequency distribution maps given by MacDonald[10] it can, however, be seen that the 5% isopath extends further from the coast of Africa in the southern hemisphere winter than it does in summer, that is, the winter dust loadings which we have obtained are likely to be an average upper limit for the yearly South Atlantic dust concentrations in this area.

To assess fully the effect of the Kalahari and Namib deserts on dust loadings of the SE trades, collections must be made southwards of St Helena towards the Namib coast of South

Table 3 Mineralogy of Some of the Dusts

Dust	Clay mineralogy* (<2 µm fraction)				>2 µm fraction†								
	Montmorillonite	Illite	Chlorite	Kaolinite	Mica	Talc	Amphiboles	Chlorite	Kaolinite	Quartz	K-feldspar	Plagioclase	Dolomite
SH1 SH2 SH3 SH4	Only small amounts of these samples were collected, and the <2 µm fractions contained a high proportion of X-ray amorphous material				×	×	—	Trace	Trace	× × ×	—	× × ×	—
					Trace	—	—	Trace	Trace	× ×	—	× × ×	—
					×	×	—	×	?	× ×	—	× × ×	—
					×	×	—	Trace	Trace	×	—	× × ×	—
GH1	Trace	43	57		×	× ×	Trace	—	—	× ×	—	× × ×	—
GH2	Trace	46	31	23	×	×	—	×	—	× ×	—	× × ×	—
GH3	2	25	11	62	× ×	×	—	×	×	× × ×	× × ×	—	—
GH4	20	38	14	28	× × ×	×	×	× ×	× ×	× × ×	× × ×	× × ×	—
GH5	43	22	17	18	—	—	—	—	—	—	—	× ×	—
GH6	10	41	17	32	×	—	Trace	×	×	× × ×	× × ×	× ×	× × ×

* The analyses are in terms of a 100% clay sample.

×, Present; × ×, abundant; × × ×, very abundant; —, not found.

West Africa. Our data does indicate, however, that in mid-ocean areas the deposition of eolian material from the SE trades probably has a much smaller effect on deep-sea sedimentation in the South Atlantic than it does from the NE trades of the North Atlantic.

We thank HE the Governor of St Helena for permission to use the island as a sampling base, Mr A. W. Mawson and Mr A. Thornton for assistance, the directors of the Union Castle Line for permission to collect dust samples on board their ships, and the master, officers and crew of the RMMV Good Hope Castle for making collections and the Royal Society and the Air Pollution Control Office, US Environmental Protection Agency for financial support.

R. CHESTER

Department of Oceanography,
The University,
Liverpool L69 3BX

H. ELDERFIELD

Department of Earth Sciences,
The University,
Leeds LS2 9JT

J. J. GRIFFIN

Scripps Institute of Oceanography,
La Jolla,
California 922037

Received July 19, 1971.

[1] Delany, A. C., Delany, A. C., Parkin, D. W., Griffin, J. J., Goldberg, E. D., and Reinmann, B. E. F., *Geochim. Cosmochim. Acta*, **31**, 853 (1967).

[2] Parkin, D. W., Phillips, D. R., Sullivan, R. A. L., and Johnson, L. R., *J. Geophys. Res.*, **75**, 1788 (1960).

[3] Prospero, J. M., and Bonatti, E., *J. Geophys. Res.*; **74**, 3362 (1969).

[4] Ferguson, W. S., Griffin, J. J., and Goldberg, E. D., *J. Geophys. Res.*, **75**, 1137 (1970).

[5] Chester, R., and Johnson, L. R., *Nature*, **229**, 105 (1971).

[6] Chester, R., and Johnson, L. R., *Marine Geol.* (in the press).

[7] Griffin, J. J., Windom, H., and Goldberg, E. D., *Deep-Sea Res.*, **15**, 433 (1968).

[8] Biscaye, P. E., *Geochemistry Technical Report*, **8** (Department of Geology, Yale University, 1964).

[9] Bricker, O. P., and Prospero, J. M., Abstract No. M99, *Trans. Amer. Geophys. Union*, **50**, 176 (1969).

[10] MacDonald, W. F., *US Dept Agriculture Weather Bureau Publication* No. 1247 (1930).

Recognition of Upper Miocene Neogene Zone 18, Experimental Mohole, Guadalupe Site

CORRELATIONS of marine strata using the convenient numerical zones of the Neogene[1,2], based on planktonic foraminiferans, represent an important advance in refining and developing greater precision in biostratigraphic correlations. This zonation is most effective in tropical and subtropical regions where the nominate planktonic taxa are best developed; progressive difficulty is encountered in applying it away from equatorial areas as these taxa disappear and are replaced by temperate and then by cold-water populations in polar areas. The experimental Mohole (Fig. 1), drilled near Guadalupe Island off Baja California, is significant as far as tropical-temperature planktonic foraminiferal populations are concerned because it is located in a subtropical area in which there is a commingling of tropical and temperate planktonic species[3].

Although studies have already been made of the experimental Mohole foraminiferans[3–5], the section was reanalysed in terms of Neogene Planktonic Zones (Fig. 2). The uppermost Miocene zone, Neogene Zone 18, is of special importance for three chief reasons which are relevant to the geologic history of the eastern Pacific. First, it is the uppermost zone of the Miocene and it is now possible to correlate Neogene Zone 18 of the standard numerical system for the tropics with the experimental Mohole section and in turn with the uppermost Miocene of California—the uppermost Mohnian and Delmontian Stages—using concurrent ranges of the tropical species *Globorotalia tumida plesiotumida* Banner and Blow (Fig. 3) and its descendant *Globorotalia tumida tumida* (Brady)[6]. Neogene Zone 18 can also be defined in the uppermost Messinian, the type upper Miocene of Italy[7,8]; this is a critical aspect for interregional correlations. Second, Neogene Zone 18 has been correlated with the upper Gilbert and lower Gauss Magnetic Epochs[9], the upper boundary being approximately at 3 m.y. or perhaps slightly more. This is in agreement with the 4.3 m.y. dating[10] for the Delmontian of the Mohole (Fig. 2). Third, the opening of the Gulf of California about 4 m.y. ago[11] allowed late Miocene–early Pliocene planktonic biofacies to enter the Gulf and to penetrate to the head of the Gulf before the end of the Miocene; the development of the northern Gulf before the opening at the mouth, as suggested by Larson and others[11], is not required.

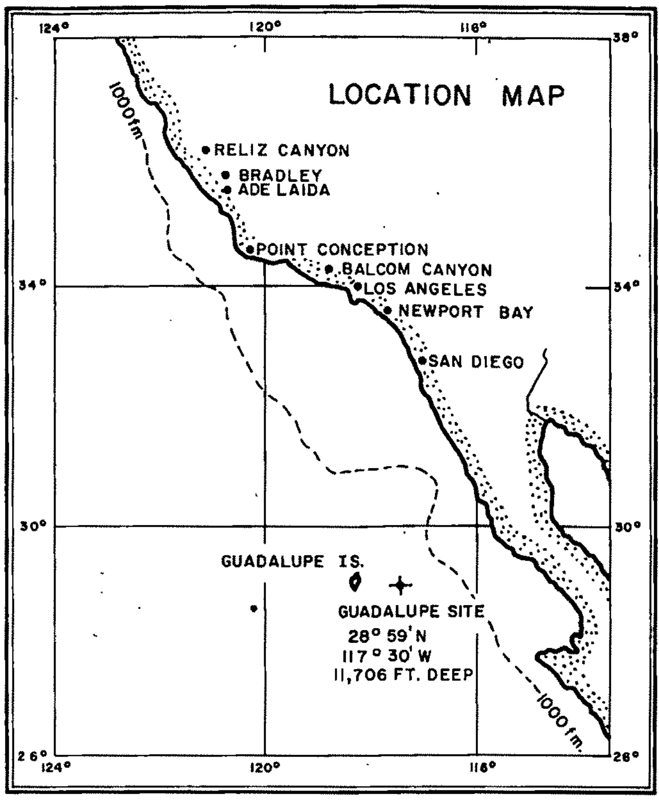


Fig. 1 Location of the experimental Mohole (Guadalupe site).

The marine deposition rates of many land-based sections are related to the definition of the upper limit of Neogene Zone 18, uppermost Miocene, with respect to southern California geology. The Ventura Basin[12], for example, has between 5,000 and 6,000 m of Pliocene–Pleistocene strata and the Los Angeles Basin[13] has not more than 3,000 m of section in the Pliocene–Pleistocene. Correlation of Neogene Zone 18 with the uppermost Gilbert and lower Gauss Magnetic Epochs suggests that there is a deposition rate of between 1,000 and 2,000 m per million years[14]. These basins, which are actively subsiding and have nearby sediment sources, exhibit rates from two to more than four times greater than those suggested for the Plio–Pleistocene of New Zealand[15]. They are compatible with rates indicated for the modern Santa Barbara Basin[16] and are many times greater than those computed for the Miocene of California.

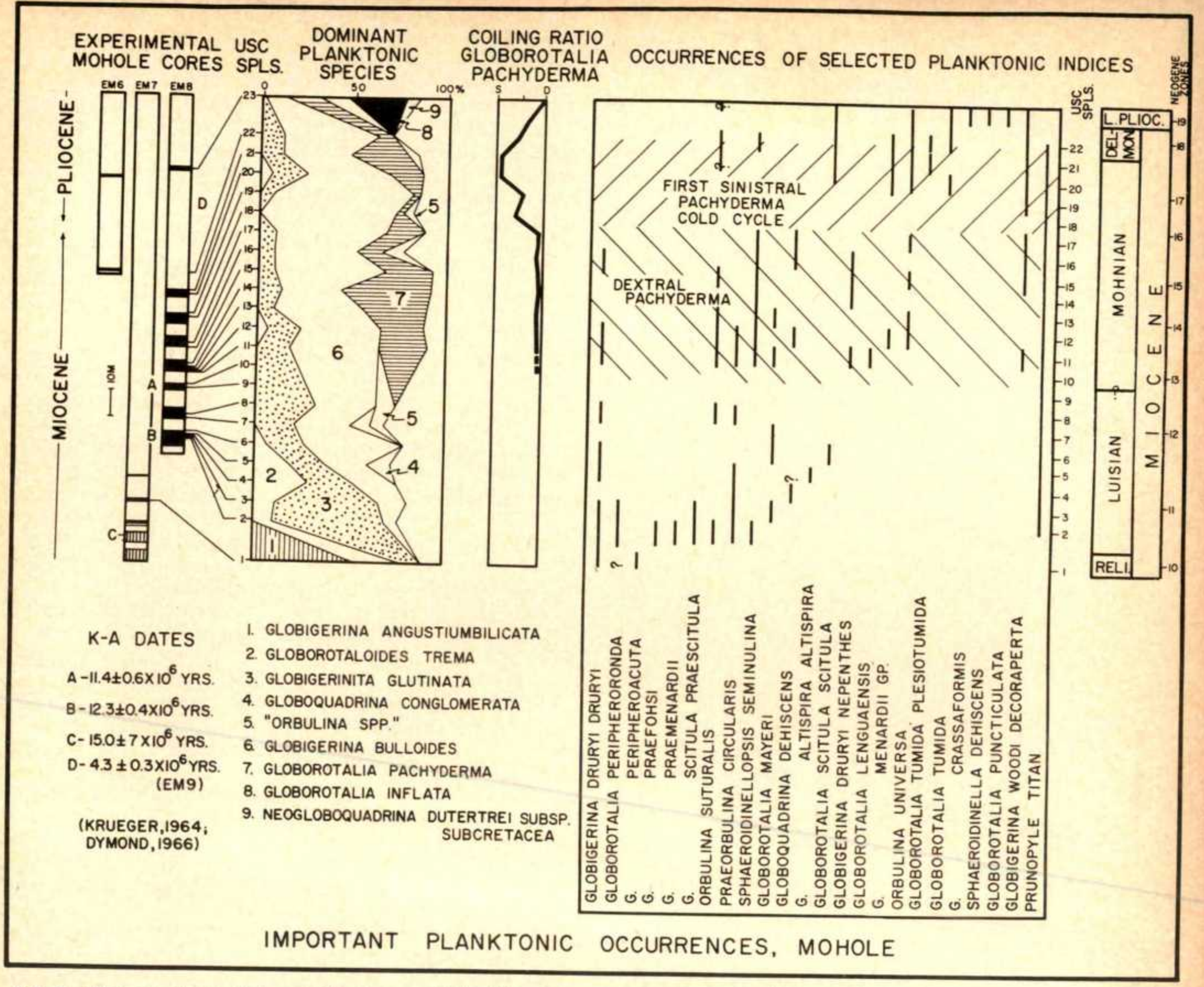


Fig. 2 Neogene planktonic foraminiferal zones (right side of figure) with important planktonic indices of the experimental Mohole showing coiling ratios of *Globorotalia* (*Turborotalia*) *pachyderma* (S, sinistral coiling; D, dextral coiling), the frequency distribution of common and abundant species, and the relative rare species as a bar graph. All are planktonic foraminiferans except *Prunopyle titan*, which is a radiolarian. K–A dates A, B, and D are those of Dymond[10] and C is from Kreuger[17]. This figure is from Bandy[8] as restudied and modified from that of Bandy and Ingle[3]. As noted in more complete analyses[8], Neogene Zone 13 marks the development of *G.* (*T.*) *pachyderma* just above the *Discoaster kugleri* zone; Neogene Zone 14 is defined by the concurrence of *Globorotalis* (*T.*) *mayeri* and *Globigerina druryi nepenthes*; Neogene Zone 17 is the level of maximum cooling, indicated by the dominance of sinistral populations of *G.* (*T.*) *pachyderma;* and Neogene Zone 18 is defined by the concurrence of *G. tumida tumida* and *G. tumida plesiotumida*.

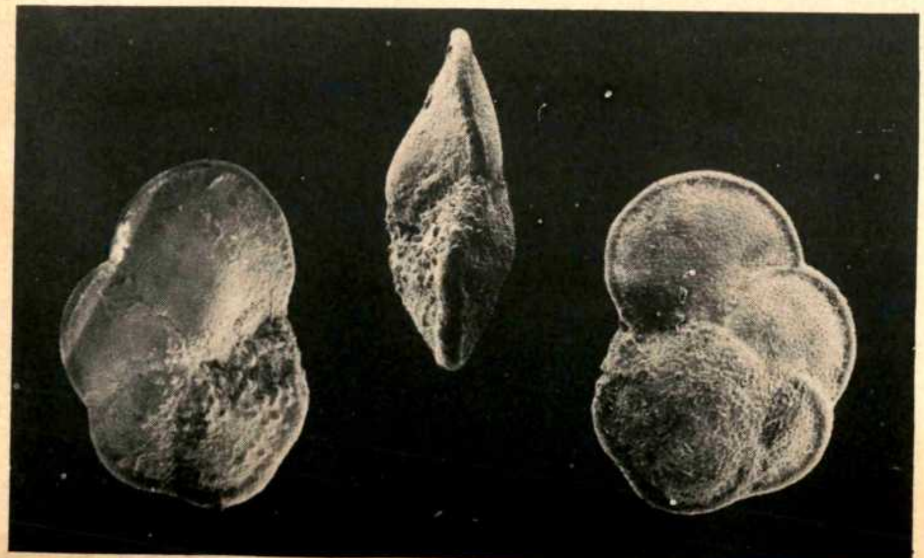


Fig. 3 *Globorotalia tumida* (Brady) subspecies *plesiotumida* Banner and Blow from USC sample 21, experimental Mohole section, Guadalupe site (Fig. 2). Specimen has a maximum diameter of 0.43 mm.

I thank Fritz Theyer for assistance in making the SEM photographs of *Globorotalia tumida plesiotumida* and the US National Science Foundation for financial support.

ORVILLE L. BANDY

Department of Geological Sciences,
University of Southern California,
Los Angeles, California 90007

Received May 11, 1971.

[1] Banner, F. T., and Blow, W. H., *Nature*, **208**, 1164 (1965).
[2] Blow, W. H., *Proc. First Internat. Conf. on Planktonic Microfossils*, **1**, 199 (1969).
[3] Bandy, O. L., and Ingle, jun., J. C., *Geol. Soc. Amer. Spec. Paper*, **124**, 131 (1970).
[4] Parker, F. L., *J. Paleontol.*, **38**, 617 (1964).
[5] Ingle, jun., J. C., *Bull. Amer. Palaeontol.*, **52**, 217 (1967).
[6] Banner, F. T., and Blow, W. H., *Nature*, **207**, 1351 (1965).
[7] Colalongo, M. L., *Giornale di Geologia*, **37** (II), 515 (1970).
[8] Bandy, O. L., *Twenty-fourth Intern. Geol. Congr.* (in the press).
[9] Bandy, O. L., Casey, R. E., and Wright, R. C., *Amer. Geophysical Union, Antarctic Res. Ser.*, **15**, 1 (1971).
[10] Dymond, J. R., *Science*, **152**, 1239 (1966).
[11] Larson, R. L., Menard, H. W., and Smith, S. M., *Science*, **161**, 781 (1968).
[12] Natland, M. L., and Kuenen, Ph. H., *Soc. Econ. Palaeontologists and Mineralogists, Spec. Publ.*, **2**, 76 (1951).
[13] Wissler, S. G., *Calif. Div. Mines and Geol. Bull*, **118**, 209 (1943).
[14] Bandy, O. L., *Calibration of Hominoid Evolution, Symposium 52* (Wenner-Gren Foundation, New York, in the press).
[15] Kennett, J. P., Watkins, N. D., and Vella, P., *Science*, **171**, 276 (1971).
[16] Bandy, O. L., *J. Paleontol.*, **34**, 671 (1960).
[17] Kreuger, H. W., *J. Geophys. Res.*, **69**, 1155 (1964).

BIOLOGICAL SCIENCES

Allosteric Preconditioning: Role of Allosteric Ligands in Promoting the Maturation of Enzymes

THE tertiary structure of an isolated polypeptide chain may differ from its most stable conformation as a component of an oligomer. The assembly of free subunits into an oligomeric structure may therefore involve a relatively unstable oligomeric intermediate state in which the conformation of the subunits corresponds to that of the free polypeptide chains. Such a metastable intermediate state may be separated from the stable oligomer by a substantial energy barrier, and the marked temperature dependence of subunit association reactions has indeed been attributed to the existence of a "conformational transition state"[1].

We wish to focus attention on the surmounting of this energy barrier, that is, the transformation of an immature precursor of the enzyme, devoid of activity, into a form capable of catalysis (in the case of apoenzymes, a form capable of combining with the prosthetic group to yield an active enzyme). This process, which can be termed preconditioning, often seems to be affected by the ligands that also regulate the activity of the mature enzyme. We believe that this process, allosteric preconditioning, represents another general mode of regulation by allosteric ligands. Five specific examples reveal fundamental differences between the manner in which allosteric ligands affect preconditioning and the way in which they modulate the activity of the mature enzyme.

(1) Threonine deaminase, the biosynthetic enzyme from *Salmonella typhimurium*, can be reconstituted from the apoenzyme and pyridoxal phosphate. The resulting holoenzyme is, however, inactive until subjected to a maturation process. The rate of this process, especially at *p*H 7.5 and above, is greatly enhanced by valine or isoleucine, which are positive and negative effectors, respectively, of the active enzyme. On the other hand, the simultaneous presence of both effectors blocks the maturation process[2,3].

(2) Muscle phosphofructokinase can be inactivated by various means, including mild acidification and prolonged storage (refs. 4–6, and manuscript in preparation). The basis for the inactivation by mild acid is considered to be a dissociation into two subunits[4,6]. Activity is restored slowly at *p*H values above neutrality, but is markedly accelerated by certain ligands. ATP and ADP (which serve both as substrates and allosteric modifiers of this enzyme) are effective in the restoration of activity, but less so in the presence of Mg^{2+}. The positive effectors AMP and cyclic 3,5′-AMP also promote restoration. That it is the inhibitory site for ATP and not the substrate site that is involved in the preconditioning process is suggested by the interference of Mg^{2+} and by the fact that ITP, which is an excellent substrate but a poor inhibitor of this enzyme[7], is much inferior to ATP in reactivating the enzyme (manuscript in preparation).

(3) The behaviour of glycogen synthetase can be modified by phosphorylation and dephosphorylation, but the enzyme is also susceptible to the kind of conditioning we are concerned with. Thus, it has been shown with the liver enzyme that there are two mechanisms for the conversion of the D-form (dependent on high concentrations of glucose-6-P) to the I-form (relatively independent of glucose-6-P). One mechanism, which has been termed "direct", presumably involves phosphatase action. The "indirect" conversion, which is relevant here, proceeds through an intermediate, inactive state (produced by incubation at moderate temperatures in the presence of EDTA at alkaline *p*H[8]). Maturation of this to a catalytically active enzyme resembling the I-form is rapidly promoted by Mg^{2+} and $SO_3^{=}$, and by the allosteric effector of this enzyme, glucose-6-P[8,9].

A similar situation is observed in the case of pyruvate kinase. The enzyme from adipose tissue, which is dependent on FDP, can be rendered independent of that modifier by preconditioning in the presence of FDP. Interestingly, ATP, an inhibitor of the enzyme, interferes with the FDP-mediated preconditioning[10].

(4) Pyruvate carboxylase seems to provide an example of the preconditioning of an apoenzyme. A specific synthetase catalyses the transfer of biotin to apo-pyruvate carboxylase isolated from *Bacillus stearothermophilus*. This reaction is strongly dependent on acetyl CoA, which is also a positive effector of the holoenzyme. On the other hand, L-aspartate antagonizes the effect of acetyl CoA both in the synthetase reaction and in the carboxylation *per se*[11,12].

(5) Tryptophan pyrrolase provides a classical example of substrate-mediated enzyme maturation[13]. The rate of production of active holoenzyme from apoenzyme is enhanced by tryptophan about ten-fold[14], but the concentration of tryptophan required for half-maximal rate of apoenzyme combination with haemin is considerably below the K_m for tryptophan of the holoenzyme; moreover, α-methyltryptophan, neither a substrate nor an inhibitor of the holoenzyme, can effectively promote maturation[14]. This suggests that preconditioning of tryptophan pyrrolase involves the binding of tryptophan to an "allosteric" site rather than to the active site of the enzyme.

These examples reveal an important general feature of allosteric preconditioning: although allosteric ligands may promote the process, it can also occur in their absence, albeit much more slowly. Moreover, the continued presence of the maturation-inducing ligands is not required for the maintenance of the mature state, that is, maturation is essentially irreversible and thus the mature state must represent the thermodynamically more stable form of the enzyme. Allosteric ligands must therefore promote preconditioning not by stabilizing the mature enzyme but by enhancing the rate of conversion of the immature to the mature state (Fig. 1). This role of allosteric ligands differs fundamentally from their role in allosteric activation or inhibition of enzyme activity, which

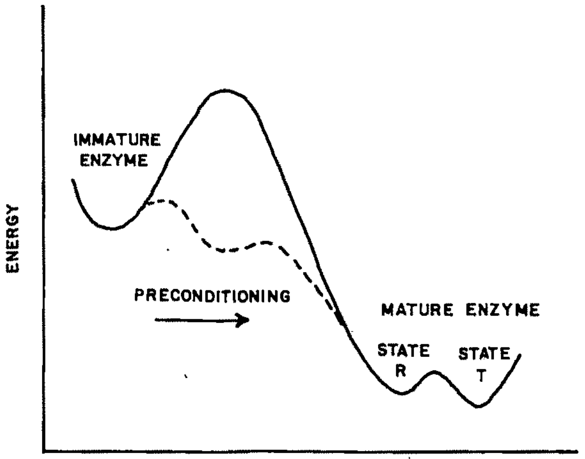


Fig. 1 Preconditioning of an enzyme in the absence (———) and in the presence (- - -) of a maturation-promoting ligand.

is mediated by the preferential stabilization of either an active or inactive form of the enzyme (that is, states R and T in Fig. 1)[1]. This difference is also illustrated by the fact that preconditioning, unlike allosteric inhibition or allosteric activation, can be promoted by either positive or negative effectors. Thus, either the positive effector valine or the negative effector isoleucine induces maturation of threonine deaminase, and phosphofructokinase is reactivated by adenine nucleotides including both positive and negative modifiers. Yet, in spite of the different mode of action of effectors in allosteric regulation of enzyme activity and in promoting enzyme maturation, it seems likely that in both processes the effectors bind to the same sterospecific site(s) on the enzyme. Direct evidence for this hypothesis must come from the study of the preconditioning of enzymes in which allosteric sites are altered by chemical modification or mutation. The observation that ATP promotes the conditioning of phosphofructokinase more effectively than ITP, which is in accord with the relative affinity of these nucleotides for the inhibitor site, supports such an idea.

Since allosteric preconditioning and allosteric regulation seem to involve the same site on the enzyme, it is important to distinguish allosteric preconditioning from the frequently observed stabilization of enzymes by allosteric ligands. The latter can be attributed to the fact that the catalytic centre of an enzyme may be more stable in one of the allosteric states of the enzyme (usually the inactive state), and thus any ligand that binds preferentially to this state will enhance the stability of the enzyme. The observation that either valine or isoleucine can promote the maturation of threonine deaminase, in spite of the fact that the positive effector valine (unlike isoleucine) reduces the stability of the enzyme[15], lends validity to this distinction.

If preconditioning or maturation were indeed obligatory in the biosynthesis of an active enzyme, as seems to be the case at least with threonine deaminase, pyruvate carboxylase and tryptophan pyrrolase, one might conclude that the primary function of allosteric sites is the binding of maturation-promoting ligands. Once such a site has evolved on a protein, the mature form of which could exist in several conformational states that differ in catalytic activity, it would be likely that the affinity of this site for its ligand would differ for each state, a situation that would subject the catalytic activity of the enzyme to regulation by the maturation ligand. In other words, allosteric regulation would be the frequent outcome of ligand-promoted preconditioning. Nevertheless, enzymes should exist where this evolution of preconditioning sites into regulatory sites has not yet occurred. Such an example may well be provided by liver tryptophan pyrrolase, where α-methyltryptophan promotes maturation by binding to a separate site but yet has no influence on the activity of the holoenzyme. On the other hand, in the enzyme from *Pseudomonas acidovorans*, the preconditioning site seems to have evolved into a regulatory site, for here α-methyltryptophan not only induces maturation but also acts as a positive allosteric effector of the holoenzyme[16].

The presence of maturation sites that have not yet evolved into regulatory sites may also account for occasionally observed enzyme-ligand interactions that are difficult to explain in physiological terms. For example, FDP specifically increases the thermolability of *Escherichia coli* β-galactosidase[17] but has no effect on the enzyme's activity. (Yet FDP is known to be an allosteric modifier of another catabolic enzyme in *E. coli*—glycerokinase[18].) Another effect which is difficult to rationalize physiologically is that of nonpolar L-amino-acids on aspartokinase. In *B. polymyxa*, nonpolar L-amino-acids act as weak activators[19], in *E. coli* as weak inhibitors[20], and in *B. subtilis* some nonpolar amino-acids activate while others inhibit aspartokinase (manuscript in preparation). These ligands may play a role in enzyme maturation rather than in the regulation of activity, and it should be of interest to determine whether the reconstitution of the enzymes from their polypeptide chains is not enhanced by these specific agents.

Interaction of enzymes with allosteric ligands is not the only way to effect preconditioning. As mentioned, variation of *p*H or the presence of various ions can also accelerate enzyme maturation, and substrates or coenzymes can also promote this process, as observed with several dehydrogenases[21–23]. We have focused our discussion on the role of allosteric effectors because the cited examples encourage the hypothesis that the preconditioning process may often be the antecedent of allosteric regulation. Moreover, the concepts proposed would provide a unified view of preconditioning phenomena and stimulate the search for such a process among allosteric enzymes and especially among those proteins which exhibit hitherto unexplained interactions with specific small molecules.

This work was supported by the National Science Foundation and the National Institute of Arthritis and Metabolic Diseases. The authors hold research career development awards from the US Public Health Service.

JOSEPH B. ALPERS
HENRY PAULUS

Department of Biological Chemistry,
Harvard Medical School,
Boston, Massachusetts 02115

Received December 17, 1970.

[1] Monod, J., Wyman, J., and Changeux, J. P., *J. Mol. Biol.*, **12**, 88 (1965).
[2] Hatfield, G. W., and Burns, R. O., *Science*, **167**, 75 (1970).
[3] Hatfield, G. W., and Burns, R. O., *Proc. US Nat. Acad. Sci.*, **66**, 1027 (1970).
[4] Ling, K. H., Marcus, F., and Lardy, H. A., *J. Biol. Chem.*, **240**, 1893 (1965).
[5] Mansour, T. E., *J. Biol. Chem.*, **240**, 2165 (1965).
[6] Hofer, H. W., and Pette, D., *Z. Physiol. Chemie*, **349**, 1105 (1968).
[7] Uyeda, K., and Racker, E., *J. Biol. Chem.*, **240**, 4682 (1965).
[8] Hizukuri, S., and Larner, J., *Biochemistry*, **3**, 1783 (1964).
[9] Steiner, D. F., Younger, L., and King, J., *Biochemistry*, **4**, 740 (1965).
[10] Pogson, C. I., *Biochem. J.*, **110**, 67 (1968).
[11] Cazzulo, J. J., Sundaram, T. K., and Kornberg, H. L., *Nature*, **227**, 1103 (1970).
[12] Cazzulo, J. J., Sundaram, T. K., and Kornberg, H. L., *Nature*, **223**, 1137 (1969).
[13] Feigelson, P., Feigelson, M., and Greengard, O., *Rec. Prog. Hormone Res.*, **18**, 491 (1962).
[14] Piras, M. M., and Knox, W. E., *J. Biol. Chem.*, **242**, 2952 (1967).
[15] Freundlich, M., and Umbarger, H. E., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 505 (1963).
[16] Feigelson, P., and Maeno, H., *Biochem. Biophys. Res. Commun.*, **28**, 289 (1967).
[17] Gest, H., and Mandelstam, J., *Nature*, **211**, 72 (1966).

[18] Zwaig, N., and Lin, E. C. C., *Science*, **153**, 755 (1966).
[19] Paulus, H., and Gray, E., *J. Biol. Chem.*, **243**, 1349 (1968).
[20] Patte, J. C., Loviny, T., and Cohen, G. N., *Biochim. Biophys. Acta*, **99**, 523 (1965).
[21] Chilson, O. P., Kitto, G. B., Pudles, J., and Kaplan, N. O., *J. Biol. Chem.*, **241**, 2431 (1966).
[22] Deal, W. C., *Biochemistry*, **8**, 2795 (1969).
[23] Kohn, L. D., *J. Biol. Chem.*, **245**, 3850 (1970).

Chromosomal Localization of the Heterochromatic Region 16qh(.76) linked to α-Haptoglobin in Man

THE structural gene locus for the alpha chain of haptoglobin (α-Hp) in man seems[1] to be on the long arm (q) of chromosome 16 linked[2] to a fragile heterochromatically staining region (h). We here report measurements localizing this region, which we designate 16qh(.76).

16qh(.76) was found to be segregating in a large family[2]. From thirty-one of the family members heterozygous for 16qh, 1,709 lymphocyte metaphases were analysed microscopically and 801 metaphases photographed. Both chromosomes No. 16 were relatively straight and free of overlap by other chromosomes in 193 metaphases. Eighty of these metaphases showed 16qh clearly and were therefore selected for measurement.

Using photographic prints with a final enlargement of ×3,840, we measured the No. 16 chromosomes with a specially designed, manually operated wheel on which a millimetre scale was directly engraved (unpublished results of H. W., W. J. K., and F. H.). Both chromatids were measured and the results averaged. We measured the length of the short arm (p) from each chromosome No. 16, q from the normal homologous 16 ($q^{\text{homol.}}$), and the euchromatic parts of q proximal ($q^{\text{prox.}}$) and distal ($q^{\text{dist.}}$) to 16qh.

We compared the length of $q^{\text{prox.}}$ to $q^{\text{homol.}}$ in each cell and found the mean length of $q^{\text{prox.}}$ to be 0.759 of $q^{\text{homol.}}$ (s.e. = 0.011). Thus, the fragile heterochromatic site is approximately 76% of the distance along the long arm of No. 16. Since other heterochromatic regions may be located on 16q, we have designated this site 16qh(.76).

Table 1 One Way Analysis of Variance of the Length of the Distal Segment of the 16qh Chromosome

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Between person	31.0	0.6631	0.0213	
Within person	48.0	0.7215	0.0150	
Total	79.0	1.3846		1.422

We then tested whether 16qh(.76) detectably affected the length of euchromatin on that chromosome, using a paired observation *t* test. The mean length of p from the involved No. 16 was not significantly different from the mean length of p from the homologue ($t = 1.68$, df = 79, $P > 0.05$). The sum of the means of $q^{\text{prox.}} + q^{\text{dist.}}$ was, however, 2.6% greater than the mean of q from the homologue ($t = 2.17$, df = 79, $0.05 > P > 0.02$).

Finally, we tested for heterogeneity between persons in the length of $16q^{\text{dist.}}$ and found none. The F ratio (between person/within person variance) was 1.42 (Table 1), which is not significant.

The foregoing cytological data should not be interpreted to mean that the structural gene for α-Hp is necessarily located 76% of the distance from centromere. α-Hp could be cytologically at some distance from h(.76) but seem to be closely linked, if the heterochromatic region tended locally to suppress crossing-over. The data also cannot reveal if α-Hp is between the centromere and 16qh(.76) or distal to 16qh(.76).

Concurrent segregation analysis of chromosome markers (for example, 16qh(.76)) and gene markers (for example, α-Hp) permits the testing of map assignments deduced from studies with chromosome rearrangements[1] and the localization of specific genes to specific chromosome regions, as in the present study. The efficiency of chromosome marker work will predictably increase as chromosome variants are recognized more frequently. In this family we have recently observed the segregation of three other potentially useful variants (3 inv, 17qh and Ginv), as will be reported in more detail.

B. T. and R. E. M. were recipients of NIH predoctoral and postdoctoral research fellowships; W. J. K. was supported by a grant from the Hill Foundation; E. W. L. and F. H. were supported by a grant from the Children's Bureau; and F. H. was in receipt of an NIH grant.

FREDERICK HECHT
BLAINE TOLBY
R. ELLEN MAGENIS
WILLIAM J. KIMBERLING
HERMAN WYANDT
EVERETT W. LOVRIEN

Division of Medical Genetics,
Department of Pediatrics, and
Crippled Children's Division,
University of Oregon Medical School,
Portland, Oregon 97201

Received February 1, 1971.

[1] Robson, E. B., Polani, P. E., Dart, S. J., Jacobs, P. A., and Renwick, J. H., *Nature*, **223**, 1163 (1969).
[2] Magenis, R. E., Hecht, F., and Lovrien, E. W., *Science*, **170**, 85 (1970).

Interpretation of Linkage in Somatic Cell Hybrids

Kao and Puck[1] have measured the linkage between human genes by studying their association in cell hybrids with Chinese hamster cells which carried mutations homologous to the human genes. Such studies are made possible by the extensive loss of human chromosomes from such hybrids. If, however, one of the human genes is retained owing to the operation of specific selection, the presence or absence of an unselected gene gives a measure of linkage between them.

In one hybrid studied by Kao and Puck the human genes were inositol independence (*inos*$^+$) and proline independence (*pro*$^+$). Among forty-two clones selected for *inos*$^+$, only seven (one sixth) were also *pro*$^+$. In another hybrid the human genes were glycine independence (*gly*$^+$) and *pro*$^+$. Among fifteen clones selected for *gly*$^+$ only five (one third) were also *pro*$^+$. Both these experiments were interpreted as evidence of non-linkage between the pair of marker genes. I submit that such associations, though well below the 50% expected between unlinked genes in sexual hybrids, are nevertheless greater than would be expected from random association of the marked chromosomes in cell hybrids. The first problem is to determine what would be the extent of random association between two unlinked genes (that is, genes on separate chromosomes). This would depend on the number of human chromosomes still retained in the hybrid. In the first hybrid, because of the method of selection one chromosome must carry *inos*$^+$. If a second chromosome is present the probability that it will not carry *pro*$^+$ is 43/45. The chance that a third and fourth chromosome will also be free of *pro*$^+$ will be 42/44 and 41/43 respectively. In general terms, if a hybrid clone has *n* human chromosomes of which one carries *inos*$^+$, the chance of *pro*$^+$ being also present will be $1 - \dfrac{43!\,.\,.\,(45\text{-}n)}{45!\,.\,.\,(47\text{-}n)}$. When $n = 2$ the association of *inos*$^+$ and *pro*$^+$ will be 0.044 and increases approximately in proportion to $(n-1)$. The observed association of 0.167 would require an average of 5 human chromosomes in all clones. The association in the second hybrid of 0.33 between *inos*$^+$ and *gly*$^+$ would require the presence of ten human chromosomes, but Kao and Puck show an idiogram with seventeen hamster chromosomes and two "human" chromosomes (judged to be 2 and 6 or X under the Denver classification) and state that other clones had "similar karyotypes", and that all stabilized clones had nineteen, or occasionally twenty, chromosomes. The original Chinese hamster cell line had twenty chromosomes, so it seems that two human chromosomes have been substituted for three hamster ones. One is forced to conclude that the human chromosomes retained in the hybrids are much fewer than five, and the association found between the genes cannot be attributed to chance association of two chromosomes. Although there is only one kind of random association of chromosomes, there is a large number of non-random associations which might be considered. The simplest and most probable form of non-random selection arises from the fact that we are sampling from a diploid set with two representatives of each kind. It would seem likely that if a sample of chromosomes contains the haploid number (twenty-three) or less, it will not contain more than one of each homologue. This would produce only a slight modification of the fully random formula given above. The association of two markers among n chromosomes would become $1-\frac{21!\ldots(23\text{-}n)}{22!\ldots(24\text{-}n)}$. For an association of 0.167, n is still 5, and for an association of 0.33 it is 9. Such non-randomness is not, therefore, capable of explaining the associations found.

Another kind of non-randomness would arise if, though the chromosomes were lost at random, certain combinations were more viable than others. In cases where one chromosome was selected, the probability of any particular second chromosome being present would vary from more than random (0.044) to less than random according to the viability of the combinations. In the hybrids considered here, the *pro*$^+$ chromosome would seem to have a greater than random chance (four-fold and eight-fold respectively) of being included with the selected chromosomes *inos*$^+$ and *gly*$^+$.

Another non-random model assumes that human chromosomes are of two kinds, compatible and incompatible with the Chinese hamster genome. The hybrid karyotype suggests that compatible human chromosomes might be those which can be substituted for hamster chromosomes. If all the compatible chromosomes had equal chances of inclusion in the hybrids and their number was x, the chromosomes carrying *inos*$^+$, *pro*$^+$ and *gly*$^+$ must be among that number. The chance of any one compatible chromosome carrying *pro*$^+$ will therefore be $2/x$. The first chromosome has been determined by the method of selection. The chance that the second one will carry *pro*$^+$ will be $2/(x-2)$. The data give us two estimates of x: 14 when *inos*$^+$ is selected and 8 when *gly*$^+$ is selected, with a best estimate of 11. In other words, out of forty-six human chromosomes only eleven are compatible with the Chinese hamster genome. The concept of incompatible human chromosomes opens up further possibilities. If the gene being selected for in the hybrid were on an incompatible chromosome, it could be retained, but only by a structural change; by being translocated onto a host chromosome, onto a compatible chromosome of the other parent, or by deletion of the "incompatible segment". It would then become very difficult to identify the chromosome carrying the human gene and even more difficult to identify the chromosome from which it was derived. Pursuing the model further, the association found between genes could be due to their presence on the same human chromosome, the degree of association being determined by the classical map distance between them; that is, by the chance that the break which enables the selected gene to be retained in the hybrid will also include the associated gene.

The karyotype presented as typical by Kao and Puck was interpreted by them as having three hamster telocentrics replaced by two human metacentrics. Another possibility is that the two new chromosomes represent rearrangements between three hamster telocentrics and one arm of a human chromosome. Such spontaneous rearrangement may seem an unlikely explanation but many spontaneous rearrangements accompanied the origin of the permanent Chinese hamster cell line, eight out of twenty chromosomes of which do not resemble any chromosomes in the primary explant. Moreover, the extreme selection pressure operating in favour of human wild type genes would fix any rare events which increased their chance of survival. This is the probable explanation of the high degree of dissociation in man–mouse hybrids between two human genes known to be sex-linked (HGPRT and G6PD) when only one of them is subject to positive selection[2]. If the association of *pro*$^+$ with *inos*$^+$ and with *gly*$^+$ is due to location on the same human chromosome, the strength of the association indicates the map distances and the gene order, which would be *pro gly inos* or *gly pro* . . *inos* (*inos* being twice as far as *gly* from *pro*).

This model fits evidence from an entirely different source. It has been argued[3] that the isolation of recessive mutants in Chinese hamster near-diploid cell lines is due to the existence of chromosome segments in the monosomic condition, so that there are no dominant wild type alleles to mask the mutations. Such mutations would be confined to few segments or even to a single chromosome segment. The location of the mutant genes *pro*$^-$ *inos*$^-$ and *gly*$^-$ on one segment of hamster chromosome would then be paralleled by the location of the genes *pro*$^+$ *inos*$^+$ and *gly*$^+$ on one segment of human chromosome.

To conclude, the association found by Kao and Puck between *inos*$^+$ and *pro*$^+$ and between *gly*$^+$ and *pro*$^+$ in Chinese hamster/human hybrids is greater than would be expected from the random association of two chromosomes. Three possible causes of such an association of the human genes have been considered.

If the associated pairs of genes are on different human chromosomes, these chromosomes must be associated, (*a*) because of some genetic interdependence, such that the presence of one increases the probability that the other will also be present; (*b*) because of some specific compatibility that both share with the hamster genome (for example, ability to substitute for hamster chromosomes); (*c*) both genes may be located on the same human chromosome, but this chromosome has a high incidence of rearrangement in hybrids. Theoretically, these explanations seem to be equally valid. They require to be tested by further genetic studies in cell hybrids combined with intensive karyotyping.

A. J. BATEMAN

Paterson Laboratories,
Christie Hospital and Holt Radium Institute,
Manchester M20 9BX

Received May 26; revised July 1, 1971.

[1] Kao, F., and Puck, T. T., *Nature*, **228**, 329 (1970).
[2] Miller, O. J., Cook, P. R., Khan, P. M., Sin, S., and Siniscalco, M., *Proc. US Nat. Acad. Sci.*, **68**, 116 (1971).
[3] Chu, E. H. Y., in *Environmental Chemical Mutagens*, **2** (edit. by Hollaender, A.) (1971).

Activation of a Central Noradrenergic Projection to Cerebellum

RECENT histochemical studies have demonstrated abundant nerve fibres containing noradrenaline in rat cerebellar cortex[1,2] terminating predominantly on Purkinje cells[2]. An inhibitory function for these fibres is suggested by the uniform inhibition of the spontaneous discharge[3] and hyperpolarization of Purkinje cells (P cells) produced by microelectrophoretic application of noradrenaline (NA)[4]. Furthermore, the firing rate of Purkinje cells is generally increased after destruction by 6-hydroxydopamine of fibres containing NA[5]. Further characterization of the natural endogenous transmitter and its effect on P cells requires experimental activation of the proposed adrenergic pathway, and identification of the origin of the NA projection to the cerebellum thus assumes critical importance. It has been suggested that the nerves containing catecholamine in rat cerebellar cortex arise largely from discrete bilateral nuclei of fluorescent cells in the dorsal medullary brain stem (the locus coeruleus, LC)[6,7]. We have reported that electrical stimulation in the area of the LC inhibits P cells[8]. We now report that this inhibitory response is evoked selectively from the NA-containing cells of the LC, that the inhibitory effects are widespread and that the response closely resembles the electrophysiological effects produced by exogenous NA.

Male albino rats (130–230 g) were anaesthetized with halothane. The cerebellar vermis and one hemisphere were exposed[3]. A fine (0.4 mm) concentric bipolar stimulating electrode was inserted through the hemisphere at an angle of 29° from the vertical into cerebellar and brain stem loci after empirical stereotaxic localization of the LC. The location of the electrode was verified histologically after each experiment. Purkinje cells, identified by their characteristic climbing fibre bursts[9], were recorded in the cerebellar vermis with extracellular micropipettes (2 M NaCl, 1–8 MΩ) or intracellular micropipettes (3 M KCl, 10–40 MΩ). Responses to brain stimulation (0.1 ms pulse duration) were recorded on magnetic tape. Mean firing rate (spikes s^{-1}) was displayed with a pen recorder[3]. Post-stimulus time histograms (PSTHs) were generated by a Digital PDP-12 computer. Mean arterial pressure was monitored during LC stimulation and showed no change.

Purkinje cells, but not cortical interneurones, showed a remarkably uniform inhibitory response to stimulation of LC with trains of pulses: ninety-four of 102 cells (twenty animals) recorded extracellularly displayed depression of spontaneous discharge rate (Fig. 1*A*, *B*). This summated response was greatest at relatively low stimulus frequencies (3–50 s^{-1}) and was markedly diminished at faster rates. Complete cessation of discharge outlasting the stimulation period by 4–65 s (mean, 21 s) could be obtained with 20–100 pulses at 10 s^{-1}. At this frequency, threshold currents ranged from 0.03 to 1.2 mA (mean, 0.35 mA).

Although the response to a single LC stimulus often escaped detection on direct visual inspection, construction of PSTHs reproducibly revealed reduced probability of spike discharge over prolonged intervals of 60 to 470 ms (mean, 293 ms) with long latent periods of 50–290 ms (mean, 148 ms). During these late inhibitions, cell discharge was often only incompletely suppressed; however, a single short burst of two to five pulses at high frequencies (20–75 s^{-1}) usually resulted in a complete pause in firing (Fig. 1*C*).

Stimulation of LC often produced short term (5–10 ms) activations of P cells (preceding the late inhibitory period), consisting either of single spikes, climbing fibre bursts, or, more frequently, simple or complex field potentials without obvious spikes. These rapid responses had latent periods ranging from 1.5–11 ms, and were often immediately followed by short inhibitory periods of 10–60 ms (mean, 18 ms). These early events may result from current spread to other brain stem areas (for example, brachium conjunctivum), because (i) many P cells exhibiting late inhibition showed no such responses, and (ii) dissociation between the long inhibitory responses and the activations and/or fields could often be produced by fine adjustment of the stimulating current or by specific drug administration (B. J. H., G. R. S., F. E. B., and A. P. O., in preparation). However, a direct connexion (via collaterals, for example) from LC to some cells of origin of mossy or climbing fibres cannot be ruled out at present.

Intracellular recording of some P cells during stimulation of LC with single shocks revealed late hyperpolarizations (not directly related to climbing fibre responses) which were usually weak or nearly lost in baseline noise (Fig. 2*A*, broad arrow). Signal averaging techniques better delineate these small late hyperpolarizing responses (0.5–2 mV), which are comparable in latency and duration to the late prolonged periods of suppression seen in post-stimulus histograms (Fig. 1*C*).

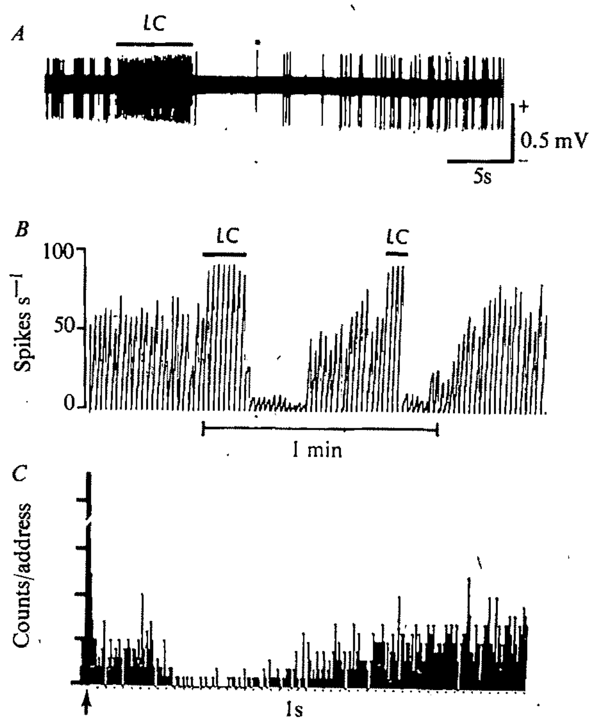


Fig. 1 Extracellular responses of P cells to stimulation of locus coeruleus (LC). *A*, Oscillograph record of action potentials; response to fifty-seven shocks (0.25 mA) delivered to LC at 10 s^{-1} (solid bar above action potentials). A climbing fibre response to a single LC test stimulus is indicated by the dot above the record. *B*, Polygraph record showing integrated discharge frequency of another Purkinje cell. Firing rate drops to zero with multiple shocks (100 and 50 pulses, respectively; 10 s^{-1}; 0.32 mA) to LC (solid bars above record). Apparent excitation seen during stimulation results from addition of the multiphasic stimulus artefact. *C*, Post-stimulus time histogram of the response of another P cell to single short bursts of shocks (0.28 mA, 3 pulses at 75 s^{-1}) delivered to the LC every 2.1 s (sixty-four sweeps). Arrow shows stimulus artefacts. Each marker on ordinate = 5 counts/address. Address or bin size = 4 ms.

With trains of pulses, large hyperpolarizations extending well beyond the stimulation period and averaging 14 mV (range, 2–39 mV) were recorded (Fig. 2*B*). An index of membrane resistance was obtained by measuring the size of climbing fibre spikes (see refs. 4 and 10)—or in obviously injured cells excitatory postsynaptic potentials (EPSPs), and by measuring the potential deflexions produced by hyperpolarizing currents (0.5–1 nA, 40 ms duration) passed through the recording micropipette in conjunction with a Wheatstone bridge circuit[11]. In all cases, input resistance, as measured by these two parameters, either increased (ten cells) or did not change (two cells; Fig. 2*B*) during the LC evoked hyperpolarizations. Hence, this hyperpolarization does not seem to result from activation of a conventional inhibitory pathway (for example, parallel fibre activation of basket cells), for a decrease in resistance would then be expected[9]. In this respect, LC stimulation exactly mimics the action of exogenously applied NA and cyclic AMP, which also produce hyperpolarizations without a decrease in membrane resistance[4]. But other mechanisms such as presynaptic inhibition, or disfacilitation, although less likely to evoke such

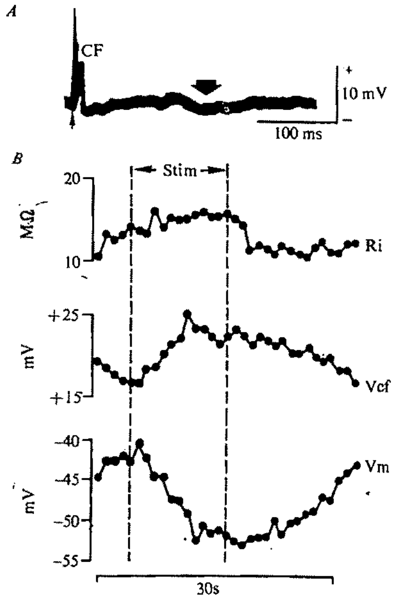


Fig. 2 Responses of P cells to stimulation of LC: intracellular recording. *A*, Oscillograph record of response to a single shock (small arrow; 0.7 mA). This supramaximal stimulus drives a climbing fibre evoked EPSP (CF) followed immediately by an early after hyperpolarization and finally a late, more prolonged hyperpolarization (broad arrow). *B*, Graph of intracellular response to stimulation of LC (Stim) with multiple shocks (0.45 mA, 120 pulses at 10^{-1}). Records were analysed at 1 s intervals by a PDP-12 computer, and the results for input resistance (Ri), size of climbing fibre responses (Vcf) and membrane potential (Vm) plotted against time. Although the input resistance did not change appreciably during this test, in the two other tests recorded in this cell, resistance increased by 164 and 295%.

large and widespread responses, might also produce the observed effects.

The anatomical discreteness of brain stem loci evoking this characteristic inhibitory response is underscored by the finding that the incidence of depression of P cell discharge falls abruptly when the stimulating electrode is placed slightly outside of LC (Table 1). In many cases, individual cells which responded when the electrode was in the LC failed to do so when the stimulation electrode was displaced from the LC by as little as 0.5 mm; recovery of response ensued with repositioning in the LC. Furthermore, when catecholamine-containing pathways were selectively and chronically destroyed by intracisternally-injected 6-hydroxydopamine[2,12], only five of sixty cells were inhibited (Table 1) when the LC was stimulated directly (seven animals) (B. J. H., G. R. S., F. E. B., and A. P. O., in preparation).

It might be argued that the late inhibitory responses to single shock stimulation of LC allow for conduction of impulses through many possible synapses, rather than the proposed monosynaptic noradrenergic pathway. A large portion of the long latent period, however, may be due to a long synaptic delay, such as those of 30–100 ms described for the slow inhibitory postsynaptic potentials (IPSPs) of sympathetic ganglia[13,14]. The slow IPSP, like the proposed LC-Purkinje cell inhibition, is conceived as an adrenergic response[15]. Moreover, the catecholamine-containing fibres to cerebellum are small C-fibres[2], which should conduct impulses very slowly. Conduction velocities for small peripheral C-fibres may range from 0.15 to 0.5 m s^{-1} (ref. 16). Finally, the neuroanatomical data suggest a direct LC projection to P cells[3,6–8].

Furthermore, the LC evoked inhibition of P cells shares many properties of peripheral autonomic systems, such as the relative efficacy of trains of pulses over single shocks[13–15,17], a diminished responsiveness at high stimulus frequencies[17], and hyperpolarizing responses without decrease in resistance[15].

In view of early reports[18] emphasizing the sparseness of catecholamine-containing fibres in the cerebellum, perhaps the most surprising finding of this study is that nearly all Purkinje cells recorded in the vermis exhibited the inhibitory response to LC stimulation. Almost all the cells in the LC of the rat contain noradrenaline[19] and noradrenaline always inhibits Purkinje cells when applied exogenously[3], so the proposed adrenergic projection from LC to cerebellar cortex may assume a greater functional significance than previously supposed. Pharmacological evidence supporting the noradrenergic nature of this projection will be reported separately.

We thank Drs R. Nicoll, S. Koslow, and A. Padjen for criticisms.

G. R. Siggins
B. J. Hoffer
A. P. Oliver
F. E. Bloom

Laboratory of Neuropharmacology,
Division of Special Mental Health Research,
National Institute of Mental Health,
Saint Elizabeths Hospital,
Washington DC 20032

Received July 26, 1971.

Table 1 Effect of Placement of Stimulus Electrode on Multiple Shock-evoked Pauses

Electrode location	Purkinje cells studied (*n*)	% Inhibited	Mean threshold current (mA ± s.e.)
Exactly within LC	102	92	0.35 (±0.037)
0.3 mm outside LC	21	62	0.65 (±0.082)
0.5–0.75 mm outside LC	15	33	—
> 0.75 mm outside LC	55	29	—
6-OHDA, in LC	60	8	—

[1] Hokfelt, T., and Fuxe, K., *Exp. Brain Res.*, **9**, 63 (1969).
[2] Bloom, F. E., Hoffer, B. J., and Siggins, G. R., *Brain Res.*, **25**, 501 (1971).
[3] Hoffer, B. J., Siggins, G. R., and Bloom, F. E., *Brain Res.*, **25**, 523 (1971).
[4] Siggins, G. R., Oliver, A. P., Hoffer, B. J., and Bloom, F. E., *Science*, **171**, 192 (1971).
[5] Hoffer, B. J., Siggins, G. R., Woodward, D. J., and Bloom, F. E., *Brain Res.* (in the press).
[6] Olson, L., and Fuxe, K., *Brain Res.*, **28**, 165 (1971).
[7] Ungerstedt, U., *Acta Physiol. Scand.*, Suppl. 367 (1971).
[8] Bloom, F. E., Hoffer, B. J., and Siggins, G. R., *Biological Psychiatry* (in the press).
[9] Eccles, J. C., Ito, M., and Szentagothai, J., *The Cerebellum as a Neuronal Machine* (Springer, New York, 1967).
[10] Frank, K., and Fuortes, M. G. F., *J. Physiol.*, **134**, 451 (1956).
[11] Araki, T., and Otani, T., *J. Neurophysiol.*, **18**, 472 (1955).
[12] Bloom, F. E., Algeri, S., Groppetti, A., Revuelta, A., and Costa, E., *Science*, **166**, 1284 (1969).
[13] Libet, B., *J. Neurophysiol.*, **30**, 494 (1967).
[14] Libet, B., Chichibu, S., and Tosaka, T., *J. Neurophysiol.*, **31**, 383 (1968).
[15] Libet, B., *Fed. Proc.*, **29**, 1945 (1970).
[16] Nishi, S., Soeda, H., and Koketsu, K., *J. Cell Comp. Physiol.*, **66**, 19 (1965).
[17] Girling, F., *Amer. J. Physiol.*, **170**, 131 (1952).
[18] Fuxe, K., *Acta Physiol. Scand.*, **64** (Suppl. 247), 39 (1965).
[19] Fuxe, K., Goldstein, M., Hokfelt, T., and Joh, T. H., *Res. Commun. Chem. Pathol. Pharmacol.*, **1**, 627 (1970).

Regeneration of Porphyropsin *in vivo*

SCOTOPIC vision is mediated by the rod cells of the retina which contain a photosensitive pigment that (according to species) can be rhodopsin, porphyropsin, or a mixture of both. The pigment content may be diminished by exposure to light, but there is no permanent loss because the light-decomposed molecules are restored by regeneration. Although the time course and some details of the mechanism of rhodopsin regeneration *in vivo* are well known[1], we have little and contradictory information about the regeneration of porphyropsin. According to Kühne and Sewall[2] and Abelsdorff[3], the porphyropsin-like pigment in the retina of the living bream (*Abramis brama*)[4,5] is regenerated within 30–40 min. Garten[6], however, who worked with the same fish, found regeneration to be complete after not less than 4 h. I have found a similar figure for regeneration of porphyropsin in the retina of crucian carp (*Carassius carassius*).

Crucian carp were used because their porphyropsin is not paired with rhodopsin[7]. In each experiment, four or eight animals of equal size (12–13 cm long) were light-adapted for 1 h in a transparent plastic tank (20 × 20 × 30 cm) illuminated by five reflector flood-lamps (light output per lamp, 1,950 lumens, colour temperature about 2,730 K). This light bleached about 70% of the pigment. The temperature during light adaptation was 16–17° C and 15.0 ± 0.3° C during subsequent dark adaptation. The dark periods lasted from 30 min to 5 h. To estimate the full pigment content of the fish eyes, some animals were dark-adapted overnight. Several precautions were taken to prevent regeneration during dissection and washing. After the fishes had been killed, the eyes were removed as quickly as possible and put on a block of solid carbon dioxide—a procedure which took about 4 min for six eyes. Subsequently, the retinas were dissected out into a Petri dish filled with an ice-cold solution (*p*H 4.5) consisting of a 1 : 1 mixture of McIlvaine buffer (*p*H 4.6) and 0.2 M hydroxylamine solution. The same cooled solution (2° C) was used to wash the retinas and the receptors. The 0.1 M hydroxylamine buffer solution served also as the solvent for the digitonin that was used as an extractant in a concentration of 3%. It was shown in some preliminary experiments that the relatively high concentration of hydroxylamine had no influence on the yield of pigment, nor did it diminish the stability of the extracts. Double extractions were made in all experiments, the first extracts contributing some 75% and the second ones approximately 20% to the total pigment yield.

The unbleached extracts exhibit two well defined absorption peaks at about 520 nm and between 355 and 360 nm (Fig. 1). The former is the easily identifiable porphyropsin peak, the latter is presumably caused by 3-dehydroretinol, the all-*trans*-isomer of which has a λ_{max} of 350 nm. The second absorbance maximum shifts to shorter wavelengths when the extracts are prepared from retinas which have been left in darkness for comparatively short periods. In these conditions the ultraviolet peak was found near 352 or 353 nm and its height was somewhat greater. Although porphyropsin and presumably impurities contribute to the ultraviolet peak, its height may be taken as roughly proportional to the amount of 3-dehydroretinol present in the extracts (line *B* in Fig. 1). The absorbance change on bleaching at 520 nm (line *A* in Fig. 1), on the other hand, indicates the amount of porphyropsin present. The sum of *A* plus *B* was fairly constant in all the extracts (($A+B$) ± s.d. = 1.2015 ± 0.1964; N = 13) with the exception of those taken from fully light-adapted eyes. These were presumably contaminated with material extracted from the pigment epithelium, for it was difficult to obtain clean retinas in these experiments. The ratio of *A* to *B* increased with the duration of dark adaptation. A/B was as low as 0.05 in light-adapted retinas, it has the value 0.16 after 3 h of dark adaptation and approaches a figure of 0.30 in fully dark-adapted retinas (Fig. 1). If it is assumed that the material represented by the data was extracted chiefly from the receptors themselves, then the values for *A* and *B* permit the following conclusions to be made : (1) the retina of *Carassius carassius* stores some 3-dehydroretinol; (2) 3-dehydroretinal released from bleached porphyropsin and reduced to the alcohol is added to the store; (3) during regeneration, the prosthetic group is supplied by this same store because the ratio A/B increases by a factor of six or so while the sum $A+B$ remains constant.

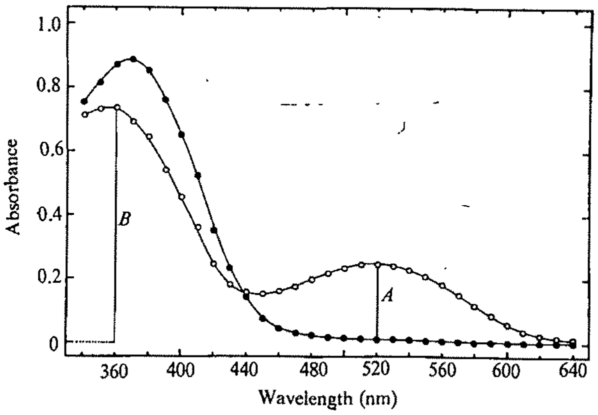


Fig. 1 Absorbance spectrum of a retinal extract from the dark-adapted crucian carp *Carassius carassius*. The data refer to a first extract from six retinas. The change from ○ (unbleached) to ● (bleached) is due to light exposure. The lengths of lines *A* and *B* are taken as respective measures of porphyropsin and 3-dehydroretinol.

Difference spectra derived from curves, like those in Fig. 1, peak at 522 nm (positive branch) and 385–388 nm (negative branch) indicating that dehydroretinal oxime is the principal product of bleaching (compare ref. 8).

The time course of regeneration is shown in Fig. 2. The points represent the amount of photosensitive material (λ_{max} = 520 nm) extracted from the retinas after various times in the dark. Although the data show appreciable scatter, the general trend is as follows: after a lag of approximately 1 h, regeneration begins slowly during the second hour. This is followed by 2 h of a fairly constant rate of increase of pigment until eventually regeneration approaches virtual completion during the fifth hour of darkness.

The mechanism that causes the long initial delay of regeneration is unknown. Regeneration of rhodopsin in the living frog is similarly delayed, but for not more than 15–20 min.

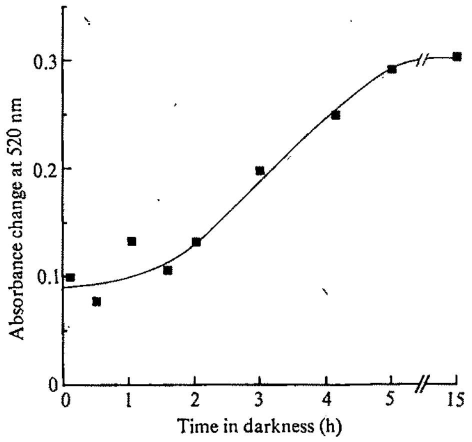


Fig. 2 Time course of porphyropsin regeneration in the retina of the living crucian carp. The ordinate refers to the amount of photosensitive material in extracts from six retinas, though two thirds of the data shown are based on double measurements with 2 × 6 retinas. Total number of experiments: fifteen.

This period, though shorter, is not of a different order of magnitude, however. Mammals, on the other hand, never show this lag[1]. Their regeneration curve begins with the rectilinear part which the fish retinas exhibit during the third and fourth hour of darkness when the pigment is re-synthesized at the rate of 14% of the bleached porphyropsin in 30 min. This figure means that the regeneration of porphyropsin *in vivo* is slow[6] and comparable with the regeneration of rhodopsin in cold blooded animals[9]. Three reactions are probably essential for the regeneration of the visual pigment: the formation of 3-dehydroretinal from the corresponding alcohol by oxidation, an isomerization of the retinal and the coupling of the prosthetic group with the protein. The first two reactions would involve two different enzymes—an alcohol dehydrogenase and an isomerase respectively. The course of consecutive enzymatic reactions is known to be complicated[10], and a curve like that in Fig. 2 could well result if the two enzymes were present in different concentrations and their respective Michaelis constants were also different.

This study was made while I was working with the MRC Vision Unit at the University of Sussex. The help of Professor Dartnall and the technical assistance of Mrs Erika Church are gratefully acknowledged.

CH. BAUMANN

Department of Experimental Ophthalmology,
W. G. Kerckhoff-Institut,
635 Bad Nauheim

Received January 20; revised May 20, 1971.

[1] Baumann, Ch., *Handbook of Sensory Physiology*, 1, ch. 10 (Springer, Heidelberg, 1971).
[2] Kühne, W., and Sewall, H., *Unters. Physiol. Inst. Heidelberg*, 3, 221 (1880).
[3] Abelsdorff, G., *Z. Psychol. Physiol. Sinnesorg.*, 14, 77 (1897).
[4] Köttgen, E., and Abelsdorff, G., *Z. Psychol. Physiol. Sinnesorg.*, 12, 161 (1896).
[5] Garten, S., *Albrecht v. Graefes Arch. Ophthal.*, 63, 112 (1906).
[6] Garten, S., *Hdb. ges. Augenheilk.*, 3, 1 (Anhang, 1907).
[7] Dartnall, H. J. A., in *The Eye*, 2, 523 (Academic Press, New York and London, 1962).
[8] Schwanzara, S. A., *Vision Res.*, 7, 121 (1967).
[9] Peskin, J. C., *J. Gen. Physiol.*, 26, 27 (1942).
[10] Haldane, J. B. S., *Enzymes* (Longmans, Green, London, 1930).

L-Asparaginase and Complement

L-ASPARAGINASE, widely regarded as a potential oncolytic agent[1–6], is known to depress both cellular and humoral immunity. Our investigation of the effect of L-asparaginase on the complement system, described in what follows, reveals a more complicated interaction—the enzyme inhibits the complement in whole serum *in vitro* but is a potent activator of the same system in certain circumstances.

Human serum (1 ml.) was diluted five times and incubated for 2 h at 22° C with 50 U of L-asparaginase (LA). The enzyme was obtained from Merck, Sharpe and Dohme and was dialysed overnight against phosphate buffer (0.005 M) before use. The serum was then diluted a further ten times and its complement (C) activity was measured using 1×10^8 sheep erythrocytes sensitized with rabbit antibody (EA)[7]. The percentage haemolysis was determined kinetically by stopping the reaction with citrate–saline at intervals and measuring absorbance at 415 nm (Table 1). The same procedure was used in the case of guinea-pig serum except that it was diluted a further 200 times and readings were taken at shorter intervals (Table 2). Both human and guinea-pig sera have less C activity after treatment with LA; after 60 min, this effect is total and irreversible in human serum but partial and reversible in guinea-pig serum.

Table 1 Percentage Haemolysis obtained with Human Serum

LA added	Min of incubation			
(U)	10	30	50	60
None	3.3	45.0	62.0	67.0
50	0	0	0	0

Table 2 Percentage Haemolysis obtained with Guinea-pig Serum

LA added	Min of incubation		
(U)	7.5	10.5	18
None	39.5	81.0	95.0
50	5.0	36.0	80.0

In a second experiment, the intermediate EAC142 (ref. 8) was prepared by incubating 5×10^8 EA cells in 1 ml. with 0.25 ml. of human R3 for 2 min 30 s at 37° C. The cells were then incubated with ethylenediamine tetraacetate-treated guinea-pig serum (EDTA–serum) diluted thirty times, a reagent having the activity of the terminal C components from C3 to C9. After 25 min, the reaction was stopped by the addition of citrate–saline and the percentage haemolysis was determined by reading absorbance at 415 nm. The results were compared with those obtained with a similar intermediate prepared with an R3 reagent (diluted 1/15) that had been incubated for 15, 60 and 100 min at 22° C with varying amounts of LA (Table 3) before being mixed with EA cells. A significant inhibition of haemolysis occurred in the tubes containing the EAC142 intermediate prepared with an R3 pre-incubated with LA. Moreover, the inhibition seemed to depend on both dose and time.

Table 3 Percentage Haemolysis after Incubation of the R_3 Reagent

Asparaginase added to R_3	Min of incubation		
(U)	15	60	100
None	66.5	71.0	75.0
5	70.0	46.0	57.0
10	62.8	18.0	31.0
25	51.0	15.0	6.0
50	27.0	11.0	6.0

In the next experiment, guinea-pig serum was diluted five times and incubated with various quantities of LA. After incubation for 120 min EDTA was added to a final concentration of 0.01 M and the reagent was used to haemolyse EAC142 cells. From the data in Table 4, it can be concluded that LA has no influence on the terminal C factors C3 to C9.

It therefore seems that the inhibitory effect of LA on C takes place early in the sequence of C fixation, at the C1, C4 or C2 steps. Cells in the EAC1 state were prepared by incubating 1×10^8 EA cells with a suitable dilution of a C1 preparation for 10 min at 37° C[9]. The EAC1 intermediate was lysed by guinea-pig serum which had been diluted forty times and treated with Na_2 Mg-EDTA to permit the action of all the C components except C1. EAC1 was prepared in the same fashion, except that the C1 preparation was pre-incubated with 50, 25 and 10 units of LA during the 45 min before contact with EA (Table 5). Surprisingly, unlike the EA cells treated with native C1, the haemolysis of the EAC1 intermediate prepared with LA-treated C1 was greatly increased

Table 4 Percentage Haemolysis of EAC142 Intermediate

Asparaginase added to guinea-pig serum (before EDTA) (U)	% haemolysis
None	81.0
50	78.0
25	82.5
10	81.0

Table 5 Percentage Haemolysis of EAC1 Intermediate

LA added to C1 before contact with EA (U)	% haemolysis
None	25.9
50	31.0
25	59.5
10	67.0

after addition of Na_2 Mg-EDTA–serum. Moreover, the smaller the quantity of LA the greater the potentiation of haemolysis.

In other experiments, the use of cells in the EAC4 state enabled us to locate the LA action at the C1 stage. EAC4 cells were prepared after elution of C1 by EDTA from EAC14 cells[10]. To three tubes, one containing EAC4 (1×10^8 cells), one a suitable dilution of C1 and one a suitable dilution of C2, 75 U of LA was added. The tubes, together with tubes containing equivalent volumes of EAC14, C1 and C2 without LA, were incubated at 37° C for 60 min. From the pre-incubated samples, various mixtures of EAC4 and C1 were made and incubated for 20 min at 37° C (Table 6). C2 was added after centrifugation. The EAC142 so formed was centrifuged and incubated with guinea-pig serum diluted twenty-five times and treated with EDTA. The results confirm that LA produces its potentiating effect only in the presence of free C1. Whenever C1 is present in free form, LA behaves as a potent activator, the best results being observed with C1 which has been pre-incubated with LA (line 3 of Table 6). Moreover, fixed C4 is unaffected by LA-activated C1.

Table 6 Percentage Haemolysis by EDTA–Serum

Formation of the EAC142 intermediate*	% haemolysis
EAC4+C1+C2	10.3
EAC4LA+C1+C2	40.8
EAC4+C1LA+C2	51.8
EAC4+C1+C2LA	8.0

* The reagents pre-incubated with LA are designated EAC4LA, C1LA, C2LA.

At first glance, the results seem contradictory. Although LA inhibits C in whole serum, it behaves as a potent activator of the same system provided its action is limited to the first step in C fixation, that is, to the formation of cells in the EAC1 state. This can be explained, however, if one assumes that LA activates C1 with the subsequent formation of a potent C1 esterase. This, in turn, would cause an increased destruction of fluid phase C4 and C2[11,12] leading to a decrease in haemolysis in whole serum. On the other hand, when LA is incubated with C1 which is, after activation, used for the preparation of cells in the state EAC1, the intermediate produced shows a high reactivity. In this case, since the EAC1 cells are centrifuged before the addition of Na_2-mg-EDTA–serum, no free fluid-phase C1 esterase is present in significant amounts to inactivate C4 and C2. This situation is not unlike that in hereditary angioneurotic oedema[13], in which a decreased concentration of the inhibitor of C1 esterase results in an overactivity of the first component of C and a subsequent fluid phase destruction of C4 and C2.

The observations described here may well have their counterpart *in vivo*. They might, for example, give a clue to the severe anaphylactic reactions noted in some patients on administration of the drug[14]. An exploration of the behaviour of C in the blood of leukaemic patients treated with LA might throw some light on the mechanism of action of this enzyme and clarify the role of complement in therapeutic oncolysis. Moreover, human serum seems more sensitive to the action of LA than gp serum, a finding that is of course not unexpected.

This work was supported by the Medical Research Council of Canada.

J. M. DELAGE
J. SIMARD
G. LEHNER-NETSCH
A. BARRY

Department of Haematology,
Hôpital du Saint-Sacrament and
Department of Medicine,
Faculty of Medicine,
Laval University,
Quebec (10)e

Received March 31, 1971.

[1] Broome, J. D., *Nature*, **191**, 1114 (1961).
[2] Astaldi, G., Burgeo, G. R., Krc, J., Genova, R., and Astaldi, jun., A., *Lancet*, i, 423 (1969).
[3] Ohno, R., *Proc. Amer. Assoc. Cancer Res.*, **10**, 66 (1969).
[4] Schwartz, R. S., *Nature*, **224**, 275 (1969).
[5] Ohno, R., and Hersh, E. M., *Blood*, **35**, 250 (1970).
[6] Ohno, R., and Hersh, E. M., *Cancer Res.*, **30**, 1605 (1970).
[7] Mayer, M. M., in *Experimental Immunochemistry* (edit. by Kabat, E. A., and Mayer, M. M.) (Thomas, Springfield, Illinois, 1961).
[8] Leon, M. A., *J. Exp. Med.*, **105**, 403 (1957).
[9] Gigli, I., and Austen, K. F., *J. Exp. Med.*, **129**, 679 (1969).
[10] Pickering, R. J., Naff, G. R., Stroud, R. M., Good, R. A., and Gewurz, H., *J. Exp. Med.*, **131**, 803 (1970).
[11] Haines, A. L., and Lepow, I. H., *J. Immunol.*, **92**, 468 (1964).
[12] Lepow, I. H., Ratnoff, O. D., Rosen, F. S., and Pillemer, L., *Proc. Soc. Exp. Biol. Med.*, **92**, 32 (1956).
[13] Donaldson, V., and Rosen, F. S., *J. Clin. Invest.*, **43**, 2204 (1964).
[14] Oettgen, H. F., Stephenson, P. A., Schwartz, M. K., Leeper, R. D., Tallal, L., Tan, C. C., Clarkson, B. D., Golbey, R. B., Krakoff, I. H., Karnofsky, D. A., Murphy, M. L., and Burchenal, J. H., *Cancer*, **25**, 253 (1970).

Carboxymethylpachymaran, a New Water Soluble Polysaccharide with Marked Antitumour Activity

WE have reported that the polysaccharides lentinan[1,2] and pachymaran[3] strongly inhibit the growth of sarcoma 180 transplanted in mice, and that they could be considered as excellent immuno-accelerators of cell-mediated responses[4,5]. It is difficult, however, to dissolve them in water and to label them with isotopes, and their use in biological investigations can therefore be troublesome. We have now prepared a water soluble polysaccharide with marked antitumour activity, known as carboxymethylpachymaran ($[\alpha]_D^{20} = -2.8°$; $c = 1$ in H_2O; solubility, 6.7 g/100 ml. H_2O at 30° C) which can be labelled at the carboxymethyl residue with ^{14}C so that its distribution at the cellular and subcellular level can be investigated.

The results of the antitumour assays of carboxymethylpachymaran on sarcoma 180, Ehrlich carcinoma, MM-102 adenocarcinoma[6], CCM carcinoma[7] and NTF lymphosarcoma[8] are shown in Tables 1 and 2.

Antitumour activity was measured by the method described before[2]. A 7 day old ascites tumour was transplanted subcutaneously into the right groin of a mouse. This produced solid tumours at the site of inoculation. Carboxymethylpachymaran was injected intraperitoneally every day for 10 days starting 24 h after tumour transplantation. In assays terminating at the end of 5 weeks, the mice were killed and the tumours were removed and weighed. Tumour inhibition ratios were calculated by comparing the experimental and control mice. The antitumour effects of carboxymethylpachymaran on sarcoma 180 and MM-102 adenocarcinoma were striking, for the tumours regressed completely in many mice. Any direct action of the polysaccharide against tumour cells similar to that manifested by lentinan was not observed, and whereas pretreatment with lentinan[5] 14–45 days before

Table 1 Antitumour Effect of Carboxymethylpachymaran on Sarcoma 180

Mice	No. of mice	Route	Dose (mg/kg × 10)	Body weight change (g)	Death/ arrived	Average tumour weight (g)	Tumour inhibition ratio (%)	Complete regression
SWM/Ms	10	i.p.	5	+3.6	0/10	0.9	90.1	5/10
	10	i.p.	1	+4.1	0/10	3.9	61.4	0/10
	10	i.p.	0.5	+2.8	0/10	10.5	−7.1	0/10
	10		Control	+4.2	0/10	9.8		0/10
ICR-JCL	10	i.p.	100	+5.4	0/10	1.0	92.3	1/10
	10	i.p.	50	+5.9	0/10	0.5	96.1	8/10
	10	i.p.	25	+5.5	0/10	0.5	95.9	5/10
	10	i.p.	10	+5.1	0/10	1.7	87.1	5/10
	10	i.p.	5	+5.7	0/10	6.1	53.4	1/10
	10		Control	+4.8	0/10	13.0		0/10
ICR-JCL	10	i.p.	25	+7.0	0/10	0.5	95.4	7/10
	10	i.p.	10	+6.0	0/10	2.1	78.7	4/10
	10	i.p.	5	+6.3	0/10	6.1	38.8	1/10
	10		Control	+7.5	0/10	10.0		0/10
ICR-JCL	10	i.p.	10	+2.5	0/10	0.7	93.2	5/10
	10	i.p.	5	+3.1	0/10	4.4	57.7	1/10
	10	i.p.	1	+2.2	0/10	5.7	45.2	0/10
	10		Control	−0.2	2/8	10.4		0/8
Swiss albino	10	i.p.	5	+1.6	2/8	0.1	99.1	6/8
	10		Control	+5.6	0/10	6.4		0/10
	9	i.m.	5	+4.4	0/9	0.2	96.5	7/9
	9	s.c.	5	+5.4	0/9	0.0	99.6	8/9
	9	i.c.	5	+4.2	0/9	0.8	86.3	7/9
	9		Control	+3.6	0/9	4.9		0/9
	10	per os	100	+2.7	0/10	2.9	14.7	0/10
	10		5	+2.5	0/10	3.1	8.8	0/10
	10		Control	+3.1	2/8	3.4		0/8

Table 2 Antitumour Effect of Carboxymethylpachymaran on Ehrlich Carcinoma, MM–102 Adenocarcinoma, CCM Adenocarcinoma and NTF Lymphosarcoma

Tumour	Mice	No. of mice	Dose (mg/kg × 10)	Body weight change (g)	Death/ arrived	Average tumour weight (g)	Tumour inhibition ratio (%)	Complete regression
Ehrlich carcinoma	SWM/Ms	6	5	+0.6	0/6	12.3	0	0/6
		6	Control	+4.5	1/5	12.3		0/5
		6	200	+4.4	0/6	15.4	−6.3	0/6
		6	100	+3.4	0/6	8.7	40.0	0/6
		6	50	+1.5	1/5	13.7	4.8	0/5
		6	Control	+2.7	0/6	14.5		0/6
MM–102 carcinoma	C3H/arima	8	5	+6.5	3/5	2.3	78.9	0/5
		8	Control	−2.3	1/7	10.9		0/7
CCM adenocarcinoma	SWM/Ms	10	5	+2.4	0/10	7.5	36.4	0/10
		10	Control	+1.2	0/10	11.8		0/10
NTF lymphosarcoma	SWM/Ms	10	5	+2.7	1/9	9.0	−2.3	0/9
		10	Control	+3.4	2/8	8.8		0/8

Carboxymethylpachymaran was injected intraperitoneally in every case. The vehicle was distilled water.

tumour transplantation produced a marked effect, pretreatment with carboxymethylpachymaran at this time was ineffective. On the other hand, carboxymethylpachymaran had a pronounced antitumour effect which was, except for oral administration, irrespective of injection route, whereas lentinan was effective only after intraperitoneal injection.

Carboxymethylpachymaran was synthesized from pachymaran[3], a β-(1→3)linear glucan. A suspension of 3 g of pachymaran in 80 ml. isopropanol was mixed with 8 ml. of 30% aqueous sodium hydroxide, and stirred vigorously. Monochloroacetic acid (3.6 g) was then added to the mixture and the reaction was continued for 2 h at 40°–50° C with stirring. The reaction product, carboxymethylpachymaran, was filtered in suction and washed with 70% methanol–acetic acid solution, 80% aqueous methanol, 100% methanol and acetone, successively, and dried *in vacuo*; the yield was 3.3 g. The degree of carboxymethyl substitution in the polysaccharide thus prepared was 0.95 mol per anhydrous glucose unit. ^{14}C-Labelled carboxymethylpachymaran could be obtained by incorporating monochloroacetic acid labelled with ^{14}C in the mixture.

The studies of the mechanisms of action of antitumour polysaccharides on organ, cellular and subcellular level are in progress using the ^{14}C-labelled carboxymethylpachymaran. Carboxymethylpachymaran with lentinan is worth considering as a useful agent in cancer research and cellular immunology.

We thank Dr W. Nakahara and Dr F. Fukuoka for their continued interest and Misses Y. Arai and M. Noda for their cooperation.

Junji Hamuro
Yumi Yamashita
Yoko Ohsaka
Yukiko Y. Maeda
Goro Chihara

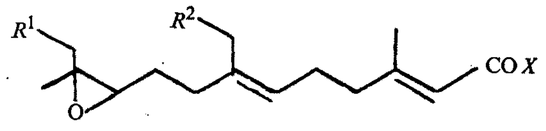
National Cancer Center,
Research Institute,
Tsukiji, Tokyo

Received May 25; revised June 21, 1971.

[1] Chihara, G., Maeda, Y. Y., Hamuro, J., Sasaki, T., and Fukuoka, F., *Nature*, **222**, 687 (1969).
[2] Chihara, G., Hamuro, J., Maeda, Y. Y., Arai, Y., and Fukuoka, F., *Cancer Res.*, **30**, 2776 (1970).
[3] Chihara, G., Hamuro, J., Maeda, Y. Y., Aari, Y., and Fukuoka, F., *Nature*, **225**, 943 (1970).
[4] Maeda, Y. Y., and Chihara, G., *Nature*, **229**, 634 (1971).
[5] Maeda, Y. Y., Hamuro, J., and Chihara, G., *Intern. J. Cancer*, **8**, 41 (1971).
[6] Irie, R. F., Koyama, K., and Hino, S., *J. Nat. Cancer Inst.*, **45**, 514 (1970).
[7] Fukuoka, F., Tokuzen, R., Hoshi, A., Egami, F., Nagasawa, K., and Kato, Y., *Gann*, **60**, 187 (1969).
[8] Tokuzen, R., and Nakahara, W., *Cancer Res.*, **29**, 730 (1969).

Terpenoid Amines as Insect Juvenile Hormones

After the discovery that an epoxide derivative of farnesenate esters (1) showed high juvenile hormone (JH) activity[1], we undertook to synthesize related compounds in an effort to find the most potent of this class of materials. The elucidation of the structure of two hormones from *Hyalophora cecropia* (2 (ref. 2) and 3 (ref. 3)) as homologues of the farnesenate structure, and the observation that products obtained from the addition of hydrogen chloride to farnesenate esters (18) also possessed hormone activity[4], led to the inclusion of analogous compounds in our programme. Here we describe results with a series of amide derivatives.



1, $R^1=R^2=H$, $X=OCH_3$

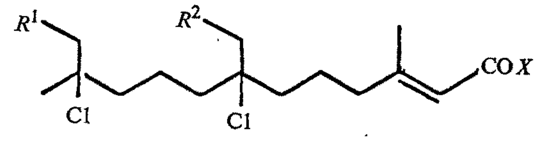
2, $R^1=R^2=CH^3$, $X=OCH_3$

3, $R^1=CH_3$, $R^2=H$, $X=OCH_3$

4–10, $R^1=R^2=H$, $X=NHR^3$

11, $R^1=R^2=CH_3$, $X=NHR^3$

12–17, $R^1=R^2=H$, $X=N(R^3)_2$



18, $R^1=R^2=H$, $X=OC_2H_5$

19, $R^1=R^2=H$, $X=NHR^3$

20, $R^1=R^2=H$, $X=N(R^3)_2$

21, $R^1=R^2=H$, $X=NHR^3$

The method used for the preparation of compounds (5) to (17) involved the phosphonate modification of the Wittig reaction[5]. Condensation of the appropriate N-substituted diethyl carbamoylphosphonate with geranylacetone, using sodium hydride as the condensing agent, gave the olefinic precursors to compounds (5)–(10) and (12)–(17). The precursor to compound (11) was obtained by a similar reaction with 6-ethyl-10-methyl-5,9-dodecadien-2-one[6]. Preparation of the intermediate N-substituted diethyl carbamoylmethylphosphonates involved treating chloroacetyl chloride with the appropriate amine to give N-substituted chloroacetamides, followed by a Michaelis–Arbuzov reaction with triethyl phosphite[7]. The precursor to the unsubstituted amide (4) was prepared by hydrolysis of the corresponding nitrile, obtained in turn from geranylacetone and diethyl cyanomethylphosphonate[8]. Epoxidations were carried out with *m*-chloroperbenzoic acid in methylene chloride, and the products were purified by chromatography on silica gel.

The dichloro derivatives (19–21) were prepared by adding hydrogen chloride to farnesenic acid or 7-ethyl-3,11-dimethyl-2,6,10-tridecatrienoic acid, followed by conversion to the acid chloride with thionyl chloride[9] and treatment with the appropriate amine. Attempts to prepare the dichloro derivatives of the amides by adding hydrogen chloride to the corresponding olefinic compounds were unsuccessful. All compounds prepared for this study were fully characterized by elemental analysis and by nuclear magnetic resonance and infrared spectrometry.

Table 1 Juvenile Hormone Activity of Terpenoid Amide Derivatives on *Tenebrio molitor*

Compound No.	R^3	Minimum active dose (μg)	% response
4	H	1.0	70
5	CH_3	0.01	100
6	C_2H_5	1×10^{-4}	100
7	C_3H_7	0.01	63
8	i-C_3H_7	0.1	100
9	C_4H_9	1.0	100
10	i-C_4H_9	1.0	100
11	C_2H_5	1×10^{-5}	70
12	CH_3	1.0	82
13	C_2H_5	0.01	68
14	C_3H_7	1.0	100
15	i-C_3H_7	1.0	100
16	C_4H_9	0.01	65
17	i-C_4H_9	0.1	85
19	C_2H_5	1×10^{-4}	100
19a	C_2H_5	0.01	95
20	C_2H_5	0.01	89
20a	C_2H_5	1.0	100
21	C_2H_5	0.1	43

Activity is minimum dose per pupa required for retention of significant pupal characteristics (equivalent to a "3" on the numerical scale of Bowers and Thompson[10]). Compounds are mixtures of all possible isomers except (19a) and (20a).

Initial screening for JH activity was carried out using freshly moulted pupae (10–20 h) of the yellow mealworm, *Tenebrio molitor*[10]. Twenty insects were used at each dose level, and minimum active doses were confirmed by three replicates. The substances were applied topically in 1 μl. of a solvent comprising 9 parts acetone and 1 part oily vehicle, usually squalene.

The presence of the oil in the topical formulations has been found to enhance the activity of many JH agents. This is particularly true of the dichloro derivatives (19–21); in the absence of the oil, these substances were active only at 100 to 1,000-fold larger concentrations. The nature of the oil was also found to be important. In general, oils with high unsaturation gave better results: exceptions were coconut oil and squalane, essentially saturated substances which afforded significant potentiation.

Of the epoxides (5–17), the N-ethyl derivatives (6) and (11) are by far the most potent of the series, and are the most potent in the *Tenebrio* screen of any published JH substances. By comparison, a synthetic mixture of isomers of *Cecropia* JH[6] had an activity of 0.1 μg, and 6,7-epoxy-3-ethyl-7-methyl-2-nonenyl-3,4-methylenedioxyphenyl ether[11] had an activity of 10^{-3} μg in the same assay procedure. Among the monoalkyl amides the JH activity falls off rapidly with decreasing and increasing size of the N-substituent. Among the dialkylamides both (13) and (16) show significant activity, but do not approach the potency of (6).

Synthesis of the dichloro derivatives (19) and (20) facilitated the isolation of pure *trans* isomers (19a) and (20a). It is interesting to note that in both cases the mixtures (19) and (20) are 100-fold more active than the corresponding *trans* isomers. The mixtures were characterized by nuclear magnetic resonance spectrometry, and were shown to be predominantly the *cis* and *trans* isomers of 7,11-dichloro-7,11-dimethyl-2-dodecenamides. Impurities were detected, however, and they could account for the enhanced activity. The *Cecropia* JH analogue (21) is much less active than the farnesenamide (19); the former is a much more complex mixture of isomers, and has not been as well characterized as (19).

The N-ethyl amides are structually very similar to the esters (1–3). Increased potencies could well be due to the different binding characteristics of the secondary nitrogen (both an electron pair and a hydrogen atom for noncovalent interactions) or to increased stability of the amide over the ester group. The only other nitrogen containing compounds with JH activity are some carbamates reported by Schwarz *et al.*[8] and some peptide derivatives reported by Zaoral and Sláma[12].

During our screening programme combinations of JH agents, or JH agents and insecticide synergists, were examined. In most cases there was little, if any, enhancement of activity, but a 1 : 1 combination of (6) and (19) produced a remarkable potentiation in the *Tenebrio* assay. Pupal characteristics were retained by the test insects at doses as low as 10^{-6} μg.

Compound (6) is effective in preventing maturation of houseflies (*Musca domestica*) when applied topically to final instar larvae. No normal adults are obtained at doses of 0.01 μg. Compound (19) was less active, affording complete control at 0.1 μg per larvae. Only slight potentiation effects are observed on houseflies with combinations of (6) and (19). Adult mosquitoes (*Culex pipiens*) fail to emerge from pupae when larvae are reared in water containing 0.1 p.p.m. of (6).

Incorporation of either (6) or (19) into the diet of the German cockroach (*Blatella germanica*) effectively controls colony growth and most of the adults die within a few days of being given a bait of starch containing 20 p.p.m. of the active ingredient. Gravid females drop their egg sacs prematurely, and the hatch, if any, is very small. Any nymphs which appear fail to survive to adulthood. The baits have retained their effectiveness for more than 6 months against exposure in a test cage.

Although this effect on cockroaches seems to be a general toxic response, it is also highly specific to this species. Other species, such as *Tenebrio molitor* and *Triboloium confusum*, survive as larvae or adults in media containing up to 100 p.p.m. of either (6) or (19). Development of the larvae is arrested (larvae ultimately die long after pupation would normally have occurred) and eggs laid by the adults fail to develop, but no "toxic" deaths are observed. Other species such as the Indian meal moth (*Plodia interpunctella*) seem to be unaffected by either compound. One can only speculate that the *Corpora allata* hormone(s) have some metabolic function in adult insects, and that the German cockroach is particularly susceptible to disruption of this function.

Acute oral toxicity tests have been carried out with both (6) and (19). Massive doses of (6) (3,200 mg/kg) are without effect on laboratory mice. Compound (19) has an observed LD$_{50}$ of 3,980 mg/kg, a value which indicates this material is relatively safe as far as acute toxicity is concerned.

We thank Mrs E. O. Johnson and Mr D. S. Pincus for technical assistance.

PHILIP A. CRUICKSHANK
RAYMOND M. PALMERE

Central Research Department,
Chemical Research and Development Center,
FMC Corporation,
Princeton, New Jersey 08540

Received February 15; revised May 7, 1971.

[1] Bowers, W. S., Thompson, M. J., and Uebel, E. C., *Life Sci.*, **4**, 2323 (1965); Cruickshank, P. A., *US Patent* 3,453,662 (1969).
[2] Röller, H., Dahm, K. H., Sweeley, C. C., and Trost, B. M., *Angew. Chem. Intern. Ed. Eng.*, **6**, 179 (1967).
[3] Meyer, A. S., Schneiderman, H. A., Hanzmann, E., and Ko, J. H., *Proc. US Nat. Acad. Sci.*, **60**, 853 (1968).
[4] Law, J. H., Yuan, C., and Williams, C. M., *Proc. US Nat. Acad. Sci.*, **55**, 576 (1966).
[5] Wadsworth, W. S., and Emmons, W. D., *J. Amer. Chem. Soc.*, **83**, 1733 (1961).
[6] Mori, K., Stalla-Bourdillon, B., Ohki, M., Matsui, M., and Bowers, W. S., *Tetrahedron*, **25**, 1667 (1969).
[7] Lomakina, V. I., Roslavtseva, S. A., Ya, A., Mandel'baum, N. N., and Mel'nikov, Zh., *Obshch. Khim.*, **36**, 447 (1966).
[8] Schwarz, M., Sonnet, P. E., and Wakabayashi, N., *Science*, **167**, 191 (1970).
[9] Sláma, K., Romañuk, M., and Šorm, F., *Biol. Bull.*, **136**, 91 (1969).
[10] Bowers, W. S., and Thompson, M. J., *Science*, **142**, 1469 (1963).
[11] Bowers, W. S., *Science*, **164**, 323 (1969).
[12] Zaoral, M., and Sláma, K., *Science*, **170**, 92 (1970).

Possible Role of the Pituitary/Adrenocortical Axis in Aggressive Behaviour

THE recent finding by Cherkin and Meinecke[1] that aggressive behaviour in previously isolated male rabbits could be suppressed for some weeks by allowing them to recover in pairs from barbiturate anaesthesia, raises an interesting question concerning the action of components of the pituitary/adrenocortical axis on behavioural responses. It is well established that most forms of anaesthesia are "stressful" in that they result in activation of the adrenal cortex, and the possibility thus exists that findings such as those by Cherkin and Meinecke are a consequence of changes in ACTH.

The role of pituitary and adrenocortical hormones on behaviour has been recently reviewed by Levine[2], and evidence has been obtained that ACTH depresses isolation-induced aggressive behaviour in the albino mouse[3,4].

Defeated animals show a pronounced increase in adrenocortical activity[5–7] which is not evident in the victor and it seems likely that this increase in ACTH and/or glucocorticoids may have a cue function in inducing subordinate behaviour by increasing the fear response and reducing the aggressive response. An alternative explanation for the result obtained by Cherkin and Meinecke is thus that the stress of anaesthesia causes both animals to assume subordination on emerging from anaesthesia with a consequent suppression of aggressive behaviour. This subordination could be specific to the animal in whose presence they recovered: dominance could be associated with the visual or olfactory characteristics of the other animal.

I thank Professor Chester Jones for encouragement and the SRC for a fellowship.

PAUL F. BRAIN

Department of Zoology,
University of Sheffield,
Yorkshire

Received June 24; revised July 15, 1971.

[1] Cherkin, A., and Meinecke, R. O., *Nature*, **231**, 195 (1971).
[2] Levine, S., *Sci. Amer.*, **224**, 26 (1971).
[3] Brain, P. F., Nowell, N. W., and Wouters, A., *Physiol. Behav.*, **6**, 27 (1971).
[4] Brain, P. F., *J. Endocrinol.* (in the press).
[5] Davis, D. E., and Christian, J. J., *Proc. Soc. Exp. Biol. Med.*, **94**, 728 (1957).
[6] Louch, C. D., and Higginbotham, M., *Gen. Comp. Endocrinol.*, **8**, 441 (1967).
[7] Bronson, F. H., and Eleftheriou, B. E., *Gen. Comp. Endocrinol.*, **4**, 9 (1964).

Myoid Conduction in the Siphonophore *Nanomia bijuga*

Myoid conduction has been suspected in the Cnidaria for some sixty years[1], but has only recently been demonstrated[2,3]. There are no recordings available of activity at the cellular level; until now potentials from cnidarian muscles have been recorded with large extracellular electrodes covering a number of active units.

The stem of the siphonophore *Nanomia bijuga* is covered with a layer of epithelio-muscular cells some 45 µm thick (Fig. 1); this is far thicker than similar cells in many other cnidarians. The muscle fibrils run longitudinally and are non-striated.

Portions of the stem, about 4 cm long, were pinned onto the wax base of a cooled chamber and covered with seawater maintained at 13° C. Cells were successfully impaled by glass microelectrodes filled with 2.8 M potassium chloride having resistances of between 60 and 80 MΩ. Resting potentials of between −50 mV and −80 mV were obtained with 63 mV being the mean of twenty penetrations. This value is in agreement with the values given for anthomedusan epithelia[3]. Many preparations showed spontaneous activity with cell interiors depolarizing 17–20 mV (Fig. 2). The rise time of these potentials is approximately 10 ms, but repolarization takes up to 3 s. These events were conducted without decrement along 2 cm of the stem with a conduction velocity of 20 cm/s as measured by a pair of extracellular suction electrodes.

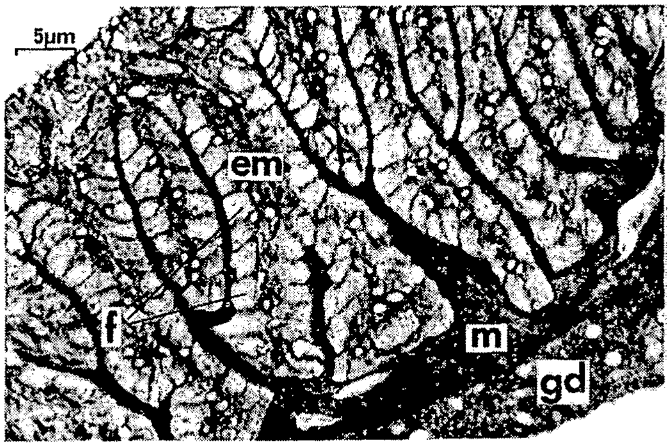


Fig. 1 Transverse section of stem of *Nanomia*, 0.5 µm 'Epon' section (Richardson's stain); em, single layer of epithelio-muscular cells; f, fibrils of epithelio-muscular cells; gd, gastrodermis; m, mesogloea.

If the preparation was shocked by square wave pulses of 1 ms duration delivered by a suction electrode to the surface of the stem two distinct types of potential changes were seen in the conducting cells. The type of potential change was dependent on stimulus strength.

The electrical constants of this preparation were not known, and so the exact current delivered below the stimulating electrode could not be calculated. With the distance between the microelectrode and the stimulating electrode at 1 cm, shocks delivered at a potential of 3 V gave no change in the potential of the cell interior (Fig. 2). At 4 V there is a distinct depolarization of the cell membrane and at 6 V an even greater change so that the interior of the cell became 4 mV more positive. In such cases the rate of increase of positive charge inside the cells is very slow. Events such as these which I have called sub-threshold pulses are not propagated but are probably conducted with decrement. At a specific stimulus strength (about 9.5 V) for any one preparation the penetrated cell will fire giving a rapid decrease in the potential across the outer membrane of the cell. This event I have called a supra-threshold pulse and it seems to be identical to the spontaneous pulses already described.

Each supra-threshold pulse causes a slight contraction of the longitudinal muscle fibres, often sufficient to dislodge the microelectrode. Contractions during spontaneous firing never caused the microelectrode to come out of the cell. If a micro-

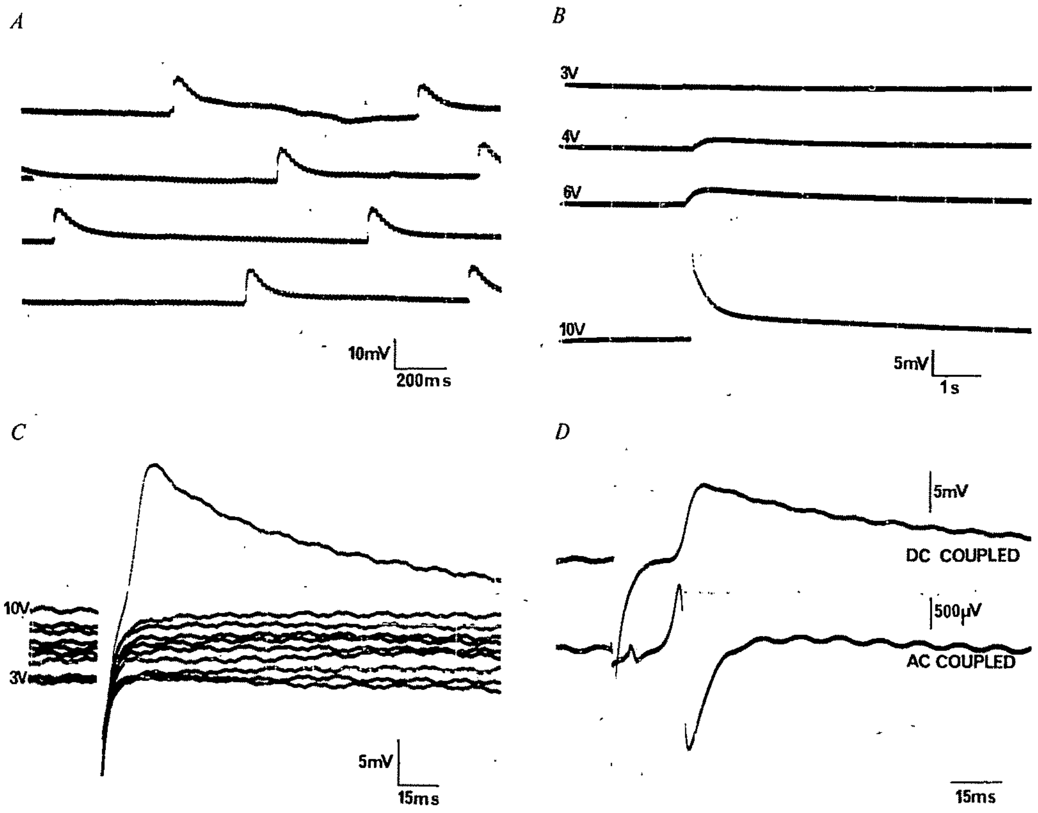


Fig. 2 *A*, Spontaneous supra-threshold pulses; *B*, electrically stimulated pulses showing change from sub-threshold pulses to a supra-threshold pulse as stimulus strength is increased; *C*, same as *B* but tracings superimposed; *D*, a stimulated supra-threshold pulse as recorded by an intracellular electrode (upper trace), and an extracellular suction electrode above the intracellular electrode (lower trace).

electrode is placed directly beneath a suction electrode one can compare the passage of a supra-threshold pulse as recorded inside and outside the cell. The suction electrode in this case is covering more than one cell. The wave-shape and duration of this pulse, as recorded by the extracellular electrode, are very similar to those recorded from other hydrozoan epithelia[3–5].

Procion yellow and Niagara sky blue 6B injected iontophoretically diffused through a number of cells and so failed to show the exact location of the electrode tip. Use of a screw-micrometer to advance the electrode indicated that the tip in most cases would have been in or just above the muscle fibrils.

The preparation continued to propagate supra-threshold pulses when immersed in a solution containing one part sea-water and one part isotonic magnesium chloride. Excess Mg^{2+} blocks nervous activity in other hydrozoans, so it seems that propagation of supra-threshold pulses does not depend on the nerve-net[3].

It is not yet possible to prove that these recordings are intracellular—they could conceivably be transepithelial pulses. Such pulses have been recorded between the enteron and surrounding water in *Hydra* but they have the opposite polarity to the pulses recorded in *Nanomia*[6]. If they are intracellular recordings then one might suppose that the cells in this conducting epithelium are connected by low resistance pathways as demonstrated by the local spread of sub-threshold excitation. This is a reasonable hypothesis since similar types of junctions have already been proposed for conducting epithelia and smooth muscles[7–9].

I thank G. O. Mackie for his help. This study was assisted by an operating grant to G. O. Mackie from the National Research Council of Canada. I am the recipient of a National Research Council (Canada) graduate scholarship.

A. N. SPENCER

Department of Biology,
University of Victoria,
Victoria,
British Columbia

Received November 30, 1970.

[1] Hadzi, J., *Arbeit. Zool. Inst. Univ. Wien.*, **17**, 225 (1909).
[2] Ross, D. M., and Pantin, C. F. A., *J. Exp. Biol.*, **17**, 61 (1940).
[3] Mackie, G. O., and Passano, L. M., *J. Gen. Physiol.*, **52**, 600 (1968).
[4] Josephson, R. K., *J. Exp. Biol.*, **38**, 579.
[5] Mackie, G. O., *J. Exp. Biol.*, **49**, 387 (1968).
[6] Josephson, R. K., and Macklin, M., *Science*, **156**, 1629 (1967).
[7] Burnstock, G., and Prosser, C. L., *Amer. J. Physiol.*, **199**, 553 (1960).
[8] Loewenstein, W. R., *Ann. NY Acad. Sci.*, **137**, 441 (1966).
[9] Mackie, G. O., *Quart. Rev. Biol.* (in the press).

Corrosion by the Sulphate-reducing Bacteria

THERE are several explanations for the aggravation of corrosion of iron and steel in oxygen-free conditions by sulphate-reducing bacteria. These are (*a*) stimulation of the cathodic part of the corrosion cell by the removal and utilization of the polarizing hydrogen by the bacteria[1]; (*b*) stimulation of the cathodic reaction by solid ferrous sulphides formed by the reaction of ferrous ions with sulphide ions produced by bacteria[2]; (*c*) stimulation of the anodic reaction, metal dissolution, by bacterially produced sulphide[3]; (*d*) local acid cell formation[4], and (*e*) formation of iron phosphide by reaction of the metal with bacterially reduced phosphates[5].

There is little evidence that (*d*) and (*e*) are significant except possibly in isolated cases, whereas (*c*) is probably important only at the start of the corrosion process due to the eventual formation of protective sulphide films in the presence of free sulphide ion[6].

Much support for (*a*), the cathodic depolarization mechanism first postulated by von Wolzogen Kühr, has come from work by Booth and Tiller[7]. Using mild steel electrodes at various controlled potentials, they observed a straight line relationship between the hydrogenase activity of the bacteria and the extra current required to maintain these potentials on the cathodes in the presence of resting-cell suspensions of sulphate-reducing bacteria, provided with benzyl viologen as electron acceptor. But the organisms used had been cultured on standard sulphate-containing medium and the resting-cell suspensions would therefore contain ferrous sulphide, both free and embedded in the mucopeptide material adhering to the cells. (The ferrous sulphide that forms during growth in sulphate media is impossible to separate completely from the bacteria except by chemical methods so severe that the bacteria themselves are destroyed.)

Using the same procedure as Booth and Tiller[7], we have observed a direct relation between the amount of chemically prepared ferrous sulphide added to a bacteria-free system, and the extra current required, as previously described. Moreover, organisms previously grown in a sulphate-free fumarate medium[8] showed little cathodic depolarization. This latter result could explain the low weight loss/time results obtained by Booth, Elford and Wakerley[2] with mild steel coupons in semicontinuous cultures of the bacteria in a sulphate-free fumarate medium. The similarity between the results of their potentiostatic experiments and those obtained by Booth and Tiller[7] suggests that there is some connexion between the rate of bacterial hydrogen uptake and the amount of ferrous sulphide present (this possibility was adumbrated by the observation of Goldner *et al.*[9] that particulate material stimulated hydrogen uptake). We have now confirmed this, using Warburg manometry to measure the rate of hydrogen uptake by bacteria grown on both sulphate-free and sulphate-containing media. When chemically prepared ferrous sulphide was added to the resting cell suspensions in the reaction flasks, substantially greater rates of hydrogen uptake were observed in nearly every case, using either sulphate or benzyl viologen as electron acceptor (Table 1).

This effect of ferrous sulphide may explain the wide variation encountered in manometric determinations of $-Q_{H_2}$ for the sulphate-reducing bacteria[10], since variable amounts of ferrous sulphide are carried over into the reaction flasks as is easily verifiable visually; the effect may also have a bearing on the observation[11] of a small hydrogen uptake by *Desulfotomaculum orientis*, a sulphate reducer usually considered to be hydrogenase-negative, and this question is being studied further.

Ferrous sulphide alone, though known to be cathodic to mild steel[2,12], is not a permanent cathode. Our weight loss/time experiments indicate that chemically or bacterially produced ferrous sulphides corrode by a first order reaction and that the eventual total corrosion is directly proportional to the

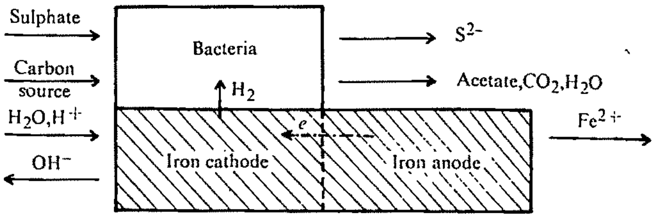


Fig. 1 Scheme illustrating the classic theory of cathodic depolarization by the sulphate-reducing bacteria.

Table 1 Hydrogen Uptake by Sulphate-reducing Bacteria (*Desulfovibrio desulfuricans* Strain Teddington R, NCIB 8312)

		$-Q_{H_2}$†	
Growth medium *	Electron acceptor for manometry	Resting cells alone	Resting cells with 1 mM ferrous sulphide added
Sulphate-containing lactate medium]	sulphate	30.7	40.6
		43.5	290
		11.3	126
Sulphate-free fumarate medium[8]	sulphate	6.8	124
		5.6	41
	benzyl viologen	627	648
		443	577

* Bacteria were suspended in 0.04 M phosphate buffer (*p*H 7.0) for manometry.

† Additions of ferrous ion (as $FeCl_2$) or sulphide ion (as Na_2S) alone did not increase the hydrogen uptake rate. Ferrous sulphide was inactive towards hydrogen for additions of sulphate or benzyl viologen in absence of the bacteria.

amount of ferrous sulphide present. Sustained high corrosion rates in such bacteria-free systems are only obtained by continual replenishment of the ferrous sulphide such as occurs in cultures in iron-rich medium where all the sulphide or bisulphide ion produced by bacterial metabolism is precipitated as ferrous sulphide[13]. In media containing minimal iron, free sulphide ions cause the formation of sulphide films which confer fair protection to the metal underneath. These films are not permanent, however, and the time for breakdown to occur is probably linked to the activity of the bacteria[13]. High rates of corrosion are observed once film breakdown occurs. It seems that once there is sufficient ferrous sulphide present to initiate the formation of the galvanic cell ferrous sulphide|Fe, then a high corrosion rate is attained and no sulphide films are formed. An observation that steel coupons in semicontinuous cultures of sulphate-reducing bacteria, grown initially on iron-rich and later on minimal-iron media, exhibit continued high corrosion rates supports this view (personal communication from D. S. Wakerley).

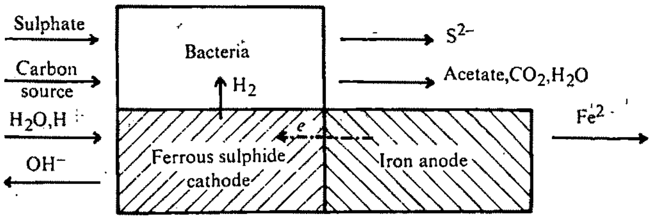


Fig. 2 Scheme illustrating the proposed mechanism of corrosion by the sulphate-reducing bacteria.

The presently accepted theory of corrosion by cathodic depolarization is represented diagrammatically in Fig. 1. We propose a new theory of corrosion by the sulphate-reducing bacteria such that the cathodic reaction, hydrogen evolution, occurs on the ferrous sulphide produced by reaction of ferrous ion with bacterially produced sulphide ion. We find that the ferrous sulphide activity is diminished with time, possibly owing to the bonding of atomic hydrogen within the ferrous sulphide crystals; but the activity of the sulphide is restored by removal of the atomic hydrogen by bacterial hydrogenase activity. This mechanism is illustrated schematically in Fig. 2.

This work was supported by the Science Research Council, from whom one of us (R. A. K.) is the recipient of a post-doctoral research assistantship.

R. A. King
J. D. A. Miller

Corrosion Group,
Department of Chemical Engineering,
University of Manchester Institute of
Science and Technology

Received April 5, 1971.

[1] Wolzogen Kühr, C. A. H. von, *Corrosion*, **17**, 293t (1961).

[2] Booth, G. H., Elford, L., and Wakerley, D. S., *Brit. Corros. J.*, **3**, 242 (1968).

[3] Wanklyn, J. N., and Spruit, C. J. P., *Nature*, **169**, 928 (1952).

[4] Goldner, B. H., and Dittman, J. B., *Amer. Petroleum Inst. Second Biennial Symp. Microbiol.* (1964).

[5] Iverson, W. P., *Nature*, **217**, 1265 (1968).

[6] Booth, G. H., and Tiller, A. K., *Trans. Faraday Soc.*, **56**, 1689 (1960).

[7] Booth, G. H., and Tiller, A. K., *Corrosion Sci.*, **8**, 583 (1968).

[8] Miller, J. D. A., and Wakerley, D. S., *J. Gen. Microbiol.*, **43**, 101 (1966).

[9] Goldner, B. H., Otto, L. A., and Canfield, J. H., *Amer. Petroleum Inst. First Biennial Symp. Microbiol.*, 16 (1963).

[10] Miller, J. D. A., and Saleh, A. M., *J. Gen. Microbiol.*, **37**, 419 (1964).

[11] Booth, G. H., Cooper, P. M., and Wakerley, D. S., *Brit. Corrosion J.*, **1**, 345 (1966).

[12] Herzog, E., *Ind. Eng. Chem.*, **53**, 64A (1961).

[13] Mara, D. D., thesis, Univ. Dundee (1970).

Infection of Schistosomes with Gram-positive Cocci

Schistosoma spindale (Montgomery) is a common trematode parasite of cattle, water buffalo, goats and other mammals over a vast area including southern Africa, South and South-East Asia. It is a close relative of the human parasite *S. haematobium* and while it has only rarely been reported from man it is a significant producer of cercarial dermatitis in rice field workers. As part of an investigation of *S. spindale* in Malaysia, a number of laboratory animals were infected in various ways with cercariae of this helminth. One young female white mouse was exposed by tail immersion to numerous cercariae of a local strain in Kuala Lumpur and killed 82 days later with 0.3 ml. of heparinized 'Nembutal' (pentobarbital sodium) injected intraperitoneally. The liver and spleen were larger than those usually found in mice with *S. spindale* infections of similar age. The spleen was soft and yellowish-white and particularly distended over about half its length. Small pieces of liver, spleen, lungs and intestine were fixed in 10% formalin, after which the mouse was perfused with citrated saline according to the method of Duvall and DeWitt[1]. Eighteen pairs of worms and thirty adult males were recovered in the perfusate; all except one pair and two males had nodular lesions (Fig. 1 *b–f*) visible to the naked eye or with low magnification. I found several additional worms, including some with such lesions, in the tissues (Fig. 1*f*), but no affected worms in fifty-seven other mice similarly exposed and perfused. The discovery of these diseased worms was unanticipated. Because their significance was not immediately appreciated, they were fixed with 10% formalin and stored in 70% alcohol as usual. On subsequent examination, it became clear that their nodular lesions were filled with great

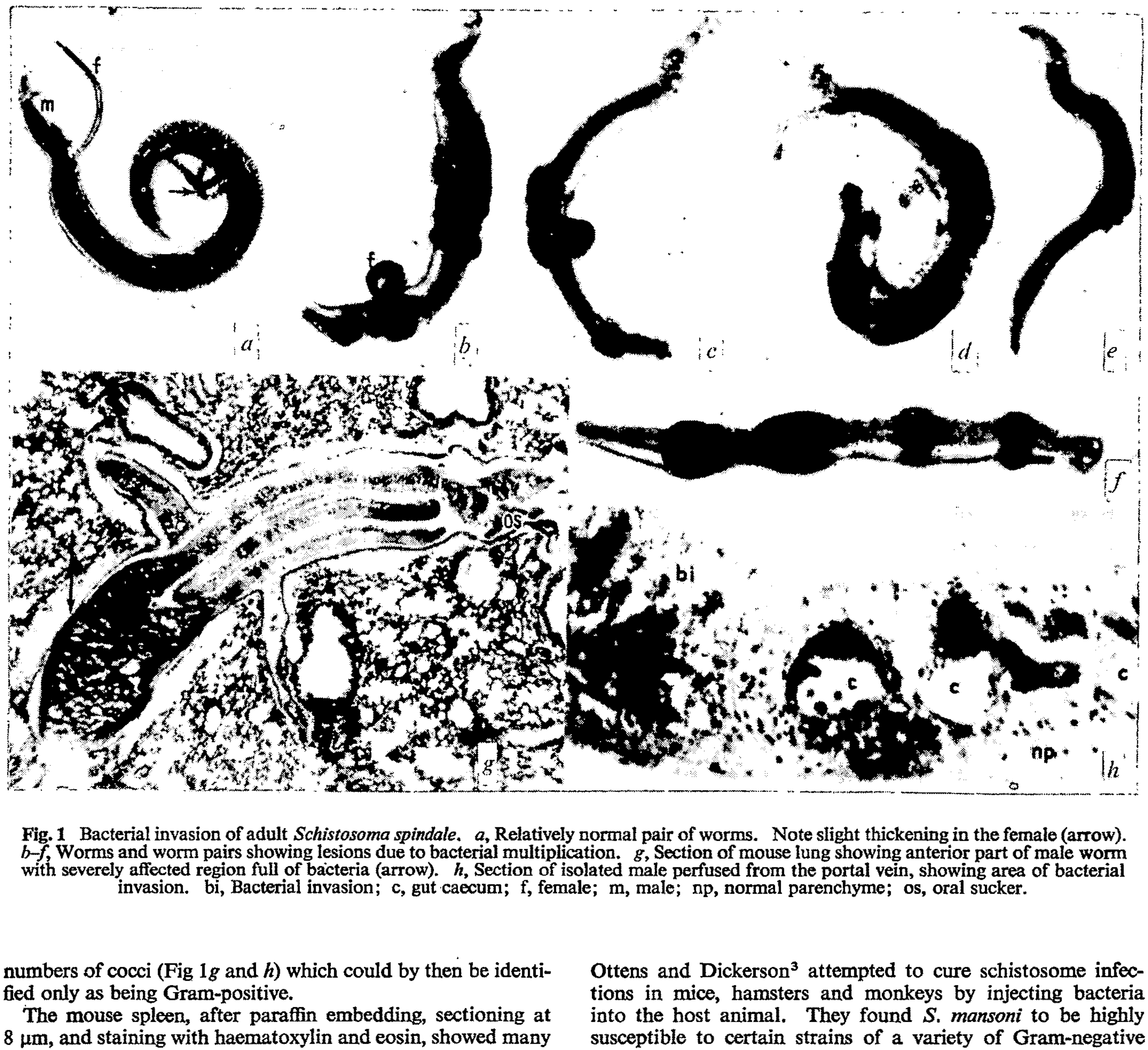


Fig. 1 Bacterial invasion of adult *Schistosoma spindale*. *a*, Relatively normal pair of worms. Note slight thickening in the female (arrow). *b–f*, Worms and worm pairs showing lesions due to bacterial multiplication. *g*, Section of mouse lung showing anterior part of male worm with severely affected region full of bacteria (arrow). *h*, Section of isolated male perfused from the portal vein, showing area of bacterial invasion. bi, Bacterial invasion; c, gut caecum; f, female; m, male; np, normal parenchyme; os, oral sucker.

numbers of cocci (Fig 1*g* and *h*) which could by then be identified only as being Gram-positive.

The mouse spleen, after paraffin embedding, sectioning at 8 µm, and staining with haematoxylin and eosin, showed many clumps of cocci in the centre of the expanded whitish region in areas of necrosis and intense cellular activity. Many polymorphonuclear leucocytes were present, together with macrophages containing phagocytosed bacteria. Numerous fibroblasts were seen in and around the region. The condition was consistent with acute splenitis of several days' duration. Bacterial invasion of the spleen was perhaps facilitated because that organ was already compromised owing to portal hypertension induced by the schistosome infection. In the liver, a small area of bacterial invasion surrounded the remains of a worm whose caeca contained masses of bacteria.

It is likely that bacteriaemia in the mouse led to infection of the schistosomes. The worms, which dwell in the intestinal and portal venous systems, had probably ingested bacteria along with their usual diet of blood. Sections and stained whole mounts of worms show that multiplication of bacteria occurs primarily in the gut caeca, which become greatly dilated; the parenchyma is then subject to massive invasion (Fig. 1*h*). The worms, in turn, act as amplifiers of the bacterial infection and serve as intense focal sources of organisms for potential invasion of adjacent tissues. Krakower *et al.*[2] found that Puerto Rican *S. mansoni* in some rats deficient in vitamin A were invaded by Gram-positive bacilli such as *Lactobacillus acidophilus*, a normal gut inhabitant presumed to have passed through the intestinal wall as a result of the malnutrition. Ottens and Dickerson[3] attempted to cure schistosome infections in mice, hamsters and monkeys by injecting bacteria into the host animal. They found *S. mansoni* to be highly susceptible to certain strains of a variety of Gram-negative bacilli and also observed bacterial invasion and lethal effects in *S. mattheei*, a member of the "haematobium" group to which *S. spindale* also belongs. These authors, moreover, evaluated twenty-four strains of Gram-positive bacteria (not specified), none of which invaded schistosomes.

This report is the first to demonstrate that Gram-positive cocci may be highly invasive and pathogenic in adult schistosome worms.

This work was done in part when I was a staff member of the University of California ICMRT at the Institute for Medical Research, Kuala Lumpur, Malaysia; supported by a US Public Health Service grant. I thank Drs S. DeWitt and K. Vosti of Stanford University for their comments.

PAUL F. BASCH

Department of Community and Preventive Medicine,
Stanford University School of Medicine,
Stanford,
California 94305

Received June 21; revised July 2, 1971.

---

[1] Duvall, R. H., and DeWitt, W. B., *Amer. J. Trop. Med. Hyg.*, **16**, 483 (1967).

[2] Krakower, C., Hoffman, W. A., and Axtmayer, J. A., *Puerto Rico J. Pub. Hlth. Trop. Med.*, **16**, 269 (1940).

[3] Ottens, H., and Dickerson, G., *Nature*, **223**, 506 (1969).

Temperature Optimum of Non-sulphur Bacteria from a Spring at 90° C

It is well established that bacteria live in hot springs at temperatures greater than 90° C depending on altitude[1-4]. We have studied the physiology and temperature optima of these organisms by growing them on coverslips and measuring directly the uptake of various radioactively labelled compounds[5]. The bacteria were obtained from an unnamed spring, known to us as "pool A", which is in the Lower Geyser Basin of Yellowstone National Park[4], and which has a temperature of 90°–91.5° C and is therefore just below the boiling point for this altitude. It has a pH of 8.6–8.9 and a low sulphide content (0.096 µg/ml.). Our work on Boulder Spring, a superheated spring with a relatively large sulphide content (3.1 µg/ml.), revealed that the bacteria in that spring incorporated radioactively labelled compounds only when sulphide was present[6], yet the uptake by bacteria from pool A was inhibited by sulphide.

Bacteria from pool A incorporated radioactive amino-acids, thymidine and lactate, but incorporation of labelled sugars was negligible. Detailed studies with radioactive leucine (Table 1) showed that isotope uptake was inhibited not only by formaldehyde but also by streptomycin, novobiocin, sodium azide, Na_2S and mercuric bichloride. Uptake of radioactive leucine is also inhibited by non-radioactive leucine and by yeast extract. It is clear, therefore, that isotope uptake is due to a vital process and not to passive uptake or adsorption. Attempts to stimulate uptake of leucine, acetate or bicarbonate by bubbling with methane, nitrogen, CO_2 or air during the incubation period were unsuccessful.

Table 1 Effect of Inhibitors on Uptake of ^{14}C-Leucine by Bacteria from Pool A

Conditions	c.p.m./coverslip
Control	4,622
+HCHO (4%)	364
+streptomycin (1,000 µg/ml.)	429
+$HgCl_2$ (100 µg/ml.)	317
+NaN_3 (1,000 µg/ml.)	153
+novobiocin (100 µg/ml.)	100
+Na_2S (150 µg/ml.)	998
+Na_2S (15 µg/ml.)	2,234
+yeast extract (0.1%)	35
+non-radioactive leucine (1,000 µg/ml.)	87

Incubation at 86° C for 1 h in synthetic D medium[8] at pH 7.0 with 0.06 µCi/ml. ^{14}C-leucine.

The optimum pH for uptake of leucine was between 7 and 7.5 (Fig. 1) irrespective of whether spring water or synthetic salts adjusted to various pH values were used. In determination of the temperature optimum at pool A, both spring water at its natural pH and synthetic salt solution at pH 7 were used as incubation media. Although the amount of isotope uptake was much greater in the latter, the temperature optima for both media were the same. A number of experiments were done to determine the temperature optimum for the incorporation of different labelled compounds. Fig. 1 shows results of a single experiment in which five compounds were run in parallel. Only 5–10 min equilibration at the various temperatures was allowed, so that there was no possibility of adaptation to the new temperatures. We assume therefore that the optima represent those which the bacteria had in the spring itself. The optima for all compounds are virtually the same, 90° C. In other experiments, optima ranging from 86° to 90° C were found. During the past 6 yr we have measured the source temperature of pool A many times and have found that it ranges from 90.5°–91.5° C. Thus the temperature optimum of these bacteria is virtually identical to their environmental temperature.

These data and those from Boulder Spring[6] suggest that these bacteria are optimally adapted to the temperatures of the springs. It would be interesting to know the temperature optima of the bacteria living in the boiling springs of New Zealand[7], which have temperatures of 99°–100° C. Clearly the bacteria found in Yellowstone springs at 90° C should not be able to multiply in the New Zealand springs.

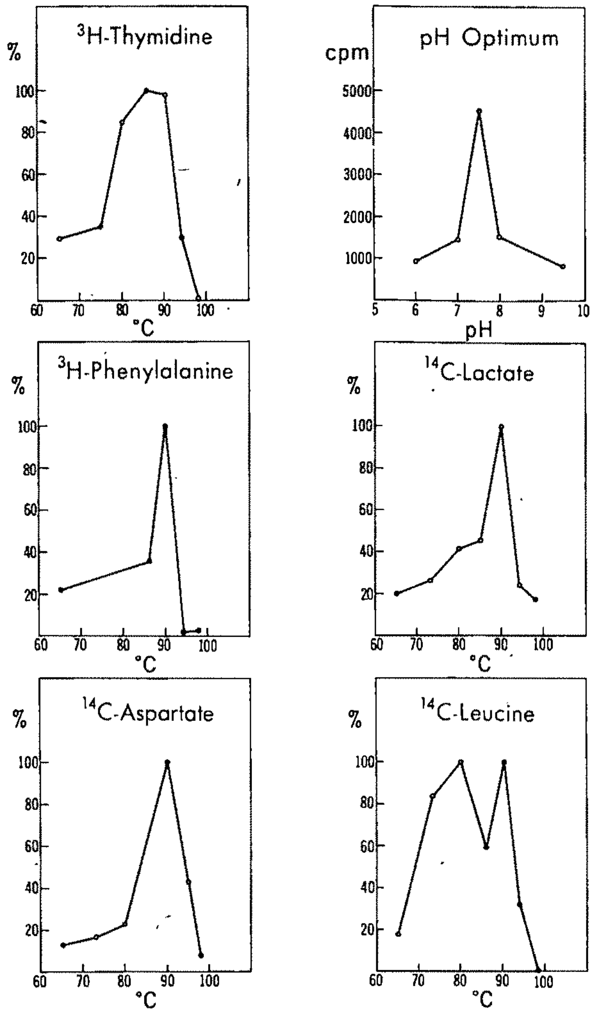


Fig. 1 Temperature and pH optima for the bacteria of pool A. Upper right, pH optimum for the uptake of ^{14}C-leucine. Incubation was at 86° C for 1 h with 0.06 µCi/ml. ^{14}C-leucine. pH values were obtained by adjusting the spring water with 1 M HCl or 1 M NaOH. Other graphs, temperature optima for the uptake of several labelled compounds, all at 0.06 µCi/ml. The results are plotted as percentage of maximum incorporation for each isotope. The 100% values in c.p.m./coverslip were as follows: ^{3}H-thymidine, 492; ^{3}H-phenylalanine, 2,224; ^{14}C-aspartic, 840; ^{14}C-lactate, 92; ^{14}C-leucine, 11,545. Specific activities of the various radioactive compounds are as follows: thymidine, 0.25 mCi/0.00368 mg; phenylalanine, 1 mCi/0.067 mg; aspartic, 3.65 mCi/mmol; lactate, 0.1 mCi/2 mg; leucine, 0.05 mCi/0.025 mg. Incubation in synthetic D medium[8], pH 7. Temperatures over boiling (92.5° C) were obtained using boiling water baths with saturated $MgSO_4$.

This research was supported by the National Science Foundation and the Atomic Energy Commission.

T. D. BROCK*
M. L. BROCK†

Department of Microbiology,
Indiana University,
Bloomington, Indiana 47401

Received December 21, 1970; revised June 2, 1971.

---

* Present address: Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706.

† Present address: Division of Research, National Jewish Hospital, 3800 E. Colfax Avenue, Denver, Colorado 80206.

[1] Brock, T. D., *Science*, **158**, 1012 (1967).
[2] Brock, T. D., in *Microbial Growth, Nineteenth Symp. Soc. Gen. Microbiol.*, 15 (Cambridge University Press, 1969).
[3] Brock, T. D., and Darland, G. K., *Science*, **169**, 1316 (1970).
[4] Bott, T. L., and Brock, T. D., *Science*, **164**, 1411 (1969).
[5] Bott, T. L., and Brock, T. D., *Limnol. Oceanog.*, **15**, 333 (1970).
[6] Brock, T. D., Brock, M. L., Bott, T. L., and Edwards, M., *J. Bact.* (in the press).
[7] Brock, T. D., and Brock, M. L., *New Zealand J. Marine Freshwater Res.* (in the press).
[8] Castenholz, R. W., *Bact. Revs.*, 33, 476 (1969).

Potential Cocarcinogenicity of Sodium Hypochlorite

SODIUM hypochlorite has been widely used as a bleaching agent and disinfectant. No particular hazard of this chemical to man has been recognized (see, for example, ref. 1) except for the poisoning of infants by the accidental ingestion of concentrated solution. We now report, however, that sodium hypochlorite is definitely concerned in cancer production in mice as a cocarcinogen[2-4].

In testing for cocarcinogenicity, a group of animals is treated with a potent carcinogen in a dose which by itself is not enough to produce a cancer. A suspected chemical cocarcinogen is also given to the same animals, either before or after. The development of a cancer offers a sensitive test for potentially carcinogenic compounds[5-7].

A commercial sodium hypochlorite solution which contained more than 10% effective chlorine was used. The carcinogen selected was 4-nitroquinoline 1-oxide[8,9]. The mice were ddN strain females maintained on water and a standard pellet diet. Before the application of the chemicals, hair was clipped off over an area 1 cm in diameter in the interscapular region where the chemicals were applied.

Three groups of forty 5 week old mice were used. One group was used in a combination experiment.

Twenty applications of 4-nitroquinoline 1-oxide alone produced neither malignant nor benign tumours. Although hypochlorite did not induce skin tumours by itself in sixty applications, skin tumours were induced in nine of thirty-two mice by forty-five applications given after submanifestational doses of 4-nitroquinoline 1-oxide. Thus, one fibrosarcoma of the skin and three squamous cell carcinomas were produced after about 240 days. Five papillomas remained until the end of the experiment. In one tumour-bearing mouse metastases to lung and kidney were observed. Besides the skin tumours, one lymphatic leukaemia was found in another mouse about 360 days after the start of the experiment. No tumours resulted from sixty paintings of aqueous sodium hydroxide at *p*H 11 ~12 combined with twenty applications of 4-nitroquinoline 1-oxide, indicating that potential carcinogenicity of hypochlorite solution is due to the hypochlorite itself and not to the alkalinity of the solution.

Solutions of sodium hypochlorite containing approximately 5% chlorine are available commercially and used widely. For bleaching, the solution is diluted up to several hundred times. Various materials, including foods, may be sterilized using a solution diluted several hundred times. Drinking water may be disinfected by a low concentration of chlorine (0.1 to several parts per million). In the treatment of some skin diseases caused by fungi, a dilute solution may be applied directly to the diseased part. Although in such conditions it seems unlikely that this common chemical constitutes a practical carcinogenic hazard, the data presented here provide a caution against its indiscriminate use.

We thank Dr W. Nakahara, director of the National Cancer Center Research Institute, for valuable discussion and pathological diagnosis and Professor T. Ukita of the Faculty of Pharmaceutical Sciences, University of Tokyo, and Dr F. Fukuoka of the National Cancer Center Research Institute for research facilities.

Table 1 Carcinogenicity of Sodium Hypochlorite in Mice when Combined with a Submanifestational Dose of Carcinogenic 4-Nitroquinoline 1-Oxide

	No. of mice	Skin tumours Malignant	Skin tumours Benign	Leukaemia
		(No. of mice)		
Group 1	32	4	5 (1)	1
Group 2	29	0	0	0
Controls	27	0	0	0

The potent carcinogen was first painted on in twenty applications of 0.05 mg in 0.25% (w/v) benzene solution[5] in the course of 50 days (1.0 mg/mouse in total). Six days after the end of this treatment, one drop (about 0.05 ml.) of the hypochlorite solution was painted on and repeated forty-five times during 245 days (group 1). Other mice (group 2) were treated only with 4-nitroquinoline 1-oxide. The control mice were treated only with the hypochlorite (sixty times in 300 days). All of the mice treated were observed for 450 days from the start of the experiment.

The number in the parentheses shows the number of papillomas that regressed.

HIKOYA HAYATSU

Faculty of Pharmaceutical Sciences,
University of Tokyo,
Bunkyo-ku, Tokyo

HIROSHI HOSHINO

Department of Radiology,
School of Medicine,
Keio University,
Shinano-cho,
Shinjuku-ku, Tokyo

YUTAKA KAWAZOE

National Cancer Center Research Institute,
Tsukiji,
Chuo-ku, Tokyo

Received June 21, 1971.

[1] Druckrey, H., *Food Cosmet. Toxicol.*, **6**, 147 (1968).
[2] Nakahara, W., *Prog. Exp. Tumour Res.*, **2**, 168 (1961).
[3] Druckrey, H., and Küpfmüller, K., *Z. Naturforsch.*, **3b**, 254 (1948).
[4] Berenblum, I., *Prog. Exp. Tumour Res.*, **11**, 21 (1969).
[5] Hoshino, H., Tanooka, H., and Fukuoka, F., *Gann*, **59**, 43 (1968).
[6] Hoshino, H., Kawazoe, Y., and Fukuoka, F., *Gann*, **60**, 523 (1969).
[7] Hoshino, H., Chihara, G., and Fukuoka, F., *Gann.*, **61**, 121 (1970).
[8] Nakahara, W., Fukuoka, F., and Sugimura, T., *Gann*, **48**, 129 (1957).
[9] Ochiai, E., *J. Org. Chem.*, **18**, 549 (1953).

Hormonal Pregnancy Tests and Neural Tube Malformations

IN 1967 Gal, Kirman and Stern[1] reported a possible relationship between pregnancy tests using pills containing synthetic oestrogenic and progesterogenic hormones and the birth of a child with spina bifida. Of the 100 mothers of children with spina bifida, nineteen gave a history of pregnancy diagnosis by this means; only four of the 100 matched control mothers gave such a history.

A similar retrospective enquiry has been made between 1968 and 1970 in three centres, but including births of the clearly related malformation anencephaly as well as spina bifida. In the London series the control mothers were those having the next baby born in the same hospital. In the Exeter series the control mothers were matched for area of birth,

Table 1 Anencephaly and Spina Bifida in Babies Born to Mothers who had had Hormonal Pregnancy Tests

		Survey cases		Controls	
		Total	Pregnancy tests	Total	Pregnancy tests
South Wales	Spina bifida	29	2	112	7
	Anencephaly	31	2		
Exeter	Spina bifida	45	3	64	7
	Anencephaly	19	3		
London	Spina bifida	75	4	75	2
	Anencephaly	72	8	72	6
	Total	271	22 (20.1)	323	22 (23.9)

The number expected in survey and control groups if no difference exists is shown in parentheses. The difference between actual and expected numbers is within the standard error.

parity and month of conception. In the South Wales series the control mothers were those who had one baby with spina bifida or anencephaly and had a subsequent normal birth during the survey period and these were thus not controlled individually. The findings are summarized in Table 1. In these three surveys there is no significant association of pregnancy tests with pills containing oestrogenic and progesterogenic hormones and the birth of children with neural tube malformations.

MICHAEL LAURENCE
MARY MILLER

Department of Child Health,
Welsh National School of Medicine,
Heath Park, Cardiff

MARY VOWLES

Paediatric Department,
Royal Devon and Exeter Hospital,
Exeter

KATHLEEN EVANS
CEDRIC CARTER

MRC Clinical Genetics Research Unit,
Institute of Child Health,
Guilford Street, London WC1

Received August 3, 1971.

---

[1] Gal, I., Kirman, B., and Stern, J., *Nature*, **216**, 83 (1967).

Effect of *Avena sativa* on Cigarette Smoking

IN 1967, in India, I came across a practitioner of ancient Ayurvedic medicine who successfully used a decoction of common oats (*Avena sativa*) to cure the opium habit. While using an alcoholic extract of the plant on a group of opium addicts, several patients reported a loss of interest in smoking. The drug is listed in the United States Dispensatory and National Formulary[1–2], yet no reference has been traced regarding its application on smokers (Library of the Pharmaceutical Society of Great Britain, personal communication). I have therefore studied the effect of this drug on a group of smokers.

The active drug was an extract of healthy, fresh plant *Avena sativa* selected just before harvest. I used 1.5 parts of the crushed whole plant by weight in 5 parts by volume of 90% ethyl-alcohol, kept at room temperature with frequent agitation for 72 h and then filtered. The active constituent has not been identified.

Twenty-six cigarette smokers including healthy volunteers and chronic patients in the chest wards of Ruchill Hospital, Glasgow, including tuberculous patients, participated in the trial. The total duration of their smoking and the average number of cigarettes smoked per day in the preceding six months were recorded.

They were told that a drug was being tested which might affect their smoking, and that they were not to make any conscious effort to alter their smoking during the trial.

Table 1 Numbers of Cigarettes Smoked during Trial

	Group I (drug)		Group II (placebo)	
	Cigarettes per day before trial	Cigarettes per day after trial	Cigarettes per day before trial	Cigarettes per day after trial
	20	20	22	19
	25	10	30	28
	10	0	18	18
	12	0	14	14
	11	0	18	17
	9	0	17	18
	35	3	17	18
	25	7	8	18
	22	0	18	18
	20	7	20	18
	25	7	15	14
	20	10	10	9
	20	10	8	8
Av.	19.5	5.7	16.5	16.7

Each patient kept a daily record of cigarettes smoked, commenting on any changes in the craving for cigarettes. By random allocation, thirteen patients received the drug and the others received placebo for 28 days. The alcoholic extract (1 ml.) was diluted to 5 ml. and each oral dose was 5 ml. of this dilution given four times a day. No psychotherapy was used. No patients were taking any other drugs which could affect smoking. The patients in both groups were comparable in age, sex and smoking history.

The results of the trial are given in Table 1. In the drug group the total daily consumption by thirteen patients was 254 cigarettes; at the end of the trial it was seventy-four. Five had stopped smoking, seven had reduced it to less than 50% and in one no change had occurred. In the placebo group the total daily consumption at the start was 215, at the end it was 217. Smoking had been stopped by none, reduced to above 50% by six and increased by three; four reported no change.

The two groups were comparable in their smoking habits, since group I "before" (mean 19.5 ± 2.0 s.e.) is not statistically separable from group II "before" (mean 16.5 ± 1.7 s.e.); $t = 1.33$, $0.30 > P > 0.20$. Group I "before" is significantly different from group I "after" (mean 5.7 ± 2.0 s.e.); $t = 5.21$, $P < 0.001$. Group II "before" is not different from group II "after" (mean 16.9 ± 1.4 s.e.); $t = 0.180$, $0.90 > P > 0.80$. The mean differences between the groups due to treatment (group I "before"–group I "after") and (group II "before"–group II "after") are naturally equally significant; $t = 5.73$, $P < 0.001$. All these calculations are based on Student's t test.

In the drug group various degrees of loss of craving for cigarettes were reported. The drug seems to reduce the number of cigarettes smoked per day, along with diminished craving for smoking. Moreover, the reduction in smoking seems to continue even 2 months after the termination of the drug. The drug has never been used in dealing with the problem of smoking and, as this was the first instance of its use in smokers, its role and significance are worthy of further investigation.

I thank Dr G. J. Addis for help with statistical analysis and Professor V. D. Sharma for extracting the drug.

C. L. ANAND

47 Clarendon Street,
Glasgow NW

Received June 4, 1971.

---

[1] United States Dispensatory, twentieth ed., part II, 1513 (1918).

[2] National Formulary of the United States, seventh ed., 60 (1942).

Possible Relationship between Smoking and Peptic Ulcer

EPIDEMIOLOGICAL studies have shown that the incidence of peptic ulcer is higher among smokers than non-smokers[1]. No reason for this association has been found. Since personality factors seem to play an important role in this disease, it could be argued that both the development of an ulcer and the habit of smoking heavily result from the same psychological traits and are therefore not related. Recently, however, Jacobson *et al.* demonstrated that nicotine markedly reduced secretion of water and bicarbonate from the pancreas by inhibiting the stimulating effect of secretin[2,3]. Since gastric acid is normally neutralized in the duodenum by pancreatic juice and, to a lesser extent, by bile, it was thought that nicotine might predispose to ulcer formation by preventing such neutralization. We have tested this possibility in rats treated with a combination of two gastric secretogogues (substances which stimulate secretion of gastric juice), pentagastrin and carbachol, which had been found to produce duodenal ulcers[4]. We have reported that when these secretogogues were administered in small doses known to produce an ulcer incidence of only 36%, the addition of nicotine raised the incidence to 90%, and the ulcers were multiple and very severe[5]. Nicotine, given alone, was without effect. This result indicated that nicotine sensitized the duodenum to the ulcerogenic effects of the two secretogogues used.

Subsequent studies demonstrated that the duodeno-ulcerogenic property of pentagastrin plus carbachol was due to the marked increased secretion of gastric juice elicited by these secretogogues[6]. We therefore investigated whether the pro-ulcer effect of nicotine was due to increased sensitivity of the duodenum to acid itself. We thought that since nicotine inhibits secretion of alkaline pancreatic juice, acid, perfused through the stomach, would not be neutralized and therefore should lead to the formation of duodenal ulcer.

A solution of hydrochloric acid was perfused through the oesophagus of rats, in amounts previously found to produce duodenal ulcers in about one-third of the animals[1]. Female Upjohn rats (derived from Sprague–Dawley strain), of an average weight of 220 g, were fasted overnight. On the following morning, a tygon tubing (22 gauge) was inserted, under ether anaesthesia, into the cervical oesophagus through a small skin incision. The tip of the tubing lay 0.5–1.0 cm above the cardiac opening of the oesophagus. On recovery from anaesthesia, the tubing was connected to a syringe mounted on a constant infusion machine, containing either HCl 0.12 N (groups I and II) or water (group III). Acid and water were perfused at a rate of 3.0 ml./h for the first 10 h, then at a rate of 1.5 ml./h for the next 14 h. The fluid flowed from the oesophagus to the stomach, then entered the duodenum through the pylorus. Group I (sixteen rats) was also infused subcutaneously with saline at the rate of 0.54 ml./h, whereas group II (fourteen rats) was infused subcutaneously with a solution

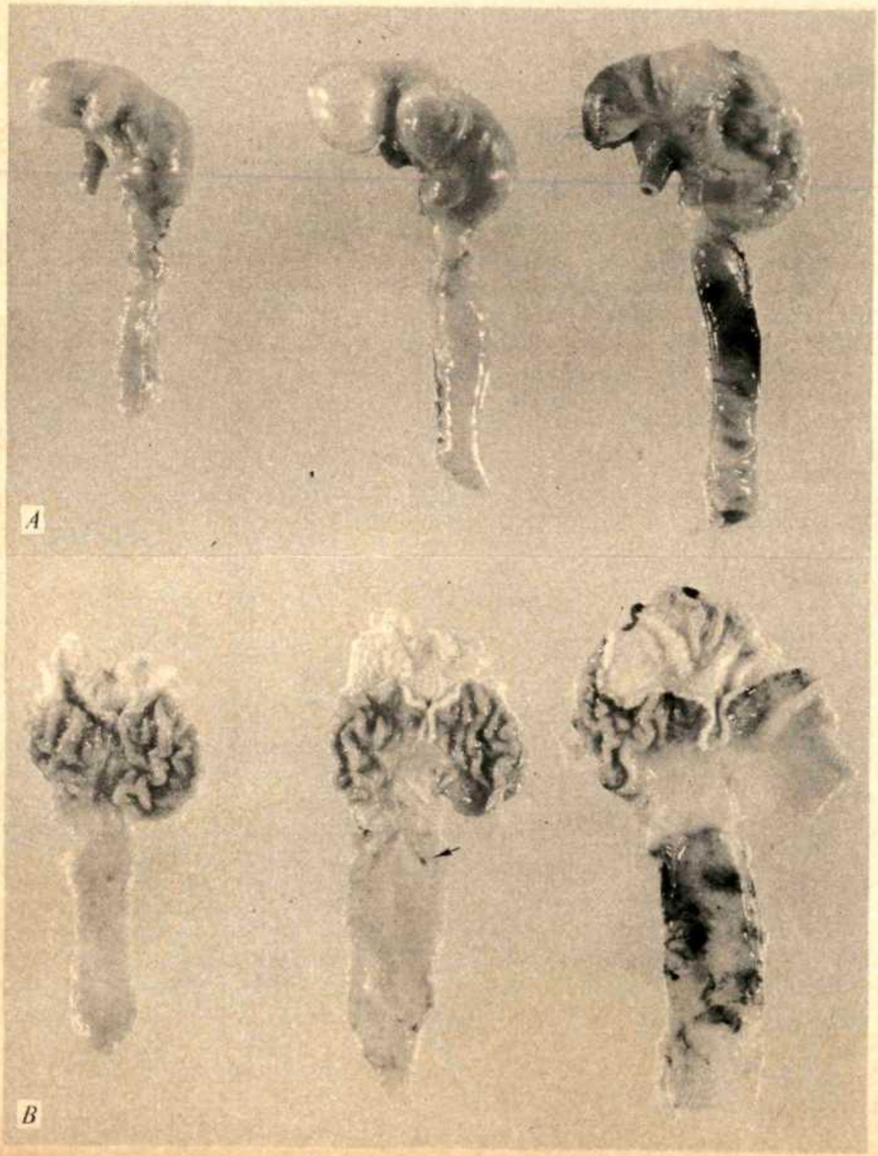


Fig. 1 Duodenal ulcers produced by acid (perfused in oesophagus) and nicotine (infused subcutaneously). *A*, External view of stomach and duodenum *B*, Same stomachs and duodenums after opening along mesenteric attachment (duodenum) and greater curvature (stomach). Left, water perfused in oesophagus, nicotine SO_4 infused subcutaneously (group III). No ulcer. Centre, acid (HCl 0.12 N) perfused in oesophagus, saline infused subcutaneously (group I). Small duodenal erosion (arrow). Right, acid (HCl 0.12 N) perfused in oesophagus, nicotine SO_4 infused subcutaneously (group II). Extensive, deep and multiple duodenal ulcers. Note that the gastric mucosa is intact.

of 20 µg/kg/min of nicotine sulphate in saline also at the rate of 0.54 ml./h. Group III (twelve rats) received the same amount of nicotine sulphate subcutaneously as group II, but water instead of acid was perfused through the oesophagus. During treatment, the animals were conscious at all times and placed in a cylindrical cage that prevented them from bending and thus from chewing the tubing. Food and water were withheld throughout the experiment. All rats were killed after 24 h of treatment. The stomach and duodenum were dissected out as a single unit and opened along the mesenteric attachment for the duodenum and the greater curvature for the stomach. They were then examined with a ×2 binocular magnifier for the presence of ulcerations.

Table 1 shows that when acid alone was perfused (group I), duodenal ulcers were found in 31% of animals. When nicotine sulphate was also given (group II), the incidence reached 93%. The ulcers in the animals treated with acid and nicotine were also more numerous and more severe than those in animals receiving acid only. Finally, nicotine alone (group III) produced no ulcer. Fig. 1 shows duodenal ulcers produced by acid in the oesophagus plus nicotine, administered subcutaneously.

Table 1 Sensitization by Nicotine to Duodenal Ulcers produced by Acid Perfusion

Group	I Acid perfused * + saline s.c.	II Acid perfused + nicotine SO_4 † s.c.	III Water perfused + nicotine SO_4 s.c.
No. of animals	16	14	12
Duodenal ulcers			
Incidence %	31	93	0
Severity 0–3+	0.3	1.5	
Ulcers/duodenum	0.4	1.6	
Ulcer index ‡	3.8	12.4	0

Animals were killed after 24 h

* Acid: HCl 0.12 N perfused through the oesophagus

† Nicotine SO_4: 20 µg/kg/min infused subcutaneously.

‡ Ulcer index: sum of (*a*) % incidence (divided by 10), (*b*) severity in pulses and (*c*) numbers of ulcers per duodenum.

These results strengthen the hypothesis that nicotine sensitizes the duodenum to the ulcerogenic property of acid flowing from the stomach. Although, in the present study, the acid was from an exogenous source, the acid concentration and the perfusion rate were close to the amount of secretion formed by the rat stomach when maximally stimulated with histamine[8]. Thus, acid alone can initiate a duodenal ulcer provided either the concentration and rate of flow are high enough (as shown by previous studies[7]), or the formation of alkaline juice from the pancreas is reduced by an agent like nicotine.

The amount of nicotine in one cigarette is about 15–30 mg. Approximately 50% of cigarette smoke can be expected to be inhaled by a heavy smoker and it has been estimated that 90% of nicotine in inhaled smoke is absorbed into the circulation[9]. If man reacts to nicotine as the rat does, it seems likely that nicotine absorbed chronically through smoking may contribute to ulcer formation and to ulcer recurrences by reducing secretion of alkaline pancreatic juice via inhibition of secretin.

André Robert
David F. Stowe
James E. Nezamis

Department of Experimental Biology,
The Upjohn Company,
Kalamazoo, Michigan 49001

Received June 14; revised August 4, 1971.

[1] *Smoking and Health*, Report of the Advisory Committee to the Surgeon General of the Public Health Service. US Department of Health, Education and Welfare, Publication 1103, 337 (1964).

[2] Jacobson, E. D., Konturek, S. J., and Johnson, L. R., *Gastroenterology*, **60**, 780 (1971).

[3] Konturek, S. J., Solomon, T. E., McCreight, W. G., Johnson, L. R., and Jacobson, E. D., *Gastroenterology* (in the press).

[4] Robert, A., Stout, T. J., and Dale, J. E., *Gastroenterology*, **59**, 95 (1970).

[5] Robert, A., *Proc. Soc. Exp. Biol. Med.* (in the press).

[6] Robert, A., Nezamis, J. E., Dale, J. E., and Stowe, D. F., *Digestion* (in the press).

[7] Robert, A., Nezamis, J. E., and Stowe, D. F., *Gastroenterology*, **60**, 709 (1971).

[8] Thompson, J. H., and Lee, Y. W., *Amer. J. Digest. Dis.*, **12**, 449 (1967).

[9] Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics*, third ed., 583 (Macmillan, New York, 1965).

Orientation-specific Masking of Letter Features

THE ability to detect or resolve a target consisting of a briefly presented bar or grating is diminished by preceding or following it with a grating of similar orientation. This orientation-specific masking is maximal when target and mask have the same orientation and the impairing effect of the mask decreases monotonically with increases in the angular difference between target and mask, reaching half its peak value at a difference of about ±15° (refs. 1–4). These findings have generally been attributed to the properties of single cortical cells which seem to act as orientation analysers and which have been shown to possess similar angular bandwidth tuning[5-7]. Such selective masking may be used to compile pattern feature lists and to elucidate their organization in the recognition of complex patterns[8]. But we know of no studies of selective masking of multi-attribute patterns. Accordingly, we have explored the feasibility of selectively masking orientational features of letters to produce predictable confusions in a typical pattern recognition task.

The recognition stimuli were eight upper case English letters: —A, Z, —Y, N, —H, T, —O, X. A backward masking paradigm was used whereby the letters were presented singly in field 1 of a Gerbrandt three-channel tachistoscope followed after a delay by a masking grating in field 2. The letters were Letraset Futura Bold (96 point) transferred to white cards. At the viewing distance of 580 mm they subtended an angular height of 2° 31′. The component bar width of the letters subtended 40′. They appeared as high contrast black letters on a white background with a luminance of 48 mLamberts and were exposed for 2 ms.

The mask formed a 10° 55′ square surrounding the location where the letter had appeared. This square was filled with a black and white square-wave grating with an angular stripe width of 12′. Mask duration was fixed at 55 ms. In the two experimental conditions the orientation of this grating was either horizontal or vertical.

A delay of mask from stimulus offset was chosen for each subject which yielded approximately 60% recognition of the letters with the vertical mask, after some practice. These delay values ranged from 12–37 ms between subjects.

Eight subjects made forced choice recognition responses to the block randomized letters with each letter occurring six times in each condition. A preliminary analysis of the data in Table 2 reveals that the letters which were least well recognized were those with components of the same orientation as the masking grating. Consider the letters Z and N which have the same form subject to a 90° rotation. With a vertical mask Z is better recognized than N, whereas N is markedly better recognized than Z with a horizontal mask. The only anomalous letter is A which is not masked by the horizontal grating. This letter, however, is unique among those studied in that even if its horizontal component is removed the residual inverted V is sufficient for correct recognition. Furthermore, this oblique feature may fall within the masking angular bandwidth of a vertical grating, as we discuss later.

The crucial test of orientation specific masking is in the nature of the confusional errors. Considering first the interaction of horizontal letter components with the horizontal mask the overwhelming proportion of errors to T is Y, the only other letter with a single vertical feature. Similarly, H is most often confused with N, the best solution for the residual two verticals, and Z with X.

When H and T are masked by the vertical grating, however, both are most often confused with Z, a solution compatible with a residual horizontal component. Y with its vertical stem masked is most often confused with X, and N with X and Y. It seems, therefore, that the pattern of confusions changes according to the orientation of the mask, and these changes are consistent with the assumption that orientation-specific masking of letter components takes place with the response determined by the best fit to the residual unmasked components.

Table 1 Percentage of Errors in Letter Recognition as a Function of Mask Orientation

Subject	Vertical mask	Horizontal mask
	Error %	
MI	14.6	2.1
ST	37.5	10.4
PH	27.0	10.4
LE	47.9	18.8
JI	60.4	22.9
MA	41.7	29.2
JA	22.9	6.3
GA	41.7	4.2
$\bar{X}$	36.7	13.0

Errors are totalled over letters.

The vertical grating was a more effective mask for every subject (Table 1). For all but one subject the overall increase in errors due to the vertical mask exceeds 250%. Furthermore, for each target letter the error total across subjects is greater in the vertical mask condition, except for the letter O which was always correctly recognized (Table 2). Because orientation-specific masking holds over a range of angular similarities, and the oblique components of the letters fall nearer to the vertical, they might be more vulnerable to the vertical mask. But with the exception of letter A which has obliques 22° off vertical and which was peculiarly vulnerable to the vertical mask, the oblique components were 32°–38° from the vertical. At this separation masking can be expected to be negligible[1-4]. Furthermore, errors to the letter T increase 50% with the vertical mask although this letter contains only one vertical and one horizontal component. The greater efficacy of the vertical mask is therefore not explicable solely in terms of the particular orientation characteristics of the letters used. Letters are usually encountered in horizontal arrays, however, and it may be that there are orientational aspects of letter processing other than the orientation of components.

Table 2 Confusion Matrix of Errors to Letters Masked by Vertical and Horizontal Gratings

Mask orientation	Target letter	Error type and frequency	Error total	Error %
Vertical	A	11X · 3Z · 3H · 1N	18	37.5
	Z	3T · 2A · 2X · 2Y · 2O · 2N	13	27.0
	Y	5X · 3A · 2T · 2N · 2Z	14	29.2
	N	7X · 7Y · 4A · 3T · 2Z	23	47.9
	H	9Z · 7Y · 7N · 5T · 3A · 2X	33	68.8
	T	8Z · 6A · 6Y · 4N · 2H · 1X	27	56.3
	O		0	0
	X	5Z · 3Y · 2H · 2T · 1A	13	27.0
Horizontal	A		0	0
	Z	5X · 2Y · 2A · 1N · 1H	11	22.9
	Y	2X · 1Z · 1H	4	8.3
	N	2H · 1X · 1A	4	8.3
	H	10N · 1O	11	22.9
	T	16Y · 1H · 1O	18	37.5
	O		0	0
	X	3A	3	6.3

Erroneous responses are listed in descending order of frequencies for each target letter. Data are totals across eight subjects.

We used a grating frequency of 2.5 c/degree, but further manipulation revealed that masking decreased steeply with increases in spatial frequency over an investigated range of 1.25 to 20 c/degree. Two explanations for this suggest themselves. Apparent contrast varies inversely with spatial frequency[9] and it seems plausible that apparent contrast determines the efficacy of a masking grating. Second, as grating frequency decreased it approached the value of the bar width of the letters. Thus spatial frequency specific masking[10] could be expected to increase, providing another source of selective masking.

A further implication of these results is that the masks used to terminate the visual persistence of letter arrays may require critical attention. Such "noise" masks seldom have the distribution of spatial frequencies and orientations which is desirable for feature masking. The controversy between integration–degradation and interruption–erasure theories of masking may well depend on the choice of mask. The common choice of a mask in terms of its efficacy when superimposed on the target may maximize integrative masking but will be irrelevant to feature masking if the results of Campbell and Kulikowski[3] can be generalized.

LESLIE HENDERSON
STEPHEN H. COLES
MICHAEL MANHEIM
JAMES E. MUIRHEAD
PHILIP M. PSUTKA

Department of Psychology,
University of Guelph,
Guelph,
Ontario

Received April 19; revised July 1, 1971.

[1] Sekuler, R. W., *J. Exp. Psychol.*, **70**, 401 (1965).
[2] Houlihan, K., and Sekuler, R. W., *J. Exp. Psychol.*, **77**, 281 (1968).
[3] Campbell, F. W., and Kulikowski, J. J., *J. Physiol.*, **187**, 437 (1966).
[4] Gilinsky, A. S., and Doherty, R. S., *Science*, **164**, 454 (1969).
[5] Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **160**, 106 (1962).
[6] Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **28**, 229 (1965).
[7] Campbell, F. W., Cleland, B. G., Cooper, G. F., and Enroth-Kugell, Christina, *J. Physiol.*, **198**, 237 (1968).
[8] Weisstein, Naomi, *Psychol. Bull.*, **72**, 157 (1969).
[9] Campbell, F. W., and Maffei, L., *J. Physiol.*, **207**, 635 (1970).
[10] Pantle, A., and Sekuler, R. W., *Science*, **162**, 1146 (1968).

Organochlorine Pesticide Residues in Harbour Porpoises from the Bay of Fundy Region

ORGANOCHLORINE pesticide residues, together with polychlorinated biphenyl compounds of industrial origin, occur in many forms of life in the marine ecosystem. These compounds are concentrated through progressive trophic levels until relatively high levels accumulate in lipid-rich tissues of predatory seals, toothed whales and sea birds[1-3].

The highest levels in marine mammal tissues published so far were in the blubber of ringed seals, *Pusa hispida*, from the

Table 1 Organochlorine Residue Levels from Blubber and Liver of Porpoises (*Phocoena phocoena*) from New Brunswick and Nova Scotia (1969, 1970)

			Average residue levels in p.p.m. in extractable fat					
Sex status	Sample size	Tissue	Dieldrin	*p,p′*-DDE	*p,p′*-DDD	*o,p′*-DDT	*p,p′*-DDT	ΣDDT
Weaned and adult males	12	Blubber	9.24 (4.4 –13.1)	121.57 (45.7 –181.0)	48.16 (27.4 –100.0)	17.84 (9.6 –26.7)	119.18 (60.6 –175.0)	306.74 (150.8 –520.0)
	3	Liver	0.09 (0.01–0.15)	1.37 (0.92–1.61)	0.98 (0.86–1.20)	0.03 (0.01–0.04)	0.35 (0.18–0.47)	2.69 (1.97–3.32)
Male sucklings	2	Blubber	5.30 (0.6 –10.0)	54.55 (36.4 –72.7)	18.50 (12.0 –25.0)	7.60 (1.2 –14.0)	50.25 (25.5 –75.0)	130.90 (75.1 –186.7)
Female suckling	1	Blubber	5.00	72.90	30.00	5.00	46.90	154.80
Immature and resting females	15	Blubber	7.75 (3.0 –12.5)	76.94 (36.9 –150.0)	39.18 (18.3 –80.0)	13.10 (5.3 –24.0)	85.04 (40.0 –200.0)	214.27 (111.6 –447.9)
	8	Liver	0.06 (0.02–0.13)	0.50 (0.25–1.28)	0.46 (0.20–0.93)	0.02 (0.01–0.08)	0.37 (0.10–1.28)	1.36 (0.61–3.57)
Pregnant and/or lactating females	6	Blubber	1.37 (0.1 –2.4)	25.50 (15.0 –40.0)	13.47 (7.3 –24.0)	2.41 (0.1 –8.0)	27.75 (14.1 –50.0)	69.03 (40.0 –122.0)
	2	Liver	0.02	0.26 (0.25–0.27)	0.14 (0.12–0.16)	0.01	0.07 (0.06–0.08)	0.48 (0.46–0.50)

Gulf of Bothnia (ΣDDT range 11–130 p.p.m., mean 120 p.p.m.), and in the blubber of grey seals from the Baltic Sea (ΣDDT range 97–310 p.p.m., mean 170 p.p.m.)[2]. One porpoise, *Phocoena phocoena*, from the east coast of Scotland contained a total residue concentration (dieldrin + DDT compounds) of 73.3 p.p.m.[4].

Sixty harbour porpoises, *Phocoena phocoena*, were collected by D. E. G. in western North Atlantic waters in 1969–70. Blubber samples from thirty and liver samples from twelve animals were chilled to sea water temperature (about 10° C) and frozen to −20° C as soon as field conditions permitted (2–7 h). A two column fractionation separated organochlorine insecticides from the polychlorinated biphenyls (PCBs). The first step consisted of a 'Florisil' column for the separation of fatty coextractives from organochlorine insecticides[5]. Up to 10 μg PCBs or 1 μg of DDT, DDE, or DDD were handled by the column to give a 90 to 100% separation. For best separation, two elutions with quantities most favourable to each group of compounds were made. Eluates were concentrated almost to dryness and redissolved in hexane for gas chromatography. Qualitative residue confirmation was achieved principally with thin-layer chromatography. Percentage recoveries were checked periodically by fortification directly into the oil obtained from hexane extraction. Average recoveries were: *p,p′*-DDE, 98%; *o,p′*-DDT, 91%; *p,p′*-DDT, 90%; *p,p′*-DDD, 95%; dieldrin, 89%. Data in Table 1 are not corrected for percentage recovery. Further study of the PCB residues is in progress. Their occurrence was, however, quite high, ranging from one to three times the levels recorded for *p,p′*-DDE.

Average dieldrin, ΣDDT levels in pregnant and lactating females were much less (1.37 p.p.m. of dieldrin, 69.03 p.p.m. ΣDDT) than in immature and resting females' blubber (7.75 p.p.m. dieldrin, 214.27 p.p.m. ΣDDT). Three sucklings had high levels (range 75.1–186.7; mean 138.8 p.p.m. ΣDDT). Residues may move into the depot fat in the foetus, or to the neonate through milk, or both.

The average ΣDDT level in the blubber of males (306.74 p.p.m., excluding sucklings) seems to be the highest published for a wild population of any mammal. The small sample size precludes speculation on residue variation at this time with respect to age, season and distribution.

These results are ominous enough to merit further immediate attention to the status of contaminants in the higher trophic levels of the Bay of Fundy food web.

Field studies were supported by a grant from the National Research Council of Canada. Other assistance was given by the Canadian Tuna Company of St Andrews and Marine Research Associates of Lord's Cove, Deer Island, New Brunswick.

D. E. GASKIN

Department of Zoology,
College of Biological Sciences,
University of Guelph,
Guelph, Ontario

M. HOLDRINET
R. FRANK

Pesticide Residue Testing Laboratory,
Ontario Department of Agriculture,
Guelph, Ontario

Received June 18; revised July 20, 1971.

[1] Robinson, J., Richardson, A., Crabtree, A. N., Coulson, J. C., and Potts, G. R., *Nature*, **214**, 1307 (1967).
[2] Jensen, S. A., Johnels, A. G., Olsson, M., and Otterlind, G., *Nature*, **224**, 247 (1969).
[3] Risebrough, R. W., Reiche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N., *Nature*, **220**, 1098 (1968).
[4] Holden, A. V., and Marsden, K., *Nature*, **216**, 1274 (1967).
[5] Langlois, B. E., Stemp, A. R., and Liska, B. J., *J. Milk Food Tech.*, **27**, 202 (1964).

Earliest Indian Human Remains found in a Late Stone Age Site

THE discovery of human remains from a Late Stone Age site in the village of Sarai Nahar Rai in Uttar Pradesh has provided the earliest available skeletal evidence of man in the Indian subcontinent. This northern Indian site, which is in the district of Pratapgarh, lies about 38 km north of Allahabad city and 33 km south-west of Pratapgarh town, connected by road. It covers about 2,700 m^2 (about lat. 25° 48′ north and long. 81° 50′ east) and is about 9 km west of Bishnathganj Railway Station on the Allahabad–Fyzabad line of the Northern Rail-

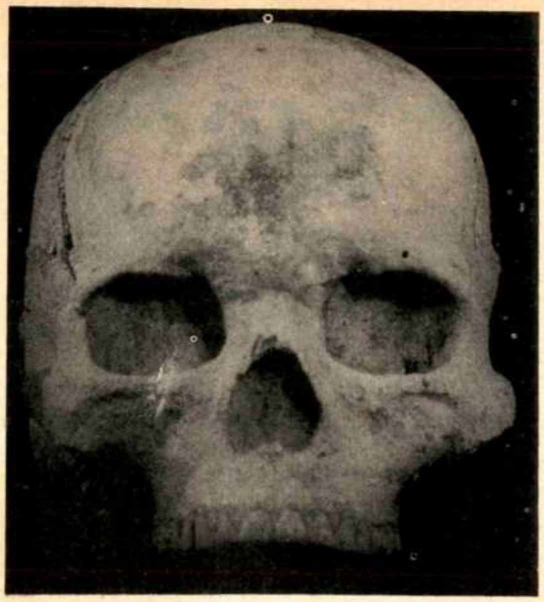


Fig. 1 Norma frontalis of skull of skeleton No. SRN-4 (photograph by courtesy of the Anthropological Survey of India).

way. It was discovered in 1968. The eastern sector of the site—about 600 m^2—had been badly eroded and there was visible evidence of a cluster of human graves, the only such example to have been reported from the plain of the Ganga.

A considerable number of microliths and waste flakes made principally of quartz were collected from the surface of the site, where there was a regular microlithic industry. The finished tools, which mostly had steep retouches, included blades, points, piercers, burins, lunates, triangles, trapezes, arrowheads and borers. Heavy tools were conspicuously absent. Chips of charred animal bones were found scattered all over the surface. Some irregular and circular markings on the eroded surface of the site were noticed. These finds represent the debris of a spot where a small group of people had camped.

In March 1970 I conducted an excavation on behalf of the Anthropological Survey of India at the request of the Archaeological Department of Uttar Pradesh, assisted by Mr Anadi Pal. Digging yielded an articulated, almost complete and undamaged skeleton (No. SRN-4) buried in a grave; only the mandible and right humerus were missing. Another eight, badly damaged skeletons were lying nearby, exposed because of heavy erosion of the top soil. The right clavicle and some fragmentary bones of one of these skeletons (No. SRN-5) were removed for examination, and other fragments were sent for radiocarbon analysis to the Tata Institute of Fundamental Researches, Bombay. Some skeletons show evidence of the early stages of petrification.

All skeletons were buried within the deposit of layer 1, and No. SRN-4 was 14 cm below the present surface. This layer is composed of compact and hard silt of a darkish colour. When I revisited the site in May 1971, accompanied by Dr D. K. Sen, director of the Anthropological Survey of India, and Mr P. Gupta, I could locate the skeletal-bearing layer 1 against an exposed section of a nearby small tributary of the Sai River flowing across the district a few kilometres to the north of the site. The layer is about 32 cm thick, resting on an under layer of about 26 cm made up of silt mixed with kankar. Immediately below it is a layer of sandy silt of about 17 cm thick. The nature of the deposit indicates minor climatic oscillations, but generally a dry phase in the post-Pleistocene period.

Clay pots prepared by coiling and baking were found embedded, possibly in some pattern, in most of the graves. The upper part of most of the pots was, however, eroded away, leaving a circular cross-section of pots projecting from the surface.

There is evidence that the dead were regularly disposed of in a supine posture, usually in a normal extended position with the head to the west (some, however, between west and south but usually within a few degrees of west).

Skeleton No. SRN-4 belonged to a male who was older than 30 when he died. The skull (Fig. 1) is virtually complete and well preserved, and lacks only the mandible. The head is sub-rounded with the keel of the vault somewhat flattened. Seen from the top, the contour is "birsoides". In profile, the forehead is steep, and the superciliary ridges are mesially developed. The face is broad and short with moderately compressed orbits, while the nose is flattish-broad with a marked depression at the base. The mastoids are strong. The cranium has a complete upper dentition which is highly attrited. The wear of the upper incisors clearly indicates that the owner of the skull had the habit of biting in an "edge to edge" manner. The cranial index is 76.04 (mesocranic), nasal index 53.61 (chamaerrhine) and orbital index (left) 74.70 (chamaekonchic).

The radiocarbon analysis of a bone sample (index No. TF-1104; depth 5 cm; layer 1) has provided an absolute date of 10,050 ± 110 (10,345 ± 110) BP (the first date is based on half life of radiocarbon of 5,568 ± 30, and the date parentheses are based on the value of 5,730 ± 40 for the half life of radiocarbon)[1].

These remains clearly predate by about 5,800 yr the previous earliest record of man, from the Late Stone Age site at Lekhania in Uttar Pradesh and dated by radiocarbon.

A detailed report on these finds will be published later.

PRATAP C. DUTTA

Anthropological Survey of India,
Government of India,
Indian Museum,
Calcutta-13

Received June 1; revised July 21, 1971.

[1] Agarwal, D. P., *C^{14} Date List—March 1971*, Tata Institute of Fundamental Researches, Bombay (1971).

More about Dowsing

THE article on "Dowsing Experiments" by Foulkes[1] describes some work with which I was concerned and reflects the considerable effort involved in the planning and subsequent analysis of the trial. It does not, however, bring out some of the things which went wrong, which both modify and add to Foulkes's account and which illustrate problems which future experimenters would presumably wish to avoid. My part in this work was the carrying out of the statistical analysis and data presentation which Foulkes reports and it is with these aspects that I am concerned. My remarks are made after reference back to notes I made during the analysis period.

First, the pattern of objects illustrated in Fig. 1 was, indeed, that plan which was originally produced and the objects were nominally laid out in that manner. During some trials with a conventional mine detector, however, subsequent to the dowsing experiment, it was found that row E had not been laid out exactly as planned. Specifically, the objects planned for E6 to E15 were misplaced and actually buried in E5 to E14 and the remaining "blank", planned for E5, was in fact "buried" in E15. The analysis, which was by this time complete, had to be redone and the Tables given in the later parts of the paper are correct. How this error was not discovered by at least one of the gangs of men who were responsible for burying another class of object is still a mystery.

The results of the two dowsers, experimenters six and seven, were not in Figs. 5 and 6 of Foulkes's article. The results of these experimenters were very interesting. They wandered off unsupervised (that is, without an accompanying "caddy" to record their responses). Their results were very similar for both types of ground. The results of experimenter six on the natural ground are given in Table 1.

Table 1 Dowsing Results of Experimenter Six on Natural Ground

	Object buried					
	B	M	C	W	P	Totals
Response						
M	1	10	2	3	6	22
P	2	11	5	6	7	31
O	1	19	0	1	27	48
(Blank)	36	0	33	30	0	99
Totals	40	40	40	40	40	200

B, Blank; M, metal mine; C, concrete block; W, wooden block; P, plastic mine.

Table 1 has a similar form to Table 2 in the article. On the original score card the dowser was instructed to record M for metallic mine, P for plastic mine and O for nothing present. When questioned about the response O and the blanks left on the score sheet, experimenter seven said that O had been used to mean that an object was present but its identity was unknown and that a blank had been left when a square had been dowsed and nothing found. Table 1 shows clearly a remarkable degree of association between the blank response and the presence of B, C or W and between the O response and the presence of M or P. Since the statement about the meaning of O was made "*a posteriori*" it does not necessarily indicate an ability to dowse and as the two dowsers traversed the course together their results are not necessarily independent.

The experiment was therefore repeated by experimenter seven and his results are, in fact, those attributed in the article to experimenter eleven. There was no significant association on this second attempt without experimenter six, who was not retested. This dowser was the soldier illustrated in Fig. 3 of the article, a Nigerian, who unfortunately could not be retested. These results, although inconclusive because of the lack of experimental control, do suggest that something peculiar occurred on this occasion which might be worth further investigation. Two different conclusions, all too common in this type of work, can be drawn. Either some form of ability was demonstrated (although peculiarly recorded and interpreted) or a breach of security of the trial (including the possibility of cheating) took place. It can be argued that it would be possible for an unsupervised student to have prodded the ground (for example with one of the dowsing rods) and at least detected an object present, since for control purposes small wooden pegs indicated the position in which an object, if present, would have been buried. Although this form of cheating may well have taken place on this occasion it does not explain how mines and other objects might have been differentiated. Indeed, since the basic concern was with methods of detecting underground mines, a proven ability to detect the difference between a mine and an object (for example a stone), even by prodding, could have been of considerable importance.

There is also slight evidence that a positive response was more closely associated with the presence of a wooden block than with a concrete block—especially on the raked ground. Although this may be a spurious result, the wooden block may have absorbed more moisture than the concrete block, thus altering the drainage pattern and hence the surface appearance of the ground.

DAVID G. SMITH

Ministry of Defence,
Defence Operational Analysis Establishment,
Parvis Road,
West Byfleet,
Surrey

Received May 7, 1971.

---

[1] Foulkes, R. A., *Nature*, **229**, 15 (1971).

Dowsing Experiments Criticized

My photograph appeared in Foulkes's article, Dowsing Experiments[1], without prior reference to me, and I would like to make the following comments on the article.

The experiments carried out at the Military Engineering Experimental Establishment (MEXE) did not involve true "dowsing" as they were not water divining. Although experienced dowsers can locate buried objects such as cables, pipes and the like, these experiments were a search for objects unfamiliar to those taking part. Those I spoke to made no claim to achieve success, but agreed to the trial as an interesting experiment that might be of practical use. It was also evident to all that, to be of practical use, map dowsing must be used when walking over a live mine field. I agree that this experiment was a failure although one man was successful far beyond the bounds of chance.

The "flow in a pipe" experiment is not one which I would attempt, although I could probably locate the pipe either full or empty. No mention is made of the dowser's experience in this particular kind of search. I consider this test valueless, if he had no such experience.

I would like to emphasize that I was not instructing anyone in dowsing during the exercise at Chatham. The object was to find a number of men who could usefully develop into proficient water diviners. I do not think it possible to instruct anyone. Of those involved, 10%, not 25%, found accurately my second flow line; their judgment coinciding with mine and confirming my classification of them as sensitives.

I still maintain that the well referred to was correctly sited on one of the flows in the upper chalk. I predicted that the water would be near the bottom of the upper chalk and detectable by drilling through the clay to the chalk. This has not been done, as the drilling rig was removed before it had passed through the clay stratum. This was, therefore, not a dowsing failure.

The test over the 42 inch pipe seems to have been made by some of the 10% of "sensitives", who had no real dowsing experience. Even over natural fissure flows I would not have expected these novices to assess quantity.

Foulkes's description of the V rod movement was inaccurate. If the rod is held firmly in the normal way, another person can press the tip up or down, and, when released, the rod will return to its original position without any additional effort by the holder.

The Rocard experiment proves nothing. There are many dowsers who cannot dowse in rubber soled shoes. If Rocard, thinking he had used magnetized iron in his elbow joints, actually used non-magnetic iron, the experiment could have had the same result. It is a pity that Foulkes does not seem to have consulted any other authority than Rocard.

K. W. MERRYLEES

Blaize House,
Lavenham,
Suffolk

Received May 7, 1971.

---

[1] Foulkes, R. A., *Nature*, **229**, 15 (1971).

BOOK REVIEWS

Social Biology

Social Implications of Biological Education. Edited by Arnold B. Grobman. Pp. 134. (Darwin: Princeton, New Jersey, April 1971.) $5.95.

THIS book was planned by a Committee of the American National Association of Biology Teachers for those who would welcome thoughtful reviews of pressing social problems associated with advances in the biological sciences. It is the report of a national conference of NABT held in 1969, in which papers prepared by distinguished biologists, who have exhibited a concern for significant social problems, are followed by the prepared statements of three panelists, which support, contradict, extend or restrict the major papers. The three members of each panel include at least one additional specialist in the field of the major speaker and one practising school teacher. The statements of the panels are followed by accounts of the question and answer sessions.

Four major topics, medicine, behaviour, genetics and population, are dealt with in this manner, and, owing to Dr Grobman's skilful editing, this technique has produced a lively and readable volume. Such questions as "Should the government support and control scientific research?", "Should euthanasia be practised?", "Is free-will an obstacle to technological progress?", "Are differences of intelligence a matter of race?", "Is birth control a solution to the population problem?" and "Should man control his genetic resources?" are ably discussed from differing viewpoints.

The section on medicine is introduced by a paper on "The Social Implications of Medicine" by Michael and Lois Debakey of the Baylor College of Medicine, Houston, Texas, who discuss the growing discrepancy between the achievements of research and technology and their application to human life. The importance of the science teacher's role in conveying not only basic concepts, the philosophy and methods of science, but also the social implications of medical science, so that each student may recognize his responsibility in helping to shape public policy in this field, is clearly brought out.

James V. McConnell of the University of Michigan introduces the topic of behaviour in a controversial paper on "Ethics and Behaviour", in which he discusses life from a behaviourist's standpoint, and a paper by Bruce Wallace of Cornell University entitled "Genetics and Genetic Manipulation" opens the section on genetics. This latter gives an interesting treatment of biological races, and comments on recent work on "racial intelligence", as well as considering eugenics and genetic engineering. This is followed by a stimulating paper entitled "Can Teachers Tell the Truth about Population?" by Garrett Hardin of the University of California.

The last chapter departs from the pattern adopted for the rest of the book. It is based on the banquet address by Claude Welch on "Evolution—the Reluctant Revolution", in which the nature of conceptual revolutions is discussed.

This volume is not intended to be a complete compendium of the social implications of biological education. It is selective and omits some very important problems. For instance, there is no section devoted to ecology, and the whole vital area of the control of pollution and the redressing of the environmental balance is only hinted at. The reason for this omission might well have been that one of the few social problems, which American biology teachers of a generation ago thought appropriate for their consideration, was conservation. In this area, NABT played an active part and, under the leadership of the late Dr Richard Weaver, the Association produced a *Conservation Handbook,* which has been highly regarded.

Furthermore, the treatment of those topics, which are dealt with in this volume, does not set out to present all possible points of view. The aim is rather to present a number of thought-provoking and related statements on current problems for teachers to consider. Where the chosen topics have implications, which fall into the two broad areas of social–political and moral–ethical, it is in the former area rather than in the latter that problems are explored.

This should be a particularly useful book for teachers of the life sciences, who wish to discuss with their students those "problems generated at the interface between science and society". As science becomes broadly integrated into all phases of our culture, its significance as a part of a general education becomes more important and the aims of science teaching will need to go beyond the restrictive context of the special disciplines and consider science in relation to mankind. It is clear from the discussions reported in this volume that it will meet a need for science teachers who are now expected to take on an entirely different kind of responsibility from that for which they have received preparation. ELIZABETH PERROTT

Parasitic Insects

Parasitic Insects. By R. R. Askew. Pp. xvii+316. (Heinemann: London, June 1971.) £3.50.

THIS book is divided into two sections; the first deals with insects having parasitic adults and the second with protelean parasitic insects. The author adopts a very broad view of parasitism and a wide range of diverse associations are described under this umbrella definition. The first section is introduced by a description of the mouth parts of adult parasitic insects, which possibly would have been more easily comprehensible had it been treated from the point of view of adaptive radiation and with a little more emphasis on function and the physiology of digestion of imbibed host tissue. Some treatment on the ecology of the host body environment and the alterations (for example, the histopathological changes) inflicted on it by the parasite would have been useful and may even give a lead to explain the absence of anticoagulants in the saliva of some of these species. For a parasite does establish a physiological association with the tissues on the surface of, or inside, the body of its host, even though the association is primarily to provide food for the parasite. This omission is not altogether the fault of the author, for much remains to be done in this field.

Chapters 2–5 deal with lice, fleas, blood sucking flies and Diptera pupipara and chapter 6 with bugs, earwigs, beetles and moths as a "convenient" grouping of other insects parasitic as adults. I believe that the bugs should have been dealt with in a chapter on their own. The approach to these organisms is from the ecological standpoint and there is undoubtedly a wealth of information here, with substantial reference to up to date literature. The final chapter in this section deals with blood sucking insects as vectors of human disease, but more could have been done on this aspect of the subject —for example, some reference to the factors influencing the movement of trypanosomes in the insect gut and the passage either to the proboscis or to the hindgut preparatory to transmission. The insect body is, after all, an environment for these pathogens and they must show physiological adaptability to it. In spite of these apparent criticisms there is no doubt that the author has drawn together much information of considerable value for undergraduate students and as introductory reading for postgraduates specializing in ectoparasites. In the introduction (page X) the author states that the term parasitoid will not be used in the book, but he proceeds to do so on page 111! The author is obviously very much more at home when writing on parasitic Hymenoptera (chapter 8) and presents an excellent review of their structure, function and ecology extending over seventy-three pages (compared with ninety-four pages for all insects with parasitic adults). Thirteen families of protelean parasitic Diptera are likewise covered in some detail and these chapters are eminently suitable for postgraduate reading. The characteristics of biological control of insect pests form the substance of chapter 10. Fly larvae which parasitize vertebrates (chapter 14) might appropriately have been included in the first section.

This book fulfils a real need for a variety of students, it is well composed, is illustrated with clear line drawings of the highest quality, and the whole is completed by a substantial list of relevant references.

DON. R. ARTHUR

Life on Earth

The Nature of Life: Earth, Plants, Animals, Man and Their Effect on Each Other. By Lorus Milne and Margery Milne. Pp. 320. (Cassell: London, August 1971.) £5.50.

Lorus and Margery Milne have produced a beautifully illustrated book about the life on this planet. It is not a technical book, but the text is informative and full of interesting miscellaneous facts, for example, that the shell of the foraminiferan *Globorotalia* coils clockwise when the climate is warm and anticlockwise when it is colder and that this has enabled geologists to obtain a measure of the temperature of the sea at different times.

The book covers all the continents of the world as well as the oceans, describing the geology, climate and forms of life to be found there. It would have been greatly improved by the use of good maps of the places being described. As it is, the almost complete absence of any maps means that one is constantly coming up against the names of faraway places, the locations of which are only dimly remembered from school geography.

The final chapter is about the damage which the human species has done and is continuing to do to its environment. Although the message is now a commonplace one, it is difficult not to be impressed all over again by the fact that one species of mammal and one species of bird become extinct, on the average, every eight months, often as the direct result of human activity.

The Milneses should succeed in inducing in the reader the desire to visit and explore as they have done and to see for himself the beautiful and varied faunas they describe.

MARIAN DAWKINS

A Young Science

An Introduction to Psychopharmacology. By Richard H. Rech and Kenneth E. Moore. Pp. xii+353. (Raven: New York, 1971. Distributed in the Eastern Hemisphere by North Holland, Amsterdam.) £4.65.

Psychopharmacology and the Individual Patient. Edited by J. R. Wittenborn, Solomon Goldberg and Phillip R. A. May. Pp. 256. (Raven: New York, May 1971. Distributed in the Eastern Hemisphere by Pitman Medical and Scientific, London.) £5.50.

PSYCHOPHARMACOLOGY is somewhat isolated from disciplines such as neuropharmacology, psychophysiology and neurochemistry and is not yet integrated with its most relevant basic sciences, pharmacology and experimental psychology. Only psychiatry has developed a common area with psychopharmacology, that of drug therapy in mental illness. The reasons for this state of affairs which continues nearly twenty years after the discovery of the first of the modern psychotropic drugs, chlorpromazine, lie in the recent historical development of the subject. Most, if not all, of the important new psychotropic drugs were discovered by serendipitous accident. Thus, chlorpromazine and imipramine were first noted to have potentially useful actions in patients. Psychiatrists using these drugs on the wards and in their out-patient clinics quickly realized that real therapeutic advances were being made with essentially new drug types. Pharmacologists and psychologists then sought actions of these drugs both in animals and in normal humans which would provide some rationale for their therapeutic effects.

In spite of all the research effort of the past two decades, the mode of action of most psychotropic drugs remains obscure or, more strictly speaking, the clinical relevance of the pharmacological actions which have been found remains unproven. For example, for some time after the introduction of the tricyclic anti-depressants, their mode of action was unexplained. Eventually it was found that they inhibited the reuptake of noradrenaline from the synaptic cleft. The other class of anti-depressants, the monoamine oxidase inhibitors, had from the start been assumed to owe their clinical action to that implied in their generic name. However, it now seems possible that they, too, inhibit noradrenaline uptake and that their enzyme-inhibiting properties may be less relevant.

For these and many other reasons, psychopharmacology has remained a loose federation of disciplines rather than a coherent subject, and at the clinical level it is probably the most empirical branch of therapeutics. These two multi-author American books represent two different approaches to the problem of maturing psychopharmacology from the empirical to the rational. *An Introduction to Psychopharmacology* aims at providing for the neophyte background reading in cognate subjects. Two rather breathless chapters attempt the impossible task of outlining pharmacology, psychology, neuroanatomy, and neurophysiology in eighty pages: there are many basic texts more suitable for this purpose. The remaining chapters are much more successful, covering biochemical aspects, the limbic system, electrophysiology, conditioning and animal testing of new drugs, with two clinical chapters on tranquillizers and anti-depressants. Except for the chapter on anti-depressants which is in the "in my experience" style, the scientific standard is high and the material presented is critically appraised. Useful key references and reviews are included. The chapter by K. E. Moore on "Biochemical correlates of behavioral effects" is very balanced and avoids the overstated claims of those who believe that biochemical mechanisms in adult mental illnesses are already apparent.

Psychoparmacology and the Individual Patient is disappointing and will be of interest to very few people. It comprises the proceedings of a meeting of the Prediction Study Group of the American College of Neuropsychopharmacology held in December 1968. The object of the research projects described in this book is to predict which patient will respond to which drug. A typical study follows this recipe: take about 500 patients, measure them on about seventy-five variables, clinical and demographic, treat them with one or more drugs and assess the outcome. Use complicated statistics to see which variables influence the outcome. The results are not only indigestible but only about twenty per cent of the outcome variance can be predicted, rendering the exercise unproductive. One reason for this failure may lie in the neglect by the investigators of pharmacological factors. Only in the excellent introductory chapter by S. Gershon is attention drawn to the marked variation between patients in plasma levels of many psychotropic drugs. The reluctance of many investigators to use pharmacological techniques in clinical studies will perpetuate the isolation of psychopharmacology which will be unfortunate as such techniques promise to rationalize our clinical use of psychotropic drugs.

MALCOLM LADER

Clinical Enzymology

Mattenheimer's Clinical Enzymology: Principles and Applications. By Hermann Mattenheimer. Pp. xiv+168. (Ann Arbor Science: Ann Arbor, May 1971.) $14.75.

Enzyme Synthesis and Degradation in Mammalian Systems. Edited by M. Rechcigl. Pp. xvi+477. (Karger: Basle, Paris and London. Distributed in the USA and Canada by University Park: Baltimore, 1971.) 90 Sw. francs; £9.50; 90 DM.

MEASUREMENTS of the activity of enzymes in blood serum constitute one of the most important ingredients in the explosive growth of clinical biochemistry. Beginning as a series of random observations of changes in the levels of a few serum enzymes in a limited number of diseases, diagnostic enzymology now offers systematic data of wide clinical application, based on rational hypotheses about factors which influence the levels of enzymes in the blood: this is particularly so with regard to the leakage of intracellular enzymes which accompanies acute tissue damage. However, efforts to assess the nature of intracellular events by direct measurement of enzyme activities in tissue samples have made slower progress, mainly for methodological reasons, except in the demonstration of inherited conditions which affect the metabolism of red blood cells and in certain other metabolic abnormalities. The growing importance of clinical enzymology has been accompanied by the appearance of textbooks which aim to codify and to rationalize the mass of data which appears yearly in the medical literature, and to guide clinicians and clinical biochemists in the scientific basis, selection and interpretation of enzyme tests. Mattenheimer's book fulfils this function, and in addition offers some differences in content and emphasis from other books in this field. Thus, only one chapter of twenty-five pages is devoted to interpretation of enzyme tests on blood plasma, with a further eight pages on enzyme tests on urine. The other eight chapters are devoted to brief descriptions of principles of enzymology, metabolic pathways, enzyme–hormone interactions, protein synthesis, inborn errors of metabolism, and the enzymology of cells and organs (two chapters). The last two topics, with their implications for future developments, form perhaps the most interesting and original part of the book. The subject of the last chapter, methods of estimation of enzyme activity, could have been left to specialized laboratory handbooks.

This wide range of topics inevitably results in superficial treatment of some; for example, protein synthesis and biochemical genetics, and intermediary metabolism, but the value of the book lies mainly in its correct emphasis on the unity of clinical and non-clinical enzymology. There are a few errors and omissions: alkaline phosphatase activity is probably not decreased in the intestine in hypophosphatasia (page 99); the statement that bone metastases of cancers other than prostatic carcinoma are not accompanied by elevated plasma acid phosphatase (page 128) is incorrect, and in the discussion of inborn errors of metabolism, some reference to lysosomal enzyme deficiencies in lipid storage diseases and the role of amniocentesis in prenatal diagnosis would have added an up-to-date touch. These minor criticisms should not, however, deter those concerned with clinical enzymology, especially clinicians, from reading the book.

Interpretation of enzyme activities in organs or blood is related fundamentally to a knowledge of rates of enzyme turnover and of factors which influence them. *Enzyme Synthesis and Degradation in Mammalian Systems* is a valuable collection of twelve reviews of research on these topics, particularly by reason of its mammalian, rather than microbiological, emphasis. The chapters vary in length from fifteen to sixty pages, but all are well written and are supported by extensive lists of references: these include a number dated 1969, so that the picture is essentially contemporary. The relative value of individual articles will be assessed differently by different readers, depending on their own familiarity with the topics discussed. However, the long chapter by Rechcigl on enzyme turnover is particularly comprehensive, and brings out clearly the prevailing state of mystery with regard to mechanisms of enzyme breakdown. The apparently highly selective nature of the processes involved in enzyme turnover is well exemplified by the case of lactate dehydrogenase isoenzymes (reviewed here by Vesell and Fritz), in which rates of synthesis and degradation in two catalytically and structurally similar proteins are found to differ widely within a single type of cell. Gelehrter argues cogently against the uncritical translation to mammalian systems of data on the induction of enzymes in micro-organisms, while chapters on nutritional (Freedland) and hormonal (Rosen and Mulholland) influences on enzyme activity, and its rhythmic variation (Fuller), also emphasize the complexity of enzyme control in the whole animal.

These chapters, and the others in this collection, constitute a stimulating volume but, in view of the problems which await solution, one which also cautions against over-optimism. Perhaps, however, only a few years will elapse before reviews of this nature will need to warn against too ready an extrapolation of mechanisms established for rat liver to processes of human health and disease.

D. W. MOSS

Nuffield Texts

Nuffield Science Teaching Project: Advanced Science (Chemistry). *Special Studies* (ed. J. G. Raitt): Food Science. Pp. 150. 90p. Biochemistry. Pp. 150. 90p. *Programmed Text*: *Ethanol and other Alcohols.* By Erica Glynn. Pp. 137. 60p. *Standard 8 Film Loops*: *Born–Haber Cycle; Hydrolysis of Bromoalkanes; Rates of Reactions.* £6.30 each. Penguin Education, Penguin Books Ltd; Harmondsworth, Middlesex.

THIS latest batch of Nuffield A-level chemistry materials maintains the generally high standard of its predecessors. *Food Science* is clear, direct, and stimulating; I learnt a great deal from it. The social aspects of the study are never kept in the background, and a major aim of the book is stated on page 3: ". . . The broad outline of the subject may help (readers) to consider how an understanding of the chemistry, physics and biology of foods can contribute towards the improvement of the health and well-being of mankind, and towards solving the problems of the shortage of food in the world". Nonetheless, the chapter on the world food problem fails to go far enough in posing the intense problems which must be faced within this century. The book is particularly good on technology, and there is an excellent outline of the processes occurring in foods during treatment and storage. The authors fire a salvo at macrobiotic food fanatics (page 95) in the course of an interesting chapter on food legislation and public health. A-level readers may find some of the chemistry tough going (for example, the browning reaction on page 56); but the book concludes with a good practical section.

Biochemistry seems to be written in a less easy style, although it gets much better as it goes on. It starts badly with some facile comments on DNA (page 1) which include a disturbingly naïve paragraph on genetic engineering, and a silly question about the Golgi apparatus (page 9). Assumptions are made about the "vocabulary" of A-level readers, particularly in the section on metabolism; here, Lehninger's diagrams are not sufficiently clear to give confidence to those treading these complex pathways for the first time. Able pupils may wonder why (as in *Food Science*) monosaccharide hexoses are always represented by Haworth structures rather than in the chair conformation, and why the stereochemistry of, for example, cholesterol and gibberellic acid is not shown. Fig. 49 has a mislabelled axis; the caption for muscarine is mysteriously transposed from page 51 to an innocent amylopectin chain on page 53.

The book has several excellent sections, especially on medical aspects. The agricultural section is fine, but the dangers of pesticide abuse are not mentioned. The chapter on biochemistry and commerce is compelling, particularly the section on sewage disposal. (I was intrigued to see a photograph of our local rural district council's activated sludge plant.) The experimental investigations look good.

Ethanol and other Alcohols is a straightforward, highly competent programmed text containing a large proportion of practical work.

The film loops are much better than their O-level counterparts. They limit themselves to one topic and work at it thoroughly. The accompanying notes are very lucid. *Rates of Reactions* provides a convincing demo using the Dainton/Fisher ping-pong ball machine. *Hydrolysis of Bromoalkanes* makes good use of models, and the diagrams in the *Born–Haber Cycle* are bright and clear. My only criticism is the cost of these films. MARTYN BERRY

X-Ray Diffraction

Interpretation of X-ray Powder Diffraction Patterns. By H. Lipson and H. Steeple. Pp. viii+335+3 plates. (Macmillan: London; St Martins Press: New York, May 1970.) £4.

THE textbook *Interpretation of X-ray Diffraction Photographs,* by Henry, Lipson and Wooster, itself the distillation of much teaching experience, has been a standard reading and reference work for many generations of undergraduates and of postgraduates learning to use crystallographic techniques. But since it was first published the subject has developed enormously and new methods, both experimental and interpretative, have become both available and standard.

To have revised the original book would have been to produce a textbook both too unwieldy and too expensive; and the decision was therefore made to rewrite the book in separate parts, making each complete in itself. For the practical study of many important solid materials that do not form single crystals of adequate size for individual examination, or that can only be obtained as a mass of randomly oriented crystallites less than, say, 10 μm in individual size, powder diffraction methods are essential. Moreover, X-ray photographic methods have been supplemented or, for some purposes, replaced by counter-diffractometer techniques including those of neutron diffraction. To the metallurgist and the solid state physicist, the geologist and those concerned with phase diagrams of all kinds, or simply with the identification of unknown materials, the powder diffraction pattern is invaluable. The technique is, in general, non-destructive and requires less than 1 mg of material; or it can be used with massive specimens *in situ.* It is therefore of the utmost practical importance. The preface implies that this is an undergraduate textbook: but it is much more than that.

Nevertheless, it is aimed in the first place at undergraduates and it begins therefore at the beginning, with crystal lattices and crystal symmetry, with the geometry of X-ray reflexion and the nature and production of X-rays. Some of the diagrams in this section are less than satisfactory. Fig. 3.2 would give a student a very weird idea of the spectrum from a copper target run at 35KV, and fig. 3.6 that of both the intensity of radiation and absorption of very short wavelength X-rays. These are followed by methods of recording and measuring powder patterns and of interpretation, with descriptions of standard and special-purpose equipment. It is a pity that although measurement of coefficients of thermal expansion is mentioned as an application, nothing is said about the absurdity of claiming to make accurate dimensional measurements (a limit of one part in 100,000 is mentioned) unless the temperature of the specimen, and not merely the ambient temperature, is measurable and is recorded.

The chapters on applications make one wish for more.

A new feature is the section of undergraduate problems (with solutions), but there follow the useful appendices and tables, with subject, references and author indexes that will certainly extend the use of the book beyond that of the undergraduate. KATHLEEN LONSDALE

(This review was completed by Dame Kathleen Lonsdale before she died earlier this year.)

Analysis by Activation

Principles of Activation Analysis. By Paul Kruger. Pp. xi+522. (Wiley: New York and London, August 1971.) £11.75.

RADIOACTIVATION analysis, a by-product (or should it be a spin-off to be really up to date?) of research in nuclear science, is now well established in its own right as an important tool in science and technology. In this book, Kruger discusses some of the applications in his final and longest chapter and devotes a good fraction of it to the analysis of trace elements, an appropriate choice in view of the very high sensitivity of activation analysis. In this day and age, industry and the environment were almost bound to be mentioned, and they are, but sensibly and with reasonable detail. Oxygen in metals, the logging of oil wells and on-line techniques are the main examples from industry; here, as in the other fields which I do not have the space to cover, the examples are clothed with extensive numerical information.

It is important for materials analysts, particularly those with a background in nuclear physics, to realize that the nucleus is not the only part of the atom capable of emitting identifying radiations, as I am sure the optical and X-ray spectroscopists would quickly point out. With this in mind, I was pleased to see that Kruger's section on trace element analysis includes a comparison of activation analysis with other methods; the electron probe is not among the others but, even so, the reminder to think broadly is there.

These comments have arisen just from Kruger's final chapter, but what of the rest of the book? The three chapters on the practices and limitations of activation analysis and on radiochemistry, which together form more than a third of the whole, are thorough and should be very helpful to the serious student of the subject. How to select the stimulating radiations, how to use the stimulated ones, whether to include chemical methods, how computers can help—these are the broad issues and in each case there is enough information to satisfy the budding professional. Here he can learn about the complexities of gamma-ray spectra, the problems of spectral stripping, the ways to deal with interfering reactions and a number of other features, and at the end of each chapter he has a few problems to sharpen his teeth on.

This, then, is a book with much useful information, and it is elegantly produced with clear diagrams and tables, but the cost is high for the individual buyer, at least in the United Kingdom. There is no doubt that half the book is well worth half the cost, but less introductory material and a lower total cost would have been a better balance, in my view, because this would have made the book more widely available. After all, Kruger's own excellent bibliography makes it clear that there are already several texts covering this first part of the work. Moreover, cuts in this section could have removed the few slips and ambiguities which are there (resonance scattering of neutrons is not synonymous with inelastic scattering; the relationship between thermal neutron fluxes and fast neutron yields is not as simple as implied; a conventional neutron flux, not the one defined, must be used with tabulated cross-sections to obtain reaction rates; in a Maxwellian distribution at room temperature, 0.025 eV is the energy associated with the most probable speed, not the most probable energy).

JOHN WALKER

The Chemistry Library
SCIENCE COLLEGE
92 Upper Circular Road
CALCUTTA

CORRESPONDENCE

Italian Science

SIR,—In Italy, research is almost completely in the hands of the National Council of Research (CNR), founded by Mussolini on November 18, 1923 (see *La Ricerca Scientifica*, 1945, p. 108). According to OCSE's experts, the CNR can be compared—as far as its juridic nature is concerned—to "an institution with a corporative nature, comparable with those professional orders which have multiplied during the fascist period". At present all financing has been suspended, and many applications have been rejected, even when some months ago it seemed that they had been approved. As for the applications which have been approved, the funds are still to be found on the budget of 1970 and some say that money for the research going on at present will not be paid until next spring. Young scientists and technicians are therefore obliged to abandon the universities, and projects (some international) must be cancelled in our programmes (supposing one may speak of "programmes" in Italy). And unfortunately this is not the first time we have had serious administrative trouble, though the CNR has at its disposal more employees than research workers.

One example is that of the International Biological Programme (or IBP), which for us was the first timid approach towards ecology, more especially productivity: whereas in other countries the IBP has seen and is still seeing beautiful research, which will go on until 1972, in Italy they say it is over. Whereas abroad many contributions have been published in the field of IBP, here there has been a very poor production. Even the results of the exploration of the small Italian islands, included in the CT programme of IBP, are extremely poor.

This contrasts strongly with the results Italian scientists had achieved in the field of the International Geophysical Year (1955), only 10 years after World War II. We cannot help thinking therefore that our Government's scientific policy is fundamentally very bad.

Useless to say that this has been well known abroad for several years: suffice it to quote Di Ferrante (*Science*, **161**, 451; 1968), Consolazio (*ibid.*, 133; 1968), Greenberg (*ibid.*, **167**, 1706; 1970), and Survey of Science in Europe (*Nature*, **226**, 1016; 1970) who give a very clear and exact description of Italian scientific policy, especially in calling the members of CNR "self serving advisers" (Di Ferrante, p. 451). We must not forget, however, that already in 1955 a member of CNR, Professor Di Mattei, had made a very clear critique of its policy, in which he says (*La Ricerca Scientifica*, **25**, 2372; 1955) "the Committee (for Biology and Medicine) has over all limited itself to a mere paternalistic distribution of the funds it had at its disposition, and has not confronted the great scientific problems which should have been the highest task of its financial policy".

What our responsible men are not able to do is to split *completely* the research in the universities (which are entirely state entities, depending on the Ministry of Education) and that in the CNR centres (which depend on the Presidence of Ministers), keeping two independent budgets, with no interchange or osmosis possible between the two. The full separation of powers and of men would be particularly necessary in a country such as Italy, which is famous all over the world for its "camarillas", mafia, "Baronie", clientelism, and so on. Of course the stronger the relationships between university and CNR, the easier the clientelism between the "owners" of CNR and the university institutes, which today depend for research completely on the former because practically all the members of CNR (presidents of committees, sub-committees, work groups, sections, and so on) are also professors in the chair at some university. With the last events of the CNR, the fear that all of us (university workers) have to be turned in post-lyceum teachers instead of research workers is stronger and stronger and quite justified. On the other hand, the political situation of the country (see a series of articles by P. Nourry and P. Bois in *Le Figaro*, the last of which was published on September 22) does not permit any sort of optimism in this field.

Yours faithfully,

GIORGIO MARCUZZI

The University of Padova

Suckling Etymology

SIR,—In his letter (*Nature*, **233**, 73; 1971) Mr Spratling complains that a communication[1] which several co-signatories and I sent to you some 20 years ago has been the cause of the confusion he has recently experienced in fairly extensive reading on the topic of suckling. We are apparently to blame because we used the verb "to suckle" in a manner contrary to the usage of Shakespeare and of that found in the Bible.

English usage never stands still. It is nearly 300 years since the verb "to suckle" began to be used to denote the activity of the young as well as that of the lactating mother. This extension of meaning has caused ambiguities. It was because of this long-standing ambiguity that the signatories of the letter, who were then all working in the field of lactational physiology and well aware of the difficulties that could arise, suggested that it might be helpful if authors of scientific papers were to restrict the use of this verb to denote only one activity and since its commonest usage in contemporary papers was with reference to the activity of the young we suggested that it be so restricted. Mr Spratling is, of course, equally entitled to suggest that the meaning be restricted to the activity of the mother provided that he indicates, as we did, that he uses the word in a restricted sense. To imply, however, that his choice is the only correct usage is, at the present time, nonsense. He should get an up to date dictionary and put Shakespeare on the shelf.

In Mr Spratling's description "the calf sucks the cow" the use of the verb "to suck" is unfortunate, not in this instance because of any change in usage since Shakespeare's time but because there have been some advances[2] in our understanding of the process whereby the young ruminant acquires milk from the udder of its dam. The movement of the suckling's tongue against the hard palate exerts positive pressure on the teat, stripping the milk from it in a manner similar to that of the fingers of the hand milker, suction being a non-essential component of the process. Only the milking machine which obtains milk from the cow solely by the creation of a partial vacuum may, I think, truly be said to suck the cow.

Yours faithfully,

A. T. COWIE

National Institute for
Research in Dairying,
Shinfield,
Reading RG2 9AT

[1] Cowie, A. T., Folley, S. J., Cross, B. A., Harris, G. W., Jacobsohn, Dora, and Richardson, K. C., *Nature*, **168**, 421 (1951).

[2] For references see review by Cowie, A. T., and Folley, S. J., in *Sex and Internal Secretions*, third ed. (edit. by Young, W. C.), **1**, 627 (Williams & Wilkins, Baltimore, 1961).

British Diary

Saturday, October 16

Unemployment Among Scientists and Technologists (10 a.m.–6 p.m.) British Society for Social Responsibility in Science, at the Harkness Hall, Birkbeck College, Malet Street, London WC1.

Tuesday, October 19

Extra-Sensory Perception: Fact or Fallacy? (1.30 p.m.) Dr A. J. Ellison, University of London, at Queen Mary College, Mile End Road, London E1.

Insight, Not Numbers (5.30 p.m.) Professor P. A. Samet, University of London, in the Chemistry Auditorium, University College London, Gower Street, London WC1.

New Techniques for the Determination of the Nutritive Value of Feeding Stuffs for Ruminants (10.30 a.m.) Society of Chemical Industry, Agriculture Group, at 14 Belgrave Square, London SW1.

Problems of Large Power Stations (7.30 p.m.) Institution of Chemical Engineers, jointly with the Institution of Mechanical Engineers, at the University, Nottingham.

The Tectonic and Depositional History of the Eastern Equatorial Pacific During the Cenozoic (5.30 p.m.) Professor Tj H. van Andel, University of London, in the Collegiate Theatre, University College London, Gower Street, London WC1.

Vinyl Resins in Surface Coatings (7 p.m.) Mr D. J. Silsby, Oil and Colour Chemists' Association, at the Pendragon Hotel, Southsea, Hampshire.

Wednesday, October 20

Application of Satellite Relayed Communications to Civil Aviation and Maritime Use (6 p.m.) Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Biomedical Research and the Chemist—a View and a Prospect (5 p.m.) The Rt. Hon. the Lord Todd, FRS, at the Royal College of Physicians, 11 St. Andrews Place, Regents Park, London NW1. (Merck Sharp and Dohme Research Laboratories Scientific Lecture.)

Computer-Aided Design in Electronics (5.30 p.m.) Mr P. E. Trier, Institution of Electrical Engineers, at Savoy Place, London WC2.

Developments in Water Based Surface Coatings (7.30 p.m.) Mr A. J. Becalick, Oil and Colour Chemists' Association, at the Carlton Hotel, North Bridge, Edinburgh.

Electronic and Radio Needs for Light Aircraft (6 p.m. discussion) Royal Aeronautical Society, at 4 Hamilton Place, London W1.

Engineering for Nuclear Power (10 a.m., Mr J. C. C. Stewart, Dr D. F. Seymour) Dr Gordon Brown and Mr P. H. W. Wolff, in the Faculty of Applied Science, University of Nottingham. (Bulleid Memorial Lectures.)

How to Survive on the Planet Earth (6.15 p.m.) Dr A. Peccei, United Kingdom Automation Council, in the Council Chamber at the Confederation of British Industry, 21 Tothill Street, London SW1.

Martin Lister (6 p.m.) Mr S. Peter Dance, Society for the Bibliography of Natural History, in the Men's Staff Common Room, University College London, Gower Street, London WC1.

Speech Scrambling and Security (7.30 p.m.) Mr R. C. French, Institution of Electrical Engineers, at the Mullard Research Laboratories, Redhill, Surrey.

The Training of Sandwich Course Students on Degree Courses Containing Options in Analytical Chemistry (3 p.m. discussion meeting) Society for Analytical Chemistry, Education and Training Group, at the Thames Polytechnic, Woolwich, London SE18.

Thursday, October 21

Agricultural Productivity in the Third World (8.15 p.m.) Professor Sir Joseph Hutchinson, University of London, at Wye College, near Ashford, Kent.

Pollution (7 p.m.) Dr R. B. Brown, Oil and Colour Chemists' Association, at the Beech Tree Hotel, Maxwell Road, Beaconsfield, Bucks.

The Evolution of the Geosyncline Concept (5.30 p.m.) Professor Ken Hsu, University of London, in the Collegiate Theatre, University College London, Gower Street, London WC1.

What Strings on Medical Research? (5.30 p.m.) Sir John Wolfenden, University of London, in the Beveridge Hall, Senate House, London WC1.

Friday, October 22

The Distance Scale of the Galaxy (9 p.m.) Sir Richard Woolley, FRS, Royal Institution, at 21 Albemarle Street, London W1.

The Paleomagnetism of Marine Sediments (5.30 p.m.) Dr N. D. Opdyke, University of London, in the Collegiate Theatre, University College London, Gower Street, London WC1.

The Quenching of a Diazo-Chromophore Fluorescence by Aliphatic Hydrocarbons (1 p.m.) Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A. & Canada
Nature (Friday)	**£14**	**$48**
Nature + Nature Physical Science	**£24**	**$83**
Nature + Nature New Biology	**£24**	**$83**
All three editions	**£29.50**	**$108**
Annual Index	**£1**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 29242

American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004

All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, October 15 1971

NATURE Volume 233
OCTOBER 22 1971

Waiting for the Other Shoe to Fall

By now, it is clear that the British scientific community is at once discreet and patient. For several weeks, there has been clamour for the publication of the report of the committee under Sir Frederick Dainton which is supposed to have worked out a strategy for the development of public support for university research for the next several years. To less innocent or scrupulous people, it may seem remarkable that those who know what the Dainton report consists of, and who are beating the drums for its publication more loudly than others, have not chosen simply to say what they think this now secret document contains. Is there, by any chance, a possibility that protest against the failure to publish so satisfies the wish to believe that the bureaucracy is malevolent that attention has been diverted from the alleged objective to see that information is put about ?

In practice, it is not very hard to guess the general drift of what the several participants in the game of reshaping public policy on science are likely to be saying. A year ago, when Sir Solly (now Lord) Zuckerman grabbed the tiller of the ship of state from time to time, it would have been easy to predict that the research councils would be swept away, with the Agricultural Research Council being transferred to the Ministry of Agriculture, the Medical Research Council to the Department of Health and Social Security and the Natural Environment Research Council to some place in which it would be even less easily identified. Sir Frederick Dainton has a more modest task—his committee has been asking how the universities and the research councils should respond to the growth which there has been and will be in higher education. What will happen to the character of university research if the funds available grow less quickly than the numbers of undergraduates ? Will the life of an academic become much more that of a teacher than a researcher ? What will be the relationship between the research councils and the University Grants Committee ? Is it sensible that the research councils should be the chief arbiters of the pattern of academic research ? There is no doubt that the Dainton report, when it finally emerges, will applaud the way in which the research councils have recently adopted with enthusiasm the policy of selectivity and concentration for which the Science Research Council paved the way. At the same time, the Dainton committee will be found to be urging that academics should become increasingly reconciled to the belief that research is a privilege and not a necessary accompaniment of a job. What the Dainton committee will say about the research councils and the relationship between them is less clear, although there seems no doubt that the Dainton report is a moderate document, pleading that research councils with responsibilities in applied research should devise ways of responding more sensitively to the interests of their chief customers.

On the relationship between the research councils and government departments and the universities, it has for more than half a century been conventional to recite an approximation of what is known as the Haldane principle, that in the conduct of basic research there needs to be some tension between those who carry out the research and those who in due course make use of it. What this implies, for example, is that there is some virtue in an arrangement under which the Agricultural Research Council (for example), responsible for basic research in agricultural science, should be managed separately from the chief users, farmers and their shepherd, the Ministry of Agriculture. The trouble, of course, is that a doctrine like this, invented in the middle of the First World War, cannot easily be expected to be valid both then and now, when the character of basic research, the relationship between basic research and applied research, and the scale on which the scientific enterprise is carried out have been radically transformed. Thus it has become convention to the managers of research policy within the government to proclaim that "Haldane is dead". To Sir Solly Zuckerman, Haldane was indeed quite dead. Both Sir Frederick Dainton and Lord Rothschild may in their different ways have more moderate versions of this theme —Haldane may be mostly dead.

In the last resort, of course, the future pattern of British scientific research will depend much more on what the government thinks of what Lord Rothschild has recommended than on the Dainton report, published or unpublished. Which way is the wind blowing ? The Rothschild inquiry and the Dainton inquiry overlap only at the edges, which is not to say that it is not a misfortune that the Rothschild inquiry has been confined to civil science in the strict sense—even the civil functions of the defence laboratories remain secrets to themselves alone. On the view that Haldane is dead or mostly dead, the research councils must necessarily change their relationship with government departments, and nobody should be surprised if in due course they find themselves compelled to earn at least some of their support from their customers. To be sure, the chances are that any changes which come about will be gradual. And while it is at least conceivable that the research councils responsible for agriculture and medicine could establish such a powerful claim on the interests of the corresponding ministries that they would be able to continue a useful life—perhaps even a more rewarding life—even those who fancy outlandish bets would not give much for the chances that the Natural Environment Research Council will be able to make a decent living for itself in that hard world.

In all this speculation about the future pattern of scientific research in Britain, it is possible to identify several simple truths, some of them at first sight contradictory. First, it is curious how much the reorganization now in prospect reflects opinions which have somehow become widely accepted—one of the mysteries of 1971 is that universities have reconciled themselves to a rate of growth through research expenditure which is less rapid in real terms than the rate of growth of the student population, and have done this without a murmur, for example. From this point of view, the changes which the government may eventually recommend may seem less like acts of policy than historical necessities. Second, not all the talk of the need to reorganize research expenditure in Britain has yet touched the question of how policy on defence research should be worked out. Third, there is a danger that enquiry into the management of research policy will become a substitute for policy. In the past ten years, there has been a continual struggle by the government and its advisers to optimize the mechanism for dealing with research in Britain. It is entirely possible that the time has come to leave it alone for a while.

Technology Assessment has a Long Arm

THE ministers for science from the member nations of the OECD seem to have had a useful meeting in Paris last week and, although these representatives of European governments are still shadow-boxing with each other as becomes political intervention in a dangerous field, they seem now to have got the hang of how to make a public statement of policy. The hope is that they will soon also learn how to make effective policies, for there is an urgent need that some means should be found of giving the OECD a framework within which to do sensible things.

The difficulty with last week's meeting was the concept of technology assessment (see page 512), invented originally by Mr Harvey Brooks of Harvard University. The trouble is that technology assessment is too seductive a concept for politicians to resist. The phrase itself implies two things—solicitude for technology and anxiety that the consequences of technology should be predicted. In other words, technology assessment appeals both to technologists and to those who are suspicious of technology. At the meeting in Paris last week, the ministers appear to have applauded themselves for devising a communiqué that says such things as "more basic knowledge is necessary to solve the increasingly complicated problems of modern society", which holds that "the growing importance of social objectives requires increased support for the relevant social sciences, including research" and at which ministers are said to have "agreed on the need to stimulate further improvements in the innovation process if the results of research and development are to have the maximum diffusion in society". It is true that the spirit of a successful conference is rarely embodied in the communiqué issued at the end, but on this occasion platitude seems to have had a field day.

What should the ministers of the OECD do? It is not, after all, that there are no jobs to be tackled. It is tempting to think that a call for "intensified scientific and technical cooperation" will serve some purpose, but how is the cooperation to be carried out, in what fields, by whom, and which governments will take the initiative? It is tempting to think that outsiders will be stimulated by the declaration that "a major task for science policy will be to assess the adverse and beneficial consequences of technological development and to foresee scientific and technical trends", but who are the people who will also foresee the consequences of now familiar practices, such as the dumping of untreated sewage into freshwater lakes and rivers? Which governments will accept the logic of those discoveries and require their taxpayers to provide sewage treatment plants? The trouble is that technology assessment is not merely a balm for the consciences of those who think that technology needs watching but a defence for those who hope that, if enough people worry about problems which do not yet exist, nothing need be done to tackle the problems which already abound.

Whose Head is in the Sand?

A WEEK from now, it should be apparent whether or not Britain will be joining the European Communities in 1973. The debate now under way in the House of Commons will finish on Thursday with a vote that will decide whether the British government is strong enough politically to go ahead with its intentions to accept membership on the terms which have been negotiated, at least in outline, over the past few months. It is shrewd of Mr Edward Heath, the Prime Minister, to have said that members of his own party will be allowed to vote as their inclinations guide them. He has very little to lose and quite a lot to gain by taking this course. Even so, the outcome of the proceedings in the House of Commons remains uncertain. Mr Heath will get his majority, but it remains to be seen how large it is or, perhaps more important, how it is constructed and thus how strong it will appear to outsiders. This is why it may not be entirely out of place to recall that the present difficulty about British membership of the European Communities stems almost entirely from the way in which the leadership of the British Labour Party, with one or two exceptions, has gone back on its own commitment to membership.

The shabby way in which this has been done is a conspicuous scandal. There is at present no reason to suppose that Mr Harold Wilson's government would have been able to negotiate better terms for membership than those agreed last summer in Brussels, and no reason to expect that the previous Labour government would not have accepted them. The argument which now prevails that the terms could have been better is hard to deny, but to use this complaint as a serious criticism of the arrangements now proposed is a little like the way in which would-be members of golf clubs might complain that they will join only if the annual subscription is made cheaper. It is equally unconvincing now to complain that the policies of the European Communities will prevent the British government from pursuing its own schemes for regional development and the like and will thus infringe British sovereignty—for one thing, there has never been any doubt that the Treaty of Rome would inhibit the freedom of governments to bolster up uneconomic parts of their economy so as to increase their competitiveness artificially, but equally it is well established that the European Communities as a whole have a responsibility for precisely the kinds of regional problems which the Labour Party is now saying will be neglected.

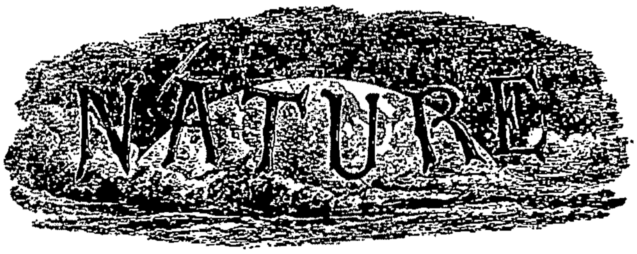
The most alarming contrast between the position taken by Mr Harold Wilson in his previous incarnation as Prime Minister and that which he now holds is that his old brave words about technology in Europe have been almost entirely stifled. In all the declarations of the past few weeks, nothing has been heard from Mr Harold Wilson or his close colleagues of the opportunities for technological collaboration between members of the European communities. Yet many will still recall the Guildhall speech in which he painted a glowing picture of the opportunities that the enlargement of the Common Market would provide. At that point, there were schemes by means of which European governments, the British prominent among them, would collaborate on technical projects such as the development of aircraft or large computers and common research and development in telecommunications. The first principle seemed to be that the combined resources of the nations of Western Europe would be better able to keep up with the pace of technical development elsewhere, the United States, for example. The second principle, chauvinistic perhaps, was that Britain had invested such comparatively large sums in research and development that extra skills had in the process been acquired, with the result that British expertise in technological development might be regarded as a kind of dowry, helping to smoothe the way for membership.

For what it is worth, Mr Wilson's first vision was far too rosy, just as his present view appears to be too misanthropic. In the past twenty-five years, there has been plenty of evidence that the success of technological development must depend on the existence of a market. Research and development will not bring prosperity unless the products are commodities or machines that people are prepared to buy. Most European countries have by now a lengthy catalogue of research and development programmes which have turned out differently. Even now there are devices such as the Concorde which may never be commercially viable and which may even fail to provide a platform on which more advanced (and saleable) technical developments may be founded. In Mr Wilson's earlier talk of the wonders of European technology, there was too much tendency to regard research and development as a kind of magic wand. But equally, there are great benefits to be derived from a state of affairs in which the developers of new products are able to spread their initial costs over a market the size of that which now exists in Europe. Indeed, once the economic integration of Europe has gone a little further, there should be a rapid stimulation of research and development as manufacturers begin to recognize that they can afford to pay for it. By contrast, if Britain were to remain economically detached from the mainland, as Mr Wilson now appears to think it should, the difficulties that haunted his government and its predecessors in planning for public support of technological development will persist. Time and again, governments will find themselves investing too little in huge and ambitious undertakings. The total cost of publicly supported research and development will remain a large proportion of the total, but both the sponsors and the research managers will be left with the impression that important work is being skimped. Even if Mr Wilson himself is not any more aware of how European economic integration offers opportunities for this kind of development, his right-hand man, the syndicalist Mr Anthony Wedgwood Benn, should have enough experience from his spell at the then Ministry of Technology to know how important it is to rationalize the planning of research and development in Britain.

There are, of course, lessons to be learned in mainland Europe on this same theme. Luckily, the wind now seems to have gone out of the sails of the plans for mounting collaborative research programmes in important fields such as telecommunications. Instead, there seems to have grown up a proper respect for the importance of the customer in the chain of technological development. This week in Brussels, it is clear that there will be a hard fight over the research and development programme on nuclear power put forward by the European Commission in the summer. By now, it seems to have been acknowledged that the Joint Research Centre at Ispra, the most tangible and perhaps the most embarrassing survivor from Euratom, will have a sensible programme of research in the next three years. There is a budget for covering fundamental work in nuclear energy, some attempt to make a close study of the biological effects of radiation and some modest work in the environmental sciences. The question now to be decided is how the European Communities should support research and development of benefit to the nuclear engineering industry.

---

100 Years Ago



WE have to record the death, on Saturday last, at the age of seventy-nine, of Mr. Charles Babbage, the eminent mathematician and mechanician. The most important events of his life, as well as some of the eccentricities of his character, are familiar to the public through his autobiographical volume, "Passages in the Life of a Philosopher." Born in 1792, he entered Trinity College, Cambridge, in 1810, and was transferred to St. Peter's the following year. At his B.A. degree he did not take honours in mathematics, not having specially pursued that subject of study as a student, and was understood to have been disappointed at not being elected a fellow. In 1828 he was however elected Lucasian Professor of Mathematics at Cambridge, a position once held by Sir Isaac Newton. He published no less than eighty volumes, but his claim to public notice rested chiefly on his invention of the Difference Engine, on which he spent immense labour and a large sum of money. Notwithstanding his eccentricities and his failings, Mr. Babbage was a mathematician and an inventor of whom England may be justly proud.

From Nature, 4, 512, *October* 26, 1871

OLD WORLD

SCIENCE POLICY

Technology Assessed

"TECHNOLOGY assessment must not become technology arrestment" is how Dr Edward E. David, President Nixon's scientific adviser, summed up the fourth meeting of ministers of science of OECD countries which took place in Paris last week. Dr David said in London after this meeting that in the seventies the quality of life is going to be a more important consideration than it had been in the sixties.

The two-day meeting discussed three main themes; trends and objectives in science policy in the 1970s, innovation in society and the economy and international cooperation in science and technology. The British delegation was led by Mr J. F. Embling of the Department of Education and Science. At the end of the meeting a joint communiqué was issued by the delegates from the 23 member countries which outlines a considerable change in official attitudes to science and technology.

The text for the conference was the Brooks report on "Science, Growth and Society" which argues that qualitative growth has been sacrificed to quantitative growth and that the risks and benefits of technology should be better assessed (see *Nature*, **232**, 6; 1971). To be sure, the ministers affirmed that "science and technology will continue to be essential elements in social and economic progress in member countries. At the same time . . . science and technology should contribute more fully to the improvement of the quality of life as well as to material well-being, and that the undesirable side-effects of their application should be controlled". Thus the ministers have accepted much of the basic stance of the report, resolving that, while research must be expanded and technological innovation stimulated, it must not be merely for quantitative growth but "to meet social needs such as environmental quality, health, education and urban development"; furthermore effective management and control of technology in the public interest must be established.

The ministers emphasized that the problems society faces are many sided and complex and underlined the need for closer links between economic, social, science and educational policy making at national and international levels. The communiqué also applauds the notion that innovation in the public sector, where market incentives may not be great, may require special measures by the government. They also call for more research into the harmful and beneficial results of new technologies.

The communiqué pays considerable attention to the promotion of cooperation between member countries, particularly encouraging cooperation in fundamental research and in the planning and utilization of expensive facilities, for example high energy physics, by international teams. On the problems of the relations between the developing members of the OECD and their richer counterparts, the ministers considered that more should be done to transfer technology to the developing members and to strengthen their scientific and technological capacity.

The policy statement is radical enough. Dr David pointed out that the fact that people having been talking along these lines for some time may lessen the apparent size of the change in policy, but he emphasized that the meeting represented a "turning point" in the application and control of science and technology.

RESEARCH COUNCILS

Pilot Fish for Dainton

A PATIENT explanation of the way in which university research is at present supported with public money has been provided by a committee of the Council for Scientific Policy under Sir Harrie Massey (*Report of a Study on the Support of Scientific Research in the Universities*, Cmnd 4798, HMSO, £0.45). The document spells out some of the questions which are answered (by all accounts) in the report of the working party under Sir Frederick Dainton, still regarded by the British government as one of those against which the Official Secrets Act is directed, but the committee says that it has not sought to answer them. For that reason, it is inevitable that its recommendations should be somewhat anodyne.

The committee commends the system under which the University Grants Committee and the research councils both help to support university research, with the former "providing the floor of support for research" and the research councils being selective. Forlornly, the committee hopes that the government will recognize the growing cost of research equipment, reckoned to have risen by between eight and eleven per cent in the past twelve years, says that contract research in the universities should be charged for at rates that take full account of overheads and suggests that the research councils should be free not merely to provide equipment or the salaries of operating personnel but any other services which may be needed, buildings for example.

The committee comes down against the so-called handover principle by means of which research projects begun by a research council are transferred to the appropriate university at the beginning of each university quinquennium together with the necessary allocation of funds, and to that extent the report is an echo of what the Science Research Council has already decided to do (see *Nature*, **233**, 298; 1971). The committee also applauds the Science Research Council's policy of selectivity and concentration, saying that it will have to go much further in the years ahead and that, in the process, there will have to be close consultation between the research councils and the universities.

The unanswered questions supposedly tackled by the Dainton committee proper are those of whether university research will be damaged by such accompaniments of the expansion of higher education as a deterioration of staff-student ratios and the shortage of technical support in administration and finance in British universities, the question whether there should be a constant ratio of undergraduates to postgraduates, what balance there should be between different kinds of postgraduate courses and what experiments should be made in varying the length of postgraduate courses as a whole. By all accounts, the Dainton report in its full splendour will be published in the next few weeks.

EMBO

One Bridge More

THE laboratory of the European Molecular Biology Organization will almost certainly be built at Heidelberg. At a meeting last week in Geneva of the European Conference on Molecular Biology (the association of states which will fund EMBO), it was decided to accept the recommendation of the working groups on the laboratory (see *Nature*, **228**, 7; 1970) that the laboratory should be at Heidelberg.

Three firm offers of a site for the laboratory were received, all of them in Germany. (Britain recently decided not to offer facilities at Culham—see *Nature*, **233**, 154; 1971.) Two sites were in Munich, one at Garching and the other at Martins Ried. The first site had the advantage that it is near the plasma physics laboratory (and instrumentation is an important theme in the prospectus of the new laboratory) but in other respects did not seem suitable. The second site, although lacking a physics laboratory, is close to the site where the new laboratories of the Max Planck Institute are to be built; this was at once an advantage in offering immediate temporary accommodation until the EMBO laboratory itself could be built, but a disadvantage in that some people have been afraid that the Max

Planck laboratories might overshadow the EMBO laboratory. Heidelberg, however, is a good choice because there are well developed physics facilities and temporary accommodation can be provided if necessary.

The agreement of the ECMB is no guarantee that the laboratory will be built. First of all, the member states must ratify the agreement to provide the budget. Nine states voted for the resolution (Denmark, Austria, France, Norway, Germany, United Kingdom, Switzerland, Spain and the Netherlands), while Sweden abstained. Israel was not present at the meeting and Greece and Italy cannot vote because the Italian government has not yet ratified its membership. Belgium, although it contributes indirectly, is not entitled to a vote.

The next step will be for a revised budget to be produced by the ECMB by December and decisions about the laboratory should finally be agreed by April 1972. Work can go ahead to establish the laboratory as soon as EMBO is sure that funds will be forthcoming ; this means that no action will be taken until 70 per cent of the budget is assured. It seems likely that agreement by sufficient states will take about a year, so that arrangements for temporary accommodation and for building the new laboratories cannot be made until April 1973. (In addition to its assessed contribution to the ECMB, Germany has offered some £1.2 million to help with capital expenditure.)

No director of the laboratory has yet been appointed, but Dr John Kendrew, the widely tipped choice for the job, has been appointed project leader. In that capacity, he will be responsible for organizing the laboratories during their opening stage ; this is of indeterminate length and, at some time after the laboratory is functioning, a director will formally be appointed. If plans do not fall too far behind schedule, some sort of grand opening ceremony of the laboratory can no doubt be expected in 1976.

NOBEL PRIZE

Cyclic AMP Recognized

The award of a Nobel Prize for Medicine to Earl Sutherland, Professor of Physiology at Vanderbilt University School of Medicine in Tennessee, will be universally acclaimed among biochemists. Professor Sutherland, who is fifty-five and has been studying hormone action for twenty-five years, is the forty-third American to receive the award, now worth £36,000. This is the first time in ten years that the prize has not been shared. A generation of biochemists had now been brought up on Professor Sutherland's theory of hormone action, which stems from his discovery in 1957 of what is now known to be a ubiquitous biological molecule, cyclic AMP.

Professor Earl Sutherland.

Sutherland's group were in 1957 trying to find out how the hormones glucagon and adrenaline mobilize glucose from glycogen, its storage form in the liver. When they added either hormone to an extract of liver cells, the production of this unknown monophosphate derivative of adenosine was easy to detect. Biochemical analysis subsequently showed this to be 5′, 3′ adenosine monophosphate, with the phosphate making an inelegant and unexpected bridge between the third and fifth carbon atoms of the ribose component.

Much more refined kinetic studies revealed that the hormone-induced increase of cyclic AMP in liver cells occurs before glucose mobilization is apparent, which strongly suggested that cyclic AMP is an effector, rather than a by-product, of the mobilization process. It was on the basis of these extensive studies that Sutherland proposed his novel concept for the molecular basis of hormone action. Glucagon or adrenaline acts to correct low levels of blood glucose by stimulating the enzyme adenyl cyclase of liver tissue to convert ATP to cyclic AMP. Cyclic AMP then behaves as a "second messenger" within the cell to promote the release of free glucose. In a complex series of reactions, cyclic AMP causes the conversion of the enzyme glucose-1-P phosphorylase from its inactive to active form, which breaks down glycogen by pyrophosphorolysis.

More recent work has not only verified this idea but has also shown cyclic AMP to be the second messenger for several other hormones, including those that mobilize lipids from fatty tissues and those that provoke the synthesis of steroids. Because of these and other function ascribed to it, cyclic AMP has become something of a wonder molecule. It is implicated not only in the stimulation of various enzymatic activities, as in glucose mobilization, but also in the control of gene expression both in bacteria and in the cells of higher organisms.

But even as research on cyclic AMP burgeons and new findings continue to change old ideas, it seems that Dr Sutherland's proposal for its action as second messenger stands out as clearly as his discovery of the molecule itself. The practical implications of this discovery are difficult to assess, but one application is likely to be the use of derivatives of cyclic AMP which can overcome deficiencies in certain hormones by stimulating specific functions.

FOOD

Mercury Miscellany

It seems that the average Briton has no need to worry about any mercury he ingests from food. This balm appears in a report issued by the Ministry of Agriculture, Fisheries and Food after a nine-month investigation into the level of mercury in food which was prompted by the ban placed on the sale of tuna fish in the United States last December (*Survey of Mercury in Food,* HMSO, £0.20). The working party finds that the amount of mercury in the average diet of a British person is "extremely low and in most cases barely detectable". The level of mercury in canned and fresh fish and shellfish is also low but higher than in other foods.

Mr James Prior, Minister of Agriculture, Fisheries and Food, wholly endorsed the report last week and added that it had not been possible to identify in Britain any group that had suffered from ingesting mercury. The investigation showed that even with a group of six fishermen, there was not an inordinate amount of mercury in the hair, but blood analysis showed that they had more mercury in their blood than did six control subjects—the fishermen had between 0.01 and 0.03 mg kg^{-1} compared with the control subjects who had at most 0.005 mg kg^{-1}.

Mr Prior has recommended that mercury in food should still be monitored and that other heavy metals—lead and cadmium in particular—should also continue to be monitored. Methyl mercury will get special attention. An implication of the report is that monitoring for mercury will continue at a reduced rate, but special attention will be given to fish caught in coastal waters where the chances of mercury pollution are high.

One of the reasons given for not banning the sale of canned tuna in Britain, as was done in the United States last December, was that the average amount of tuna eaten per person in Britain is only one tenth that of the USA. The report shows that the amount of mercury found in canned tuna in Britain is similar to that found in the United States and of the varieties of foods tested tuna contained most mercury—an average of 0.18 mg kg^{-1} compared with canned salmon that was found to contain between 0.01 and 0.04 mg kg^{-1} of mercury. No detectable mercury was found in flour, cheese, beef, chicken meat, eggs, bacon, ham, rice, sugar, and breakfast cereals while small but detectable amounts were found in cabbage, potatoes, apples, carrots and imported tomatoes (all less than 0.004 mg kg^{-1}).

FRENCH SCIENCE

Budget Plans

from *La Recherche*

WITHIN the next few weeks the French Parliament will be approving the country's research budget for the coming year. This action is significant because 1972 will be the first year of the sixth five-year plan, and will thus be evidence of the long-term intentions towards research.

There has been nothing innovative so far in the method of determining the French research budget, although the *enveloppe-recherche,* or grouped project approach, has been refined. This idea is that most of the sums voted for civil research and development underwritten by various ministries are examined in advance each spring by an interdepartmental group. Thus, the money to be spent on the *enveloppe recherche* in 1972 is in close agreement with the overall *enveloppe* approved for the comprehensive 1971–5 plan.

The total vote for 1972 is 3,848 million francs (almost £287 million) in research programme authorizations. This represents an increase of 15.5 per cent on the year. If this increase appears to be unusual, it is nonetheless quite in line with the minimal projections set forth in the plan. The breakdown of the budget conforms, of course, with the major options set forth in the plan so that, for example, the 54 per cent increase awarded to the Health Ministry and the 46 per cent increase for agriculture reflect the priority being given to research and development in the life sciences. The sums voted for work on pollution and the environment under the aegis of the new Ministry of the Environment increase from 4.2 to 13 million francs (£1 million). The vote for aid to industrial research will also jump sharply while the earmarked appropriations for industry—*l'aide au développement*—are being augmented by an impressive 35 per cent.

Among the research organizations responsible to the Ministry of Industrial and Scientific Research, only the oceanological CNEXO centre will enjoy an increase in its vote (25 per cent). The 1972 sums confirm a levelling out of expenditure at the Commissariat à l'Energie Atomique and a marked braking of the momentum of space research. The space agency, CNES, will be voted a modest increase of 2.3 per cent (581.5 to 595 million francs). There could be a supplementary vote for CNES if the European launcher becomes a reality, but the future of the French space agency is increasingly in doubt.

Operating expenses for research organizations will be raised by about 15 per cent, but there will not be a commensurate increase in the research establishment. In 1971 there were 432 new jobs created throughout the ministries ; there will be 280 next year. At the National Scientific Research Centre (CNRS) there will be 130 new jobs, whereas this year there have been 250. This lack of new blood in the year to come is a difficult problem in France's current scientific research effort.

The situation concerning basic research remains precarious. The 15 per cent increase of operating expenses at CNRS will be devoted largely to adjust compensation in the career development process and to give deserved merit increases to the staff. Elsewhere, the modest sums voted (an 8 per cent increase) for research at the level of higher education is a reversion to past practice. University laboratories face a difficult situation because they depend on the sums voted them directly and in many cases they do not themselves receive support from the CNRS.

So will next year's financing of research amount to nothing more than catching up, to compensate for other, leaner years in French research? In certain research areas, there will definitely be marking of time for another year. Perhaps M. Ortoli, the Minister of Industrial and Scientific Research, will be able in the days ahead to resolve some of the uncertainties undermining French research policy today.

ARCHITECTURAL ASSOCIATION

Still Alive

THE Architectural Association's School is now "active and healthy and well" in spite of the traumas of the past eighteen months, according to Professor Alvin Boyarsky, the newly appointed chairman of the school and its academic board.

The recent history of the school has been far from healthy. In February last year, plans for a merger with Imperial College, precipitated by the school's financial difficulties, fell through after six years of negotiation (see *Nature*, **225**, 575; 1971). Other proposed mergers went the same way and the school was left with the old problems and a lease that expires in 1976 and is unlikely to be renewed on the present favourable terms. There were fears that the school would have to be run down and closed. In spite of the problems, however, the AA's members and students at the school were determined to keep the school open and approaches were made to the Department of Education and Science (DES) and to local education authorities (LEAs) resulting in a rise in the fees from £460 to £580 and a fresh intake of 57 students this October.

Along with the new lease of life has come a reorganization of the school's structure. The post of principal, previously held by Mr John Lloyd, has disappeared and Professor Boyarsky is now responsible to a nine-man forum elected by the students, staff, association council and the membership.

Professor Boyarsky, a former staff member, is enthusiastic about the school's potential. Having departed in 1965 before the troubles began in earnest, he is far more concerned about future plans than past battles. He confesses to being "stunned" by the amount of activity in the school, sees its problems merely as "a challenge", plans to expand the school's evening work to involve more outside people and hopes to enlarge the school's already considerable international role.

But if things are brighter than they were, the clouds are still on the horizon. The school's fees of £580, paid by local education authorities, are much higher than the £60 to £75 paid to send students to other colleges and universities. In real terms, however, the school claims that the total cost of sending a student to the school is half to two thirds of that of sending him elsewhere—unlike other institutions, the AA is independent of central government finance. Although the Department of Education and Science has agreed to the steep increase of fees, it is insisting that there must be a viable plan for the future before the 1972 intake will be supported. What Mr John Smith, president of the Architectural Association, wants to see is a partnership between school and state, but he also feels that "to maintain our purpose we think it is important that we remain independent". Professor Boyarsky says that the biggest problem is not in finding the lump sum to renew the lease or find new premises (an appeal is to be run during the next year to help raise the money for this) but in finding the money for day to day expenses. He points out that an annual grant of £250,000 would solve the AA's problems by bringing the fees for the 485 students down to the same level as for other colleges and "to keep this cloud over this vibrant situation for so long for such a small sum of money is outrageous".

But whatever solutions are tried to remove the problems of running costs, further plans for mergers are not among them. The AA has come to value the very independence which causes its problems and even what sometimes seems to be this school's function as a "rogue elephant".

MUSEUMS

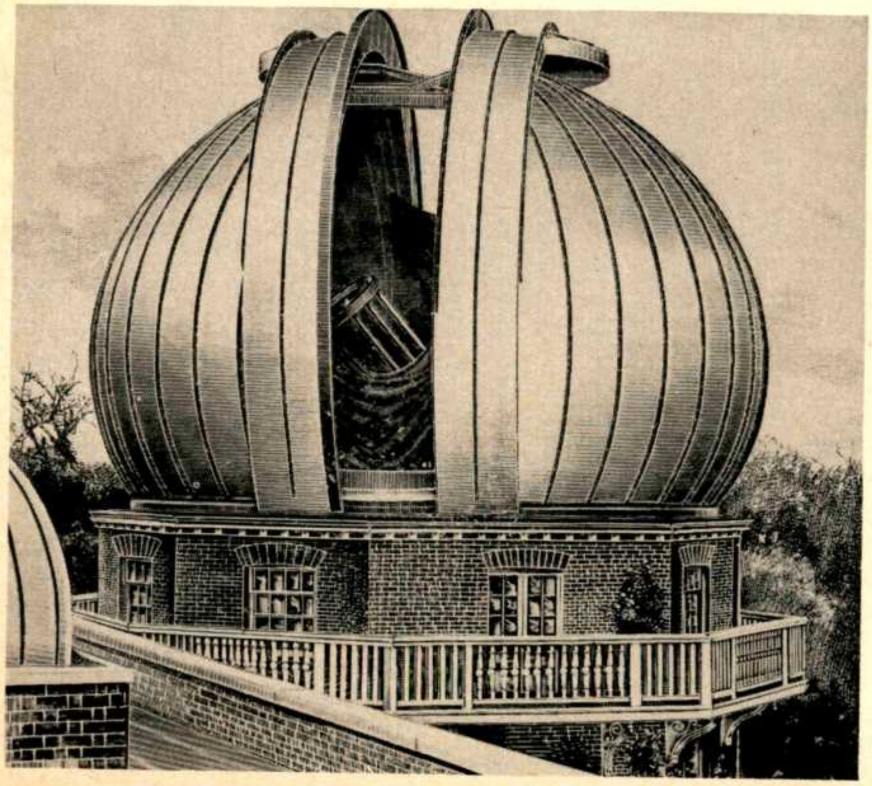
Telescope Transported

from a Correspondent

THE 28-inch refracting telescope that was in use at the Royal Observatory at Herstmonceux until a few months ago is being moved this week to its original site in the old Royal Observatory buildings at Greenwich. The telescope will become part of the museum of astronomy that is being established at Greenwich, and by late 1973 it should be available again for use by amateur and professional astronomers.

Removal of the telescope from the dome at Herstmonceux—which is needed for a new wide-angle telescope —and transportation and re-erection at Greenwich is an awkward engineering job. As well as the 27-foot telescope tube, the plan calls for the transport to Greenwich of the 26-foot polar axis and two 24-foot cast iron piers. But the chief task will be the reconstruction of the onion dome in which the telescope used to be housed while it was at Greenwich. The reconstructed dome will be built during 1972 and 1973, well in time for the celebrations in 1975 marking the tercentenary of the founding of the Royal Observatory. In the meantime the telescope and its mounting will be protected by a plastic drum which has already interrupted the familiar skyline of Greenwich.

The 28-inch telescope was built during the expansion of the observatory that occurred during the second half of the nineteenth century as a result of the appointment of the Cambridge mathematician George Airy as Astronomer Royal, although Airy retired before the telescope came into use, and he is not thought to have observed with it. Demand for the telescope arose through the so-called Neptune scandal: mathematicians in France and Cambridge independently predicted the existence of Neptune and the planet was observed by French astronomers, but there was no instrument in England capable of detecting it. As a result of the rumpus that this caused in scientific circles and in Parliament, the Admiralty—then responsible for the Royal Observatory—agreed to the commissioning of a 28-inch telescope to replace the 12.8-inch refractor, but insisted on economic grounds that the same mounting be used. But in order to accommodate the extra length of the new telescope, an onion-shaped dome had to be constructed—of *papier mâché* on an iron frame—that remained a feature of the Greenwich skyline until 1954.



National Maritime Museum

The original onion-shaped dome of the 28-inch refractor at the Royal Observatory, Greenwich.

RESEARCH ASSOCIATIONS

Where Next?

THE Sira Institute seems to be swimming against the economic tide. The annual report, now published, shows that income increased from £536,510 to £588,115 over the year just past in spite of a decrease in grant from the Department of Trade and Industry of £30,790 to £227,270. Dr Sydney Jones, chairman of Sira, is cheerful enough about this modest increase in business but it seems clear that the research associations will in future be much more keen to sell their services in the open market. No doubt a research association concerned with scientific instruments has more scope than most.

The position of research associations is still far from clear. Sira itself is in the second year of a five-year plan during which the government grant will be steadily decreased, and there is no guarantee of continued support thereafter. Dr Jones's optimism about the immediate future does not change the view that, in the long run, government aid in some form will be necessary if the research associations are to survive. Many of them, Sira included, are hoping to attract government money in the form of research and development contracts rather than continuing grants.

In the past year, Sira seems to have benefited a little from the way in which economic pressures have forced companies to take a more hard-headed view of research and development expenditure. There have been some occasions when companies have put research and development contracts with Sira instead of trying to do the work themselves.

Sira's income from general subscriptions last year was the same as during 1969–70 but it was achieved by smaller subscriptions from a larger number of companies, possibly reflecting this trend. Dr Jones added that the increase in Sira's income came from an 11 per cent increase in project contributions and a 27 per cent increase in contract income, together with a 5 per cent decrease in expenditure achieved by reducing the staff by over 14 per cent to its present level of 190 employees.

Two German companies, Jenaer Glaswerk Schott and Gen. of Mainz and Siemens AG, have joined and are cooperating on projects concerned respectively with the image control of fibre plates and ion selective electrodes for analysis and monitoring. Another sign of the times as far as research institutions are concerned is that Mr S. S. Carlisle, director of Sira, is a member of a committee of the Department of Trade and Industry that coordinates collaboration between the instrument making industry in Britain and that of Eastern Europe.

NEW WORLD

Rogers May Yet Win Cancer Stakes

by our Washington Correspondent

A UNANIMOUS vote in the House Subcommittee on Public Health and Environment last week may ensure that the National Institutes of Health is not broken up by Congressional efforts to help find a cure for cancer. The subcommittee has approved a bill designed to give the National Cancer Institute a total of $1,600 million over the next three years, but to keep it as an integral part of the NIH. The bill, which is in many important respects the same as that introduced in September by Paul G. Rogers, chairman of the public health subcommittee, and five of his colleagues (see *Nature*, **233**, 228; 1971), is also believed to be acceptable to the White House—a factor which could greatly ease its passage through the rest of the Congressional mill.

Mr Paul G. Rogers: "I anticipate no fight."

Before Mr Rogers introduced his bill in September, it seemed that the House would follow closely the footsteps of the Senate and agree to a cancer cure programme administered by an agency which, to all intents and purposes, would be independent of the NIH. But there is now a very good chance that Mr Rogers's bill will prevail over the Senate version, and that the vastly expanded cancer research programme will be administered by the NIH. Last week's important vote in the subcommittee sends the bill to the full committee from which, if approved, it will be reported to the House. The House is expected to vote on the bill early in November.

Even if the House passes the bill in its present form, however, some of the most crucial decisions on the future organization of cancer research would still have to be taken, for at that stage the differences between the House and Senate versions of the cancer cure bill would have to be reconciled. If the House version is unacceptable to the Senate, the whole issue would have to be decided behind the closed doors of a conference committee composed of representatives of both legislative chambers, and it is at this stage that the Rogers bill is now most likely to be emasculated. Mr Rogers said last week, however, that he expects no "committee, floor or conference committee fight".

Mr Rogers bases his optimism on several important factors, chief among which is that the bill is unlikely to provoke spirited opposition from either the White House or from the lobbyists who successfully inveigled the Senate into passing a bill designed to set up an independent cancer cure agency. Rogers took the far-sighted precaution of having representatives from the White House and from the Department of Health, Education and Welfare participate in the committee's deliberations when it marked up the final version of the bill, and he believes the Administration to be favourable to its objectives. One of the most influential lobbyists for the Senate version said last week that he believes "we have got a good bill". The fact that the bill was approved unanimously by the subcommittee may also ensure that it is not substantially altered by the full committee, whose chairman, Harley O. Staggers, introduced the Senate version of the bill into the House, but who declared at the opening of hearings in the subcommittee that he had not then formed any set convictions on the optimum organization for cancer research.

Mr Rogers's committee has held four full weeks of hearings on the bill, during which a string of witnesses from the biomedical community testified against the idea of setting up an independent agency. Although few new arguments emerged during the hearings, the subcommittee was evidently impressed by the fact that apart from the American Cancer Society, no major scientific organization supported the main points of the Senate passed version of the bill.

The members of the subcommittee were, nevertheless, under great pressure from skilful lobbyists, and Rogers said last week that the bill had been approved by his committee "in spite of the pressures that have been mounted". Only two days before the subcommittee approved the bill, for example, the American Cancer Society and a group calling itself the Citizens Committee for the Conquest of Cancer found it necessary to take full page advertising space in twenty-four newspapers (including papers in each of the ten congressional districts of the members of the subcommittee) to explain the virtues of the Senate version—an enterprise which drew from one member of the subcommittee the remark that the money could have been put to better use in cancer research.

As a result chiefly of the intensive lobbying, and in part of testimony received during the hearings, the original bill submitted by Rogers and his colleagues underwent some surgery. No vital parts were removed, but a few extra items were grafted on by the subcommittee.

The bill would give the National Cancer Institute a budget of $400 million for the 1972 financial year (which started on July 1)—an increase of more than $60 million over the amount appropriated by Congress last month and some $170 million more than the institute received last year—rising to $500 million next year and $600 million in 1974. The NCI's budget would be drawn up by the institute's director (who would be promoted to the rank of associate director of the NIH), and transmitted directly to the President. The director of NIH, the secretary of HEW and the Advisory Cancer Council would be able to comment on but not alter the budget.

Those provisions were part of the original bill introduced by Rogers, and survived intact the subcommittee scrutiny. But the bill reported out of the subcommittee also contains four important additions to the original bill, none of which, however, alters the philosophy that underlies its chief intentions.

First, the new bill would create 15 clinical research centres which could each receive block grants of up to $5 million a year from the NCI, and this grant arrangement is also extended to other existing cancer research centres, such as the Sloan-Kettering Institute in New York.

The idea behind the proposal is that individual institutions would be given greater flexibility in their research arrangements since they would not have to come to the NCI with thirty or forty applications for grants for specific projects.

Another proposal designed to speed up grant applications is a provision in the bill which would allow applications for grants of $35,000 or less to be studied by peer groups and approved by the director of the NCI without first being studied by the National Cancer Advisory Council.

The original bill had offered this proposal only for grant applications involving $20,000 or less, and the idea is to cut out a bottleneck which arises because the National Cancer Advisory Council meets only three times a year. The proposal would lift 40 per cent of the grant applications from its shoulders, and greatly increase the time available for the council to plan overall strategies for cancer research without being bogged down with what amounts to formal decisions.

The new bill also specifies a committee of three as a liaison between the President and the director of the National Cancer Institute. The suggestion for this committee is reported to have come from Mr Ancher Nelsen, ranking Republican on the Rogers subcommittee, as a means for helping the President to exercise his control over the programme by offering scientific reports and advice (two of the three members of the committee would be practising scientists or physicians).

Also included in the new provisions is a call for reactivation of federally funded cancer control programmes (pap tests, oral and breast examinations and so on) that were phased out by the Office of Management and Budget last year. The bill asks for $20 million this year, rising to $40 million in 1974 for this purpose.

If Rogers does manage to steer his bill through the rest of the Congressional mill, he will have achieved the distinction of defeating proposals put forward both by Senator Edward M. Kennedy and by the Administration. Kennedy introduced a bill earlier this year which called for an independent cancer research agency, on the lines suggested by a panel of cancer researchers and businessmen (see *Nature*, **228**, 1133; 1970).

The Administration, anxious to prevent the NIH from being broken up by the proposal, introduced a bill designed to keep the cancer effort within a National Cancer Institute elevated to the position of a bureau within NIH, but later entered into a compromise with Kennedy which in effect put the Administration's name on Kennedy's original proposals. That "compromise" went through the Senate with only Senator Gaylord Nelson of Wisconsin registering his dissent. Nelson's opposition to the Senate bill as much as Rogers's tenacity in spite of intense pressure has helped to steer the congressional debate back on realistic lines.

HEREDITARY DISEASE

Congress Against Sickle Cell

by our Washington Correspondent

ALTHOUGH Congress may be close to deciding how to spend several hundred million dollars a year on cancer research (see page 516) it stands little chance of running out of diseases to conquer. The latest object of potential Congressional largesse is sickle cell anaemia, which afflicts about one in every five hundred blacks, killing up to half its victims before they reach twenty. Identical bills introduced into the Senate and the House of Representatives last week call for a total of $90 million to be spent over the next three years on genetic counselling, screening, public education, research and treatment for sickle cell anaemia. Apart from the £1,600 million being requested for cancer research, the sum being asked for sickle cell anaemia may seem paltry, but compared with the $1.2 million budgeted last year for research and treatment of the disease, it is a huge increase.

Introduced into the Senate by John V. Tunney of California, and into the House by Walter Fauntroy, the District of Columbia delegate, the bills have attracted an impressive list of cosponsors including Edward M. Kennedy and several members of his Senate health subcommittee. One bill calls for a national programme aimed at the prevention of sickle cell anaemia, while the other seeks to institute a pilot programme in the District of Columbia, which has a larger percentage of black people than any other city in the United States. The chief aim of the programmes outlined in the bills is greatly to increase federal funding for screening and genetic counselling.

The National Sickle Cell Anemia Prevention Act, as it is labelled, would make available $25 million a year for grants to public and non-profit-making enterprises for the establishment and operation of screening and counselling programmes, and a further $5 million a year for research into the treatment and diagnosis of the disease. When he introduced the bill into the Senate last week, Senator Tunney said that "compared with other serious diseases, sickle cell anaemia has received only minimal attention and research". Citing the fact that diseases such as phenylketonuria and cystic fibrosis, which predominantly affect whites, consistently attract large slices of research money, while there is not even a national volunteer organization to raise money for sickle cell anaemia, Tunney called for a vastly expanded effort to make up for the neglect.

Similar sentiments were expressed by President Nixon in February, when, in his health message to Congress, he labelled sickle cell anaemia a targeted disease for concentrated research. "It is a sad and shameful fact that the causes of this disease have been largely neglected throughout our history," he said. "We cannot rewrite this record of neglect, but we can reverse it." With those words, he requested a 500 per cent increase in the budget for research and treatment of sickle cell disease, to a new total of $6 million.

Welcome as this new emphasis on sickle cell anaemia is, simply counting dollars devoted to sickle cell research can offer a misleading impression, for, as many workers are quick to point out, basic research on haemoglobin is often directly applicable to sickle cell research. One scientist engaged on haemoglobin research said last week, for example, that "there is no disease about which we have more sophisticated knowledge".

National programmes for counselling and screening have, however, not kept pace with research into the underlying causes of the disease. It is estimated that about seven per cent of the American black population carry one defective gene, and that one in every five hundred children born of black parents will receive a defective gene from each parent and develop sickle cell anaemia. The disease could, of course, be prevented if partners who both carry the sickle cell trait avoid having children, which requires an effective screening and counselling programme.

As with all such programmes, however, there is the problem of advising partners against having children, when there is only a one in four chance of their offspring developing the disease. Nevertheless, a national programme aimed at providing education and proper guidance on sickle cell anaemia will be a great improvement on the present situation in which the first many people hear about the disease is when one of their children develops it.

Dr C. Lockhard Conley, professor of medicine at Johns Hopkins Hospital in Baltimore and a specialist in haematology, said last week that he believes $25 million a year could be well spent on sickle cell anaemia. Pointing out that the money appropriated recently by Congress for research on sickle cell anaemia would provide each patient with only about $250—enough for about two days in hospital—and that there have been several recent advances in treatment of the sickle cell crisis, Dr

Conley said, "If I had to select a genetic disease for which there is a real and well founded hope of progress, it would be sickle cell anaemia".

President Nixon also used the opportunity to give publicity to his $100 million dollar request for additional funds for cancer research, and to the Administration's cancer cure programme, which was passed by the Senate in July. "I again urge the House of Representatives to act promptly on this matter so we can get on with this important work," he said.

CANCER RESEARCH

Fort Detrick Lives Again

by our Washington Correspondent

A NEAT solution of the twin problems of what to do with a redundant laboratory and of how to spend the vastly increased budget for cancer research was provided this week when President Nixon announced that the former biological warfare research station at Fort Detrick would be turned over to the National Cancer Institute for cancer research. The complex of nine laboratories at the Maryland research station were once the most sophisticated facilities for research on bacteriological weapons in the United States. But all that was stopped in 1969, and the future of Fort Detrick has since been in the balance.

Last year, the Senate voted to give the National Institutes of Health the $15 million needed to convert Fort Detrick's laboratories from bacteriological warfare research to a variety of biomedical research problems. It was then believed that the laboratories would be used chiefly by the National Institute of Allergy and Infectious Diseases and by the National Cancer Institute, for research on hazardous viruses and on suspected tumour viruses. The special facilities at Fort Detrick, which include negative pressure rooms to prevent escape of dangerous organisms and excellent culture and storage facilities, are expensive and ideal for virus research of all kinds.

The Senate's decision, the result of an amendment introduced by Senator Charles Mathias of Maryland, was, however, reversed by a conference committee which asked that the proposals should be further studied by the Department of Health, Education and Welfare. In the meantime, the research station's complement of microbiologists, whose future had been uncertain since the decision to renounce offensive bacteriological weapons was taken in 1969, began to leave the station in droves.

The decision to beat a sword into a ploughshare by transferring Fort Detrick from warfare research to study the problems of cancer was announced last Monday when President Nixon visited the establishment. Pointing out that the transfer would "help advance important public goals even as we alleviate the economic burdens which threaten idle workers and their families", Nixon said that he hopes the conversion would be completed by early 1972. The research station may provide work for up to 700 cancer researchers.

Short Notes

Justification for NASA

A RECENT address by Dr James C. Fletcher, Administrator of NASA, brought out the following juggling of figures to justify NASA's budget: "Let us put NASA's budget figure in perspective along with the rest of the federal budget. $3,300 million is actually less than one and a half cents of the federal budget dollar. We are already spending 42 cents of the federal budget dollar on human resources, including health, income, security and veterans benefits. And we are also spending 34 cents of the federal budget dollar on national defence. So even if the space programme were abolished entirely—which would be a calamity for this country—the amount available for other programmes, one and a half cents, would be relatively insignificant.

"Looking at it another way, $3,300 million is less than one third of one per cent of the gross national product and it is only 16 dollars for each person in this country." Looking at it another way, $3,300 million is $700 million more than Congress appropriated for housing and urban development.

Cannikin

CRITICS of the AEC's plan to explode a five megaton nuclear weapon under Amchitka Island this month were disappointed in their expectation that President Nixon would announce cancellation, or at least postponement, of the test, when he met Emperor Hirohito of Japan recently. The White House is in fact now saying that President Nixon may not have any intention of calling the test off, that an announcement to go ahead can be expected soon now that Mr Kosygin's visit to Canada is over. The test is designed to monitor the X-ray output of the warhead of the Spartan anti-ballistic missile that forms the long-range component of the Safeguard ABM system.

NASA

DATA gathered by the Earth Resources Technology Satellite (ERTS-A) and the Earth Resources Experiment Package (EREP) carried on Skylab will be used to provide information on such diverse topics as vegetation damage from highway construction in Maine to the movement of sediment in San Francisco. ERTS-A will be launched in spring 1972, and Skylab is scheduled for launch early in 1973. These Earth monitoring and data gathering satellites have drawn the greatest number of proposals ever received by NASA for any scientific satellite—700 had been submitted by June this year, of which some 430 have initially been selected.

The satellites, which are intended primarily to study the feasibility of remote sensoring from satellites of natural resources, will provide multispectral images of the Earth's surface. Similar techniques were also employed aboard a high-flying aircraft recently used with some success in an experiment conducted jointly by NASA and the Department of Agriculture designed to gather information on southern corn leaf blight in the United States. The aircraft surveyed 72,000 square kilometres of cornlands between June and September with infrared photography.

Methotrexate

THE Food and Drug Administration has decided to allow the controversial drug methotrexate to be used for severe cases of psoriasis, a debilitating skin disease. The latest issue of the *FDA Bulletin* includes an outline of the cases in which the drug should be used: they include only those cases of psoriasis that have proved to be resistant to conventional treatment, and the drug should be dispensed to patients only by the physician.

This announcement follows three days of hearings on the FDA's handling of methotrexate by the House Intergovernmental Relations Subcommittee, after which Benjamin S. Rosenthal, a vocal critic of FDA policies, and Mr L. Fountain, chairman of the subcommittee, accused the FDA of extreme negligence in taking no action to stop physicians from illicitly prescribing the drug. Mr Fountain warned the FDA that since there had been 71 adverse reactions to methotrexate reported during the eight years that it had been on trial, the drug ought not to be licensed for use against psoriasis.

Pure Chemistry Library,
SCIENCE COLLEGE.
92 Upper Circular Rd
CALCUTTA

NEWS AND VIEWS

Time Lost in Air Travel

No airline has yet used the argument, in its advertising, that ageing is less in high-speed travel because of the relativistic time-dilatation phenomenon. Travel agents should therefore know that J. Hafele of Washington University and R. Keating of the Naval Observatory, Washington, have taken a pair of atomic clocks on a round-the-world air trip to show that this minute effect is indeed measurable (in clocks, if not in people). Time dilatation is, of course, a familiar feature in experimentation, but such a direct test, if intended to settle the "clock paradox" controversy, is something rather different. Followers of this controversy will know that one school has always maintained that a direct test is essential to decide the issue; another school would accept the argument of W. Cochran (*Nature*, **179**, 977; 1957) that π^+ meson experiments, like one performed by R. Durbin and colleagues of Columbia University using the Nevis cyclotron, are equivalent. It is understood that Hafele and Keating used Hewlett-Packard caesium beam atomic clocks, which would presumably be acceptable to everybody.

Relativistic effects at aircraft speeds are mostly well beyond the limit of measurement. If acceleration is negligible, a clock travelling with speed kc (c=speed of light) is slowed by a factor $f=\sqrt{(1-k^2)}\sim 1-\frac{1}{2}k^2$ for small k, when observations are made by a "rest" observer. If the speed of transport is 600 m.p.h., f is about $1-(4\times 10^{-13})$, so that an accuracy of one or two parts in 10^{13} is needed for an experimental test. This is just about the limit of the caesium clock's ability under reasonable conditions. The total time "saved" over a 36-hour trip at the same speed is roughly 5×10^{-8} s.

The next problem that faces experimenters using transported clocks is that of acceleration. An "ideal" clock in relativity theory is one which is not directly affected by acceleration, or more precisely, one for which the clock hypothesis is valid. The clock hypothesis is that the slowing-down factor f still has the value $\sqrt{(1-k^2)}$ at each instant, even when the speed ratio k is variable. It is difficult to establish theoretically whether any but the simplest types of clock obey this hypothesis because the behaviour of the governing mechanism, especially in its rigidity, would need detailed analysis. The easiest way for the experimenter to overcome the problem is to ensure in some way that acceleration effects are kept within known bounds, of a smaller order of magnitude than that of the effect sought. Fortunately, this is possible for the caesium clocks in question, because experience has already been gained in transporting similar clocks by land and air (for example, in Europe). After short journeys, where time dilatation will not have been significant, it has been found that normal handling, take off and landing accelerations, and modest turbulence in flight do not introduce discrepancies in total travel time as recorded by these and laboratory based clocks.

Temperature and pressure variations during their flight should likewise have posed no very great problems for Hafele and Keating. On the other hand, the clocks are sensitive to magnetic field: because each is inherently dependent on the existence of a steady magnetic field, care has to be taken that one does not affect the other when two are placed close together. It is naturally supposed that adequate screening will have been possible.

Finally, there is the most important "extraneous" effect, which, being relativistic in character, must raise doubts as to the validity of the whole experiment or, at very least, as to its importance. This concerns the gravitational "blueshift" predicted by general relativity or, more simply, by the principle of equivalence. Einstein's principle of equivalence asserts that any gravitational field is locally indistinguishable from an equivalent acceleration of the reference system, and so the behaviour of a clock at rest in a permanent gravitational field can be determined by treating it as though it were instead being accelerated in gravitation-free space. Identical clocks, A and B, fixed on a rigid rod which accelerates uniformly in the direction AB do not keep in synchronization; B is observed to run fast, at A, because periodic light signals from B to A are received at an increased rate owing to Doppler shift. The shift arises because A's speed increases during the time the signals are travelling. Therefore, in the equivalent situation of clocks at rest at points with different gravitational potentials, the one (B) at higher potential gains over the other (A) at lower potential. For modest field variations and a small separation of clocks, the ratio of rates is $F=1+U/c^2$, where U is the difference in potential at A and B. Near the Earth's surface, $F=1+gh/c^2$, for clocks vertically separated by a height h. R. V. Pound and G. A. Rebka, jun., of the Lyman Laboratory at Harvard University verified this formula in 1960 using the Mössbauer effect.

When B is an airborne clock and A is at rest on the ground, the combined effects of time dilatation and gravitational blueshift results in a rate ratio fF for $B:A$ (observed from the ground). Taking the flight speed to be 600 m.p.h. the two effects cancel ($fF=1$) when $F\sim 1+(4\times 10^{-8})$, corresponding to a modest flying height of 2¼ miles. If the aircraft flies at a height of 6 miles, the gravitational effect is more than twice as great as the opposing one of time dilatation, and the airborne clock gains about 5×10^{-8} s during a 36-hour trip. It would be easier to separate the two phenomena using an artificial Earth satellite in place of an aircraft, as has been proposed in the past.

In places such as Denver, Colorado (at a height of 1.6 km), it is becoming common in high-precision timekeeping to apply a correction for gravitational potential. This procedure is unexceptional when relativistic time effects are not in dispute. But because similar large-order corrections will have to be applied to the experimental data of Hafele and Keating, their experiment cannot be expected to silence dissidents in the clock paradox controversy. Because time dilatation itself is so commonplace it is hard to see what other purpose the experiment has. No doubt the published account will eventually tell us.

A New Theory of Muscle Contraction

One of the outstanding mysteries of molecular biology is the mechanism whereby chemical energy is converted into work during muscular contraction. In spite of significant advances in the past few years in knowledge of the details of muscle structure and of the biochemical reactions involved in contraction, no comprehensive theory has yet been proposed which satisfactorily explains the molecular events involved in this process. A major difficulty lies in the complete ignorance of the nature of the basic mechanism involved. Mechanisms which have been proposed range from mechano-chemical processes, as suggested by Davies (*Nature*, **199**, 1068; 1963), to the electrostatic mechanisms proposed by Elliott, Rome and Spencer (*Nature*, **226**, 417; 1970).

Experiments on muscle contraction tend to fall into one of three distinct categories—structural, biochemical or physiological—and these apply also to contraction theories, which may explain observations from one area, but frequently give only a superficial consideration to observations from the other areas. Because results obtained in one category normally complement results obtained in the other two, a complete explanation of the contraction process can only come when results from all areas are melded into a single theory. Data from structural studies can provide the framework in which biochemical processes producing the energy conversion can take place. It is physiological data, however, which can test the relevance of any structural or biochemical theory to living muscle and its normal behaviour.

On page 533 of this issue of *Nature*, A. F. Huxley and R. M. Simmons propose a theory of contraction which combines observations from two of the three categories—structural and physiological. Their theory has evolved from A. F. Huxley's theory (*Progr. Biophys.*, **7**, 255; 1957) which has been reworked to take account of recent structural and physiological observations.

Huxley's original theory followed the discovery in 1954 of the sliding filament mechanism for muscle contraction. Huxley suggested that contraction resulted from the formation of actin–myosin links with specific elastic properties between the two types of filaments. The rates of making and breaking the links governed the extent and speed of the muscle's contraction. Calculations based on this theory could account for several known physiological relationships in muscle. Details of the structural components and of the biochemical reactions could not, however, be specified.

Recent experiments by Huxley and Simmons on transient tension effects in single fibres of frog muscle after quick stretches or releases have enabled them to specify certain properties of the actin–myosin links. Although Huxley and Simmons state that their general proposals can be applied to several structural models and are similar to a much earlier theory of visco-elastic behaviour of high molecular weight polymers proposed by Eyring (*J. Chem Phys.*, **4**, 283; 1936), they have based their theory on H. E. Huxley's recent structural model (*Science*, **164**, 1356; 1969).

In H. E. Huxley's model, myosin and actin filaments slide past one another (and thus cause shortening) through the formation of "cross-bridges" between the two types of filaments. Each cross-bridge results from the attachment of a projecting part of the thick myosin filament (myosin "heads", equivalent to heavy meromyosin subfragment one) to specific sites on the thinner actin filaments. Shortening at each site is produced by a change in the angle that the cross-bridge makes with the filament axis so that the actin filament is pulled relative to the myosin filament. Individual cross-bridges are formed cyclically to produce a smooth shortening motion. The head of the myosin molecule is able to bridge the variable gap between the filaments by swinging out from a hinged region part way along the straight and otherwise rigid shaft of the molecule. A cross-bridge is formed when the myosin head attaches to an actin filament. The part of the myosin molecule which hinges outward corresponds to heavy meromyosin subfragment two.

The experiments of A. F. Huxley and Simmons suggest the existence of two elastic elements in series which they identify with the cross-bridge structure. A stiff, undamped elasticity is assigned to the straight, hinged part of the myosin molecule (subfragment two), and a damped elasticity, with a time constant of a millisecond or less, to the myosin head–actin complex. During contraction each myosin head–actin attachment shifts through a series of stable positions (probably three), each with a lower potential energy than the last. The motion so produced, when coupled with the elasticity of the straight connecting link, can give a smoothly-changing force between the filaments. The authors have observed in experiments that a rapid change of length in the muscle fibre is associated first with a rapid tension change which parallels the length change (an undamped elasticity) and then with a (damped) recovery to a tension intermediate to the initial tension and that at the end of the length change. The tension at the end of a length change, that after the rapid recovery, and the rate of tension recovery were measured as a function of the length change imposed. The curves obtained were fitted closely by curves calculated from the theory.

To this extent, then, the theory agrees well with experimental observation. Huxley and Simmons imply, but do not demonstrate here, that their theory can also account for such established physiological data as force-velocity curves and heat production. This aspect will doubtless be covered in a fuller treatment promised for the future. The theory should also be checked quantitatively against such other observations as the length transients appearing after rapid tension changes measured by Podolsky and his colleagues (see *Proc. US Nat. Acad. Sci.*, **64**, 504; 1969). Equally important, is the theory compatible with the biochemical knowledge of muscle contraction—the energy requirements during contraction, and the roles of adenosine triphosphate, phosphocreatine and calcium?

The success of any theory ultimately resides in the future experiments it stimulates. If the Huxley and Simmons theory enables physiologists and others to develop meaningful experiments to test its predictions against existing structural and biochemical knowledge, it will have done valuable service to muscle research, whatever its fate in the archives of contraction theories.

GLYCINE

In Nerve Terminals

from our Neurochemistry Correspondent

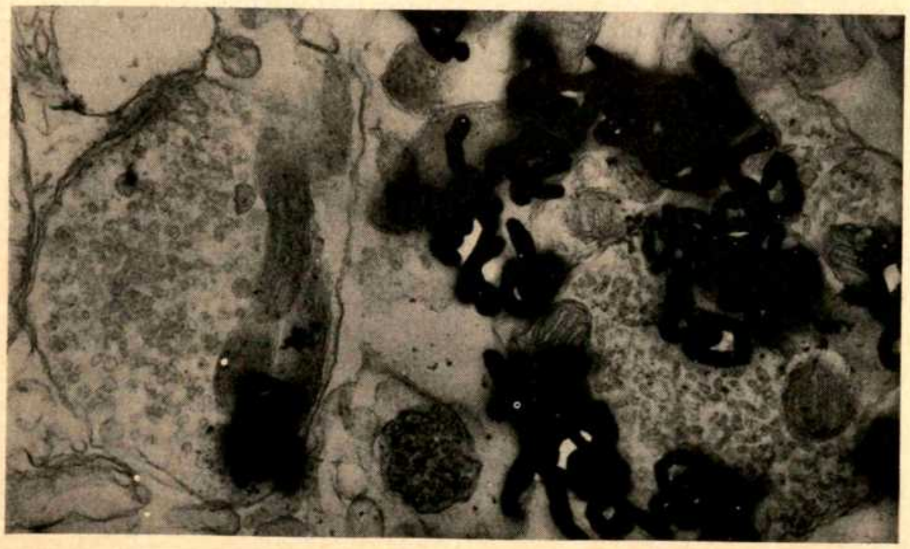
By one of those strange coincidences which occasionally arise in the world of scientific research, the current issue of *Brain Research* contains reports of two studies carried out independently by T. Hökfelt and Å. Ljungdahl at the Karolinska Institute, Stockholm, and by A. I. Matus and M. E. Dennison at University College, London, who have demonstrated by autoradiography that tritiated glycine, after incubation with slices of rat spinal cord, becomes associated predominantly with nerve terminals (*Brain Res.*, **32**, 189 and 195 ; 1971).

Glycine, apart from its universal role as an essential component of proteins, is believed to serve an additional function in the spinal cord as a mediator of transmission at inhibitory synapses. Inhibition in the nervous system plays a crucial part in the regulation of neuronal activity ; in the spinal cord, for instance, the motoneurones of the ventral horn, which give rise to axons transmitting information to skeletal muscles, are subject to a variety of inhibitory influences. Evidence implicating glycine as a spinal inhibitory transmitter (reviewed by Hebb in *Ann. Rev. Physiol.*, **32**, 165 ; 1970) arose from electrophysiological studies showing that iontophoretic application of glycine to certain cells in the cord causes an increase in the membrane potential of those cells similar to that occurring during natural inhibition. This hyperpolarization is blocked by the convulsant alkaloid, strychnine, which also blocks certain spinal inhibitory pathways. Furthermore, the distribution of endogenous glycine in the spinal cord suggests that it is concentrated to an extent greater than would be required for a general "metabolic" function in areas where inhibitory neurones may be present.

If glycine is a neurotransmitter, a mechanism should also exist for its rapid removal from extracellular sites after it has exerted its physiological effects. Transmitter inactivation may be achieved by enzymatic destruction, by uptake into adjacent cells or nerve endings, or simply by diffusion from the site of action. It has been demonstrated that slices of rat spinal cord can accumulate exogenous radioactively-labelled glycine from an incubation medium by a high-affinity active transport process, but until recently there was little evidence as to the precise cellular sites responsible for this uptake. In the cerebral cortex, where γ-aminobutyric acid (GABA) is believed to be the principal inhibitory transmitter, there is an analogous uptake system and subcellular fractionation studies have shown that a proportion of the labelled amino-acid taken up into slices may be recovered in a fraction containing nerve endings. Moreover, Bloom and Iversen (*Nature*, **229**, 628 ; 1970) were recently able to demonstrate by electron microscope autoradiography of cortex slices or homogenates after incubation with ^{3}H-GABA that the label becomes localized over certain nerve terminals and preterminal axons. The present studies, by Hökfelt and Ljungdahl and Matus and Dennison, were an attempt to localize similarly the sites of uptake of ^{3}H-glycine in slices of spinal cord using autoradiography at both the light and electron microscope levels.

The results from both groups are strikingly similar. Light autoradiograms showed a higher concentration of grains over grey than white matter, and, at the ultrastructural level, heavy accumulations of grains were seen over certain terminals, whereas others showed no activity. Matus and Dennison suggest that those terminals which were heavily labelled are those containing "flat" as opposed to round vesicles (see figure), although this possibility is not discussed by Hökfelt and Ljungdahl. This suggestion is, however, of considerable interest in view of the hypothesis of Uchizono (*Nature*, **207**, 642 ; 1965) that the shape of vesicles present in terminals may be correlated with the physiological role of those terminals—excitatory endings containing round vesicles and inhibitory endings with flattened ones.

Previous studies have shown that of the labelled glycine taken up by spinal cord slices, only 2 per cent is incorporated into protein, the remainder being recoverable as free glycine. It has also proved possible to demonstrate the release of labelled glycine from previously loaded tissue slices by electrical stimulation (J. M. Hopkin and M. J. Neal, *Brit. J. Pharmacol.*, **40**, 136 ; 1970). This suggests that exogenous glycine may enter the same sites as are occupied by the endogenous transmitter, and the present results may thus be interpreted as evidence that glycine is present in certain inhibitory nerve terminals of the spinal cord, thus adding considerable weight to the hypothesis that glycine is a spinal inhibitory transmitter.



Electron microscope autoradiograph of a selected area from the ventral horn of rat spinal cord, after incubation in tritiated glycine, showing heavy accumulation of grains over a "flat" vesicle synapse on the right as compared with one containing round vesicles on the left. ×27,200. (From *Brain Res.*, **32**, 195 ; 1971.)

TROPICAL ECOLOGY

Man-made Changes

from a Correspondent

Three areas of concern were highlighted at the meeting of the Tropical Ecology Group of the British Ecological Society at University College, London, on October 4: first, the deterioration in the resources for cattle ranging following successful animal health control measures in Africa; second, the replacement of polyspecific forest and grassland by forest monocultures; and, third, the effects of tourism on tropical islands. The medical implications of large-scale irrigation for rice development in Kenya were also considered.

Contributions on cattle ranching in tsetse-fly infested savanna in Tanzania, by Mr J. Ford (University of Oxford), and on the effects of animal health control measures against rinderpest, by Mr A. Blair Rains (Land Resources Division, Overseas Development Administration), both illustrated very clearly, and with dramatic photographs, that increases in cattle numbers are followed by encroachment of shrubs, such as *Acacia* species, into grassland areas. Mr Ford's examples came from a large-scale attempt to ranch cattle, using drug prophylaxis against trypanosomiasis, started in eastern Tanzania in 1954; meat production increased for about ten years, then declined as range conditions deteriorated. Mr Blair Rains's examples came from Botswana and the Gambia, where control against rinderpest and other bovine diseases has been startlingly successful. Although cattle marketing is developing, large herds are still kept for traditional purposes, water supplies are no longer limiting, and inability to control stocking levels has resulted in either shrub encroachment or in the nearly complete destruction of vegetation over very large areas around water points.

Dr D. C. Dawkins (Commonwealth Forestry Institute, Oxford) spoke on the effects of forest management, with operations which range in scale from merely weeding out hollow trees, resulting in the loss of holes for tree dwelling fauna, to total clearing and replacement by forest monocultures, often of exotic species. His colleague Mr P. J. Wood then discussed ecological anxieties arising from these monocultures which are now replacing forest and grassland in many tropical lands; in the East African Highlands alone some 15,000 hectares of conifers are being planted each year. The long-term effects of these monocultures have to date been little studied, but the evidence available suggests that the resulting erosion and alteration of soil profiles present greater dangers than the introductions of pests and pathogens, or than changes in water balance or soil microflora. The "ecological spillover" effects are most in need of study. The ensuing discussion brought out the problems of ensuring that areas of indigenous forest are preserved for conservation purposes in countries which are now independent.

The unprecedented pressures of affluence and cheap travel on the limited tropical littoral resources of West Indian islands were treated in stimulating and well illustrated contributions by Mr M. A. Brunt (Land Resources Division, Overseas Development Administration) and by Dr M. E. C. Giglioli (Mosquito Research and Control Unit, Cayman Islands). In Jamaica, the replacement of mangrove swamps by man-made beaches has provided suitable breeding places for biting sand flies (*Culicoides*), with the result that there have been local infestations of these insects. In the Cayman Islands, mangrove swamps have been flooded to control the biting mosquito *Aëdes taeniorhynchus*, and cleared and filled with marl for housing development. The cleared areas have remained bare of vegetation, leaving the land exposed to damage from hurricanes which frequent this area. Dr Giglioli described aerial spraying operations, and stressed the importance of inviting local people to share in control operations to ensure the acceptance of the necessary measures by the local community.

OCEANIC RIDGES

Problems with Chemistry

from our Geomagnetism Correspondent

THE general hypothesis of seafloor spreading envisages the upwelling of peridotitic mantle material beneath mid-oceanic ridges which are known to be regions of relatively high heat flow. Partial melting of the material then takes place producing basaltic liquids which are injected into comparatively narrow regions near the ridge axis. The existence of high thermal régimes beneath ridge axes implies that beneath ridges the silicate melts are generated at shallower depths, and thus lower pressures, than would normally be the case. Because the compositions of liquids arising from partial melting of mantle material are strongly dependent upon the pressure under which such liquids are produced, the prediction is then made that the compositions of basalts injected at ridge axes should be significantly different from that of volcanic rocks produced well away from ridges. In particular, if the simple theory is valid, ridge basalts should tend to be tholeiitic whereas non-ridge basalts would be expected to be more alkaline.

But these cherished assumptions have taken a sharp knock with the publication by Bonatti and Fisher (*Earth Planet. Sci. Lett.*, **11**, 307; 1971) of compositional data for basalts from the East Pacific Rise and other Pacific sites removed from the rise. Most of the rise rocks in question were from a basalt pavement which outcrops along the axis of the rise between 14° S and 6° S though two others were obtained from 18° S and near to the equator, respectively. All were less than one million years old according to radiometric or fission track dating. The non-rise samples were collected from five seamounts lying between 450 and 1,500 km from the rise axis and all were less than five million years old, again according to radiometric or fission track dating. It is possible, of course, that the seamount samples were emplaced at the rise axis and subsequently spread away to their present positions and if this were so—and assuming a reasonable spreading rate of 4 cm a year—their ages would lie in the approximate range 11–35 million years. The measured ages rule this out, however, and suggest that, on the contrary, the seamounts were formed more or less in their present positions. The contrast between rise and non-rise basalts is thus valid.

The surprise comes from comparisons of normative compositions and the degree of silica saturation for the rise and non-rise basalts. When normative compositions are plotted on the Q-Di-

Similarities between Pulsar Models

THE two widely favoured rotating neutron star models for pulsars differ less widely than is sometimes believed, according to an article by L. Mestel, of the University of Manchester, in next Monday's *Nature Physical Science*. These two models are the axisymmetric model of Goldreich and Julian (the "non-oblique rotator") and the oblique rotator of Pacini and Ostriker and Gunn.

Goldreich and Julian found that for their model, with its relatively dense magnetosphere, the vacuum electric field ($\mathbf{D} = \varepsilon_0 \mathbf{E}$) just outside the surface of the star has a component along the magnetic field which produces electrical forces on the charges in the surface layer which are much greater than the gravitational forces. This results in a motion of electrons out of the star, so that the steady state of the model includes a charged magnetosphere. Currents produced by the bulk motions of the charge distribution determine a field structure typified by a corotating region of field lines which close within the light cylinder and a wind zone defined by the lines of force which cross the light cylinder, and allow charge to flow outwards. But even at points well within the light cylinder the magnetosphere is relativistic, in the sense that the magnetic energy density is much greater than the mass energy density.

When the angle between the magnetic and rotation axes is non-zero, the electromagnetic and plasma fields around the pulsar are non-steady. But it seems inevitable that charges will still be removed from the star, as in the Goldreich and Julian model. The details of the magnetic field structure are less easy to determine than for the axisymmetric case, but Mestel puts forward a qualitatively similar view in which, as for the Goldreich and Julian model, the field obeys the curl free (dipole) approximation to a good accuracy at points well within the light cylinder. For both models, provided that the hydromagnetic condition

$$\mathbf{E} + \frac{\mathbf{V} \times \mathbf{B}}{c} = 0$$

is obeyed, energy outflow occurs only along field lines which cross the speed of light cylinder, and classic magnetic dipole radiation from the curl free zone is suppressed. For a poloidal field the torque will vary as $(\Omega a/c)^{2n-3}$. For a dipole $n=3$, but the work of Pacini and Rees (*Nature*, **226**, 622; 1970) suggests that in the Crab pulsar $n \sim 2.6$, which tends to confirm the non-dipole nature of the energy loss mechanism.

Hy-Ol-Ne diagram it becomes clear that there is no systematic tendency for the non-rise samples to be more alkaline. Furthermore, when the Niggli Quartz Index for each sample—a measure of the amount of silica relative to Al, Fe, Mg, Na and K in the rock—is plotted as a function of distance from the rise it becomes equally apparent that there is no significant tendency for the non-rise basalts to have lower silica saturations. In short, as far as these key parameters are concerned the basalts are indistinguishable. Nor do any differences become apparent when the data are plotted on a silica-alkali diagram of the type used by MacDonald and Katsura (*J. Petrol.*, **5**, 82; 1964) to differentiate between alkaline and tholeiitic basalts in Hawaii.

Bonatti and Fisher interpret this information to mean that both rise and non-rise basalts must be produced within the same depth range, and hence the same pressure range, within the upper mantle. This then implies that the differences in heat flow between the rise region and other areas do not indicate that beneath the rise there is a higher thermal zone which reaches down to the region of magma generation. The heat source which accounts for the higher heat flow must lie above the region of magma production, though it is not immediately clear whether or not such a conclusion conflicts with the simple picture of mantle upwelling at ridges and sinking beneath trenches. An alternative interpretation—that pockets of magma produced beneath the rise are caught in, and spread with, the lower crust only to break through to the surface at a later date—seems to be ruled out by the fact that during the spreading the magma would undergo fractionation and differentiation, processes which would themselves give rise to a compositional difference between rise and non-rise basalts.

PROTEINS

Finding the Way

from our Molecular Biology Correspondent

Arguably the most interesting problem in protein chemistry at present is just how a randomly coiled polypeptide chain finds its way through the almost infinite maze of possible conformational states that it may assume to the haven of the native conformation. It can safely be asserted only that the chain cannot within any reasonable time scale approach it by a process of random wandering, but that there must be pathways determined by the sequence. A widely favoured hypothesis is that the starting points are segments that readily form an ordered structure, such as a few turns of α-helix or a β-fold. This would imply that the parts of the sequence most prone to enter these conformations in the random chain will also be α-helical in the finished article—the folded, rigid globular protein. Scheraga has given arguments, based on the helix-forming potential of particular amino-acids, compared with their occurrence in α-helixes in proteins of known structure, that such is indeed the case. Not even the most sanguine supporters of the theory would claim, however, that the evidence is conclusive, but at present there is no other to which its opponents can rally.

Scheraga and his colleagues are now attempting to place the calculations of helix stability in relation to amino-acid composition on a quantitative basis, and their strategy is outlined in a new series of articles. The stability of an α-helix is defined by two equilibrium constants, σ and s, which define respectively the initiation of an α-helix in a randomly coiled homopolymer chain, and the incorporation of a residue into an existing helix. The well known cooperativity of helix formation and breakdown carries the implication that σ is much the smaller. In homopolymers helix-coil transition profiles can be used to determine both parameths. Von Dreele *et al.* (*Macromolecules*, **4**, 396; 1971) have re-worked the statistical mechanics of the system, with some new approximations, to include polymers containing a proportion of a foreign residue with different σ and s. From the effect of such "guest" residues on the helix-coil transition profile of the "host" polymer, values of the constants can be determined, and Von Dreele *et al.* (*ibid.*, 408) report that for a test case the σ and s values extracted for a given "guest" residue agree well with those determined

Arvin as an Anticoagulant

Fibrinogen is a plasma protein which plays a constitutive and initiative part in the haemostatic mechanism of wound healing. But it is also involved in the formation *in vivo* of fibrin deposits with the grave clinical consequences of disseminated intravascular coagulation, for example, thrombosis and arteriosclerosis. The production of thrombin *in vivo* triggers the following simplified scheme:

$$\overset{\text{Fibrinogen}}{(\mathrm{A}\alpha, \mathrm{B}\beta, \gamma)_2} \xrightarrow[\mathrm{Ca}^{++}]{\text{Thrombin}} \overset{\text{Fibrin clot}}{(\alpha, \beta, \gamma)_p} \xrightarrow[\mathrm{Ca}^{++}]{\text{Factor XIII}} \overset{\text{Crosslinked fibrin clot}}{(\alpha^p, \beta, \gamma^2)_p}$$

where p denotes polymer and factor XIII ≡fibrin stabilizing factor (FSF).

In recent years it has been discovered that certain enzymes from snake venoms can induce formation of fibrin clots from plasma fibrinogen and these clots differ in stability and structure from thrombin-initiated clots. The most used venom enzymes, arvin ('Ancrod') and reptilase, remove only fibrinopeptide A, whereas thrombin removes fibrinopeptides A and B from the Aα and Bβ chains of the fibrinogen molecule respectively. Further differences in the mechanisms of these enzymes are described by Mattock and Esnouf in next Wednesday's *Nature New Biology*. They use the technique of SDS–acrylamide gel electrophoresis to explain at a molecular level the action of arvin as an anticoagulant and thus help to bridge the gap which exists between empirical clinical practice and biochemical rationale.

Mattock and Esnouf corroborate the findings of McKee *et al.* (*Proc. US Nat. Acad. Sci.*, **66**, 738; 1970) that thrombin in the presence of calcium ions activates factor XIII which mediates clot stabilization by the formation of γ chain dimers and α chain complexes of molecular weight in excess of 500,000. They show that reptilase works in a similar manner but in the absence of calcium ions and this would explain its role as a potent topical haemostatic agent. On the other hand, arvin is shown to remove 31,000 molecular weight portions of the α chain with no formation of α chain complexes. It can be suggested that the arvin susceptible areas of the α chains are those which would have taken part in amide cross-linking mediated by factor XIII. Arvin was also shown not to activate factor XIII because very little γ dimers were formed. Non-crosslinked fibrin clots are readily degraded by intrinsic plasmin to soluble non-clottable fibrin degradation products and the role of arvin as an anticoagulant by a defibrination mechanism was demonstrated at a subunit level.

It is interesting to speculate whether arvin might be thrombolytic because Mattock and Esnouf indicate that it degrades the α chain complexes produced by thrombin. Their argument for suggesting that this highly interesting proteolytic activity of arvin could not result from an additional plasmin-like enzyme in the arvin preparation is not supported by the recent evidence that plasmin produces 30,000 molecular weight fragments from the Aα chain of fibrinogen (Gaffney and Dobos, *FEBS Lett.*, **15**, 13; 1971). But in spite of this contradiction, Mattock and Esnouf have amply demonstrated the mechanism by which an arvin preparation acts as a controlled anticoagulant and they illustrate the part that biochemical techniques can play in understanding the principles underlying thrombus formation and dissolution.

directly from measurements on a homopolymer of the "guest" species alone. Ananthanarayanan *et al.* (*ibid.*, 417) have started on the long road to σ and s values for all twenty amino-acids, with glycine as guest (which, because polyglycine is not α-helical, and in any case quite insoluble in water, cannot be examined directly). Glycine turns out by this method to have low s, and should thus function as an α-helix breaking residue, as had been generally supposed.

Lewis, Momany and Scheraga (*Proc. US Nat. Acad. Sci.*, **68**, 2293; 1971) have now considered the β-bend, where a chain folds on itself to form a peptide hydrogen bond between one residue and another four positions along the chain (the third residue being glycine in the best defined form). These folds are relatively common in globular proteins, and Lewis *et al.* have examined a series of proteins of known structure, and worked out the probability that a given amino-acid will be found in each of the four positions defining the β-bend. Armed with these probabilities they have then scrutinized the sequences of other proteins in order to predict where β-bends should occur. Comparison with the X-ray structures showed (depending on how precisely the start of the bend is defined) a moderate correlation between expectation and reality. Of course this suggests only that there are steric preferences for a β-bend, not necessarily that these structures persist the denatured chain. Lewis *et al.* infer, nevertheless, that the bends may be nuclei for folding, and act by bringing remote parts of the chain into appropriate contact.

The experimental approach to the same folding problem has been pressed by Anfinsen and his colleagues. Epstein *et al.* (*J. Mol. Biol.*, **60**, 499; 1971) have now examined further the refolding of staphylococcal nuclease from the acid denatured state. Their handle on the reaction has been the change in fluorescence of the solitary tryptophan residue, which was followed by stopped-flow fluorimetry on neutralization. The reaction, it turns out, can be fitted by two first-order rate processes. The rate constant for the slower process is strongly temperature dependent, that for the faster scarcely at all. The most attractive explanation is that one process consists of the formation of nuclei for the folding, and the other the folding process itself. It is facile, but irresistible, to suggest that the temperature-independent process has the attribute of an entropy-driven nucleation. The scheme is certainly plausible, though it must be recognized that the acid-denatured form of a protein chain will not in general be a true random coil (as, for example, in concentrated guanidine hydrochloride solutions), and must be expected to contain some (though not of course necessarily the right) structural elements. Another facet of the same reaction comes to light in a high-resolution proton magnetic resonance study from the same laboratory (Epstein *et al.*, *Proc. US Nat. Acad. Sci.*, **68**, 2042; 1971). Resonances from the four histidine residues can be individually observed in the native protein, whereas in the acid-denatured state all become environmentally equivalent, and merge into one. For three of the histidines the *p*H profiles are identical, and coincide with that of trytophan fluorescence, but for the fourth there is a biphasic profile. This implies the existence of at least one intermediate, partly folded state in the equilibrium between the native and acid-denatured conformations.

CRYSTAL GROWTH

Origin for Epitaxy

from our Materials Science Correspondent

A RECENT article (S. A. Kobzareva and G. I. Distler, *J. Crystal Growth*, **10**, 269; 1971) adds a further stone to an unusual but increasingly solid edifice built over the past few years by Distler and his colleagues of the Institute of Crystallography in Moscow. Distler claims to have established a hitherto unconsidered origin for epitaxy—that is, the growth of one crystal species on a crystalline substrate of a different species, in an orientation defined by that substrate. He proposes that electric fields emanating from the surface of the substrate control the orientation, size and density of the epitaxial growths. Impressive evidence for this was published some time ago in *Nature* (Distler and Zvyagin, **212**, 807; 1966; Distler and Obronov, **224**, 261; 1969): it was then reported that a sufficiently thin amorphous intermediate layer between substrate and deposit failed to inhibit epitaxy, and furthermore the power to induce epitaxy could be "imprinted" on the amorphous layer (selenium in this instance) by irradiating it before removing it from the substrate. The imprinted amorphous layer, by itself, supported an epitaxial growth of anthraquinone. These results were met by understandable scepticism and at least one investigator failed to reproduce Distler's early findings on the NaCl/amorphous C/gold system, but subsequently C. A. O. Henning (*Nature*, **227**, 1129; 1970) did confirm the findings.

Distler points out that all ionic crystals have charged point defects and assemblies of defects which can under certain circumstances—photoexcitation being particularly favourable—induce substantial permanent dipole moments

---

Composition of Lunar Spinels

WORK on lunar samples has fostered concentrated attention on the nature and properties of several mineral groups, among them the spinels. Lunar samples have been made available to the investigators for limited periods, and rapid publication at conferences and in journals has been encouraged, so as to stimulate discussion and ideas for work on samples for future missions, and perhaps even influence the planning and location of later landings. The publications have thus had more of the flavour of progress reports than is perhaps normal. Each successful mission brings new samples, with additional spinels, so that the reports tend to be cumulative, as indeed is Haggerty's article in next Monday's *Nature Physical Science*. He reviews the compositional features reported by several investigators (including himself) of spinels from Apollo 11 and 12 missions, seeing them in the context of his new results (electron probe analyses) on spinels from Apollo 14 samples.

The major element concentrations in the Apollo 11 and Apollo 12 spinels placed them approximately in the range chromite ($FeCr_2O_4$)-ulvöspinel (Fe_2TiO_4). Apollo 12 spinels occupied each end of the range whereas those from Apollo 11 occupied the middle. Haggerty discusses whether or not this indicates the possibility of a complete range of solid solution, and in order to do this he stresses the roles of magnesium and aluminium which are also present in these spinels.

Haggerty finds that the Apollo 14 samples contain three kinds of spinel, some rather similar to the titanium chromites from Apollo 12, some comparable with the intermediate Cr-Ti spinels from Apollo 11, and some not observed hitherto in lunar samples, which are chromium-bearing members of the series $MgAl_2O_4$-$FeAl_2O_4$. He also uses extrapolations of published thermodynamic data to deduce the minimum oxygen fugacity needed for the stability of each of the spinels, and suggests an increasing fO_2 with falling temperature as crystallization proceeds.

In the absence, so far, of phase equilibrium studies on synthetic spinels of relevant composition, the "circumstantial evidence" of such natural specimens (lunar and terrestrial) as are available is the best guide to chemical relationships. In this situation, however, it is possible that the picture will change somewhat each time more spinels are analysed, particularly if they come from new samples representing possibly different chemical environments.

and consequential fields at the surface. In effect, the crystals contain electrets, the electrical analogue to magnets. Just how the electric fields control the nucleation and growth of epitaxial layers—especially when these are electrically conducting—Distler does not attempt to propose, but it must be admitted that the circumstantial evidence is increasingly compelling.

In the latest article, Kobzareva and Distler make use of a recently established technique to deduce the sign of the local electric field from the local size alignment and packing density of epitaxial anthraquinone crystals on triglycine sulphate (TGS), NaCl and mica (this time without an amorphous barrier). TGS is ferroelectric and so it would be expected readily to "radiate" strong local fields. It was found that on each substrate, two kinds of deposit morphology were found, associated (it is supposed) with positive and negative fields. This hypothesis is reinforced by the direct observation, some time ago, of electrical heterogeneity on a mica surface, using an electrometer, whereas similar heterogeneity was established by an electron microscope technique applied to NaCl (Distler, *Fizika Tverd. Tela*, **11**, 547; 1969). The implication of this latest study, taken together with the earlier ones, is that not only the nucleation but also, to some extent, the growth habit of the epitaxial deposit is controlled by electric fields emanating from the substrate. Distler insists, as he has done in his earlier work, that this makes epitaxial growth strikingly similar to some biological replication process. It now remains to discover how electric fields manage to do it!

FISH BIOLOGY

More about the Eel

by our Marine Vertebrate Correspondent

A RECENT article by E. M. Pantelouris, A. Arnason, and F.-W. Tesch (*Marine Biology*, **9**, 242; 1971), which compares the electrophoretic fractions of esterases, transferrins and haemoglobins in samples of eels from both sides of the North Atlantic, adds a little to the solution of the Atlantic eel "problem". The authors studied samples from Rhode Island and Iceland and their data show both quantitative and qualitative differences between the populations; the qualitative differences are the most striking. The absence of transferrin *D* from the American eels confirms the results of other workers. Furthermore, there are differing electrophoretic mobilities of all fractions making up zones *b* and *c* of the plasma esterases, as well as differences in enzymatic activity and the susceptibility to mercuric chloride of zone *C* of plasma esterases.

Pantelouris and his colleagues note that there were quantitative differences within the samples of the European eel (*Anguilla anguilla*) and speculate that this can be attributed to more than one cause. They suggest that, for example, the European eel may form several subpopulations which are related to different areas of spawning; alternatively, differential selection may distort the phenotype frequencies in which the samples are taken. The authors also note that the unequal susceptibility of certain homologous esterases of mercuric chloride in their samples might be attributable to mercury pollution of the habitat. Pantelouris *et al.* propose to examine this question, and they also plan to test the validity of the differences found between *A. anguilla* and the American eel, *A. rostrata.*

Current interest in the differences between the North American and the European freshwater eels stems largely from the presentation in 1959 by D. W. Tucker of a "New Solution to the Atlantic Eel Problem" (*Nature*, **183**, 495; 1959). Tucker's solution was to suggest that *A. anguilla* and *A. rostrata* are bred of *rostrata* stock in the Sargasso Sea spawning area; all European eels perish before spawning; and such differences in meristic features, such as vertebrae, used by the Dane Johannes Schmidt, and others, to distinguish between the species are caused by changes in water temperature encountered by the larvae at the spawning site. Tucker's hypothesis has gained few serious adherents, but the popular appeal of a "suicide migration" of Europe's eels (to say nothing of the implications for commercial fisheries) has attracted considerable attention.

Before his death, Anton Bruun (*Advances in Marine Biology*, **1**, 137; 1963) had nearly completed a detailed investigation into the chief props of Tucker's theory, and this work shows that the data available do not wholly support Tucker's conclusions. As early as 1961, Gemeroy and Boyden (*Serological Museum Bulletin*, **26**, 7; 1961) had examined the new solution by serological methods and their results show that the sera of eels from North America and Europe differ enough to support the specific distinction between

Flat Revertants Isolated

CELLS transformed by tumour viruses are usually insensitive to density dependent or "contact" inhibition of multiplication; as a result cultures of transformants grow to much higher densities than do cultures of the parental untransformed cells and this phenotypic character of transformed cells forms the basis of methods for their isolation. Not all transformed cells grow to high densities, however, for so-called "flat revertants"—transformed cells which grow to only low saturation densities and in this respect are indistinguishable from untransformed cells—have been selected from populations of typical high density transformants. And now Scher and Nelson-Rees describe in next week's *Nature New Biology* a method for isolating directly SV40 transformed 3T3 cells which grow only to low saturation densities. Furthermore, they have shown that these cells grow only to low densities not because they have retained their susceptibility to "contact inhibition" but because at confluence the cells die as fast as they divide.

Scher and Nelson-Rees's technique is simply to select from cultures of mouse 3T3 cells infected by SV40 those cells that are able to grow in media which lack the serum factors required for the survival and proliferation of untransformed 3T3 cells. Having isolated in this way transformants which do not depend on serum factors, they then measured their saturation densities; most clones grew to densities between 2.5 and 10.5-fold greater than untransformed 3T3 cells, but about 15 per cent of the transformed cell clones grew only to the same density as the parental cells.

In an attempt to explain the unusual behaviour of these "flat" transformants, Scher and Nelson-Rees measured their ability to incorporate thymidine into DNA and the extent of mitosis in confluent cultures. They found during a two day period between 50 and 70 per cent of the "flat" transformant cells make DNA and during a 60 hour period about 50 per cent of the cells divide. By contrast, less than 1 per cent of a confluent culture of untransformed 3T3 cells divides during 60 hours. Clearly therefore these "flat" transformants continue to divide at confluence even though the number of cells in the culture does not increase significantly. Cells must therefore be dying at about the same rate as they are multiplying. An analysis of the chromosomal complement of these directly isolated "flat" transformants reveals that although several lines have more chromosomes than the parental cells, at least two have about the same number of chromosomes as the parental cells.

These experiments once again draw attention to the fact that the phenotypic characters used to diagnose viral transformation can be dissociated from one another. They also rub home the lesson that cells may grow to only low saturation densities for reasons other than susceptibility to "contact" or density dependent inhibition of division.

the species. So different are they in fact that they approach the limits of intrageneric relationship when compared with other fish taxa. This recent article by Pantelouris *et al.* and the substantial body of literature on eels that has accumulated since 1959 all tend to support Schmidt's classical hypothesis.

ULTRASONICS

Recent Applications

from a Correspondent

THE "Ultrasonics 1971" conference and exhibition was held in London on September 28 and 29 and was the seventh of the series organized by the journal *Ultrasonics*.

On the opening morning, the session on underwater sound was notable for an account by Professor W. D. Chesterman and Dr J. C. Hopkins (Bath University of Technology) of the recording of side-scan sonar signals on magnetic tape—to be replayed later under controlled conditions or into an oscillograph system so that the pictures can be filmed to give true-to-scale survey records. In a fascinating contribution, Dr R. W. G. Haslett (Kelvin Hughes Ltd, Ilford) described the use of sound in fishing and indicated how model investigations on the echoes could be correlated with the average size and shape of fish in a shoal.

Drs M. Topp and P. Eisenklam (Imperial College, London) drew attention to the importance of ultrasonic atomizers in oil burners, as medical nebulizers, as metal sprayers and the like; their use in Britain is, however, not extensive. Ultrasonic atomizers derive their power from magnetostrictive transducers (20 to 125 kHz) or piezoelectric transducers (30 kHz to 5 MHz). One such ultrasonic atomizer involves an angular bowl-shaped concentrator which is capable of atomizing a 100 μm sheet of solid aluminium placed across the mouth, using only a 100 W generator. Although ultrasonic cavitation produces much more drastic effects in polymer solutions than other degradation techniques, Dr P. Vaughan (Letchworth College, Hertfordshire) emphasized that such processing by ultrasound still remains a research undertaking. In the biological field, Drs W. I. Coakley and C. J. James (University College, Cardiff) spoke on the activity of enzymes released from yeast by sonification. They showed that the rate constant for enzymatic denaturation is dependent on the protein concentration and that for optimum results the cell concentration should be high.

In the afternoon session certain general aspects of ultrasonic testing were considered and there was one very significant contribution by Drs E. E. Aldridge and H. G. Tattersall (NDT Centre, AERE, Harwell, who have developed the idea that use could be made of the ultrasound radiation scattered from the main wave. Although in general this radiation will not give an absolute measure of the macrostructure of the medium, it can yield useful results when correlated with other relevant information.

High power ultrasonics has come into prominence again and was the theme of one of the parallel morning sessions of the second day; two of the subjects dealt with concerned transducers (Dr R. R. Whymark, Interand Corporation, Chicago, and Dr E. A. Neppiras, University of Houston). Dr B. Largenecker (PVC Laboratory, Waldbach, Austria) showed how the application of high amplitude ultrasound reduces the external stress necessary for plastic deformation; he has made a notable contribution to the design of machines for drawing brittle materials. The second of the morning sessions was devoted to the fast growing field of medical ultrasonics. Outstanding in this section was the talk by Dr M. K. Patrick (Birmingham General Hospital) on her experience with ultrasonic therapy in clinical practice.

The final session in the afternoon of September 29 was concerned with automated non-destructive testing and the last contributions were from Drs V. M. Barborovsky and D. Marsh (Tube Investments Research Laboratories, Cambridge) on a Schlieren study of ultrasonic pulse propagation and reflexion. The aim of the work was to simulate the detection of cracks in steel tubes and thus to improve the reliability of the test method. Glass models were used in the Schlieren system to provide a transparent medium, ultrasonically very similar to steel. The pulsed light source has to satisfy the condition that its flash duration time was short compared with the period of the ultrasonic wave and a new version of the argon jet source was found to be very suitable. The apparatus had to be carefully designed for vibration isolation because the deflected light from the phase-diffraction grating, formed by the ultrasonic pulse train, gave diffraction maxima separated by only 66 arcsec from the undeflected light using sheer waves of frequency 2 MHz.

Recognition Sites for tRNA Charging Enzymes

IN *Nature New/Biology* next Wednesday, Squires and Carbon report a series of experiments which shed new light on the intriguing question of how the different species of transfer RNAs are recognized and distinguished by their specific amino-acyl tRNA synthetases, the enzymes which charge tRNA molecules with amino-acids. And into the bargain Squires and Carbon report the location on the *Escherichia coli* genetic map of the third species of glycine tRNA, $tRNA^{Gly}_3$, which translates the glycine codons GGU/C.

Squires and Carbon found that a mutant strain of *E. coli* which lacks about one-third of the amount of $tRNA^{Gly}_3$ present in wild type cells contains a new species of glycine transfer RNA, designated $tRNA^{Gly}_{ins}$, which can translate the codons GGA/G. This mutated form of glycine transfer RNA maps at about 86 minutes on the circular *E. coli* genetic map and experiments with diploid and merodiploid cells prove that this is the position of the structural gene, or rather genes for $tRNA^{Gly}_3$, of which there must be multiple copies in each wild type genome.

To prove that $tRNA^{Gly}_{ins}$ appears as a result of a mutation in one of the $tRNA^{Gly}_3$ structural genes, Squires and Carbon analysed the base sequences of the two molecules and found that they differ in only one position; the anticodon sequence of $tRNA^{Gly}_3$ is GCC and that of $tRNA^{Gly}_{ins}$ is UCC, but apart from this G→U base change in the third or "wobble" position of the anticodon the two molecules are identical.

Glycyl tRNA synthetase charges the mutated $tRNA^{Gly}_{ins}$ at about the same rate as $tRNA^{Gly}_3$. By contrast, Carbon and his colleagues, working with wild type and mutated forms of the two other species of glycine tRNAs ($tRNA^{Gly}_1$ and $tRNA^{Gly}_2$) in *E. coli*, have found that changes in either the first or second base of the anticodon drastically impair the recognition of these molecules by the glycine charging enzyme. They conclude therefore that the first two bases but not the third "wobble" base are involved in the interaction between glycyl tRNA synthetase and the glycine tRNAs.

Squires and Carbon also noticed that the base sequence of $tRNA^{Gly}_3$ is very similar to that of the valine tRNA, $tRNA^{Val}_{2B}$, which was analysed by Yaniv and Barrell. 78 per cent of the sequences of these two tRNAs are, in effect, identical and what few differences there are between the two molecules are to be found in their hydrogen bonded stems rather than their single-stranded loops. It seems therefore that the base sequences of these double-helical stems provide recognition signals which allow the glycyl and valyl tRNA synthetases to distinguish their respective tRNAs. Whether, however, this is a general rule, and the hydrogen bonded stems of all tRNAs provide recognition signals for their respective synthetases, remains very much an open question.

Aeronautics before 1919

GEOFFREY TAYLOR
Trinity College, Cambridge

Professor Taylor describes his aeronautical experiences during the First World War and just afterwards.

I HAD studied fluid dynamics as a student and was applying my knowledge to meteorology when the First World War started on August 4, 1918. Like most of my friends, I volunteered for service in the army and suggested that I might set up a weather forecasting unit in the field. The officer to whom I made this proposal did not seem to doubt that I could tell what the weather was going to be—as he might very well have done—but thought the knowledge would be of no value to the army in the field. "Soldiers don't go into battle under umbrellas, they go whether it is raining or not," was his comment. I expected then to join a combatant unit, but I left my home address in London with the officer I had interviewed in case the army had any use for the services of a scientist.

On the evening of August 5 a message came from Major Sefton Brancker, then the head of the flying wing of the army, that the Royal Aircraft Factory at Farnborough would employ me as a scientist if I offered my services in that capacity. I went there the next day and the Superintendent, Mervyn O'Gorman, introduced me to a fascinating character, Teddy Busk, who invited me to live in the Naval Airship Mess where he lived himself. Busk was perhaps the first man to make a practical study of the stability, as distinct from the controllability, of airplanes. The machine which he designed to be stable, BE2C, could be flown without hand control and a thorough study of its aerodynamic character was being made when I began work in Farnborough.

Designing Darts

My first flight in an airplane was with Teddy on November 4, 1914, in the prototype BE2C, in which he was measuring the periods and decay times of longitudinal oscillations. The next day he was continuing his experiments on the same machine when it caught fire in the air and he was killed. During my flight with him I had noticed a strong smell of gasoline and he had explained that the carburettor was supplied from a small gravity feed tank in front of the passenger's seat. At frequent intervals this had to be filled by the pilot, who pumped gasoline from a larger tank under his seat. Unfortunately there was no indicator to show the pilot when the small tank was full and gasoline poured onto the passenger's seat if pumping was not stopped in time.

The asylum for theoreticians to which I was assigned when I arrived at Farnborough was called H department. I never knew what H stood for, but it tackled odd jobs which the engineers did not care about. One job which was assigned to Melvill Jones and myself illustrates the kind of thought which prevailed at that time. The French had been dropping pointed darts called *flechettes* on troops from their planes. It was found that these whirled when dropped, even though they would point into the wind when suspended at their centre of gravity, and we were asked to design one which would fall straight. We pointed out that a bomb which projects its fragments horizontally would have a much better chance of hitting troops than darts coming vertically, but it seemed a matter of prestige that we should have our own dart and that it should be better than the French dart, so we took on the job of designing one. After we had done this we took a few hundred of them in a satchel up a steel ladder inside a disused tall chimney so that we could watch them from above as they fell. They flew quite straight.

Having designed the dart, we were asked whether the terminal velocity in free flight was big enough to damage troops, so we bored out a rifle barrel and shot the darts with reduced charge at a leg of mutton hanging as a ballistic pendulum so that we could find out the impact speed and correlate it with penetration. Finally, we were asked how the darts would spread if a bundle of them were dropped from a plane. To answer this we got a pilot to throw a few hundred over a field from a height of several hundred feet. When this had been done, Melvill Jones and I went over the field and pushed a square of paper over every dart we could find sticking out of the ground. When we had gone over the field in this way and were looking at the distibution, a cavalry officer came up on his horse and asked us what we were doing. When we explained that the darts had been dropped from an airplane, he looked at them and seeing a dart piercing each sheet remarked: "If I had not seen it with my own eyes I would never have believed it possible to make such good shooting from the air".

The darts were, however, never used, but not apparently for the reason we had originally raised as an objection—that they would be inefficient; we were told they were regarded as inhuman weapons and could not be used by gentlemen.

Learning to Fly

After I had been employed for a short time designing instruments for use in the air, I felt that I might be able to do a better job if I were taught to fly myself. When I put that point of view to O'Gorman he agreed with me, but the army refused to sanction the proposal that a scientist should be taught to fly. They told me that I only had to ask a pilot and he would tell me anything I needed to know. The fact was that pilots usually did not understand the physical causes of the sensations they experienced while flying and told, as facts, their interpretations of the causes of their sensations. O'Gorman was, however, clever at getting his way by devious schemes. He allowed me to resign from his factory so that I would be free to apply for training as an uncommitted citizen, and said he would apply for me to return to Farnborough when I had passed through the flying training school. In May 1915 I went to the school which used the space in the middle of the old Brooklands motor racing track as an airfield. I started to learn on the 1913 model Mawrice Farman biplane, then called a longhorn or Rumpety. This plane looked old fashioned but was a good training machine. Its maximum level speed was about 60 m.p.h. and it was said to stall at about 37 m.p.h. We aimed to land it at 42 m.p.h. Though the engine frequently gave out in the air, its low landing speed made it possible to land safely in many grass fields. Also, if it did crash, the staging supporting the elevator in front could act as a shock absorber. These machines were held together with piano wires, and so many were needed that the mechanics used to say that when they erected the machine they released a canary between the wings—if it got out, a wire was missing. My instructor was a flight sergeant who had been damaged in a crash caused by one of his pupils. He decided this must not happen again, so if any of them exerted any appreciable force on the dual control column he reported they were heavy handed and would never make good pilots. As very few machines were then available for training and many people wanted to join the air service, the threat that one would be turned out was to be avoided at all costs and many of this man's pupils took over control of their machines for the first time on their first solo flight. The resulting erratic manoeuvres were sometimes spectacular. I came through better than most because of the months I had spent at Farnborough; at least I knew what the controls were supposed to do.

The course at Brooklands provided a restful interval in an otherwise restless world. We would go early onto the field and, if there was more wind than would put a match out, flying was cancelled and we would go home, though we had to keep a watchful eye in case the weather improved.

When I graduated as a pilot in July 1915, I was sent on a course described as "artillery spotting". We were instructed to observe a certain farm which was marked on a map. People on the ground let off smoke puffs and we had to tap a key screwed onto the cockpit and give in Morse code the distance and bearing of the smoke puff from the farm. Wireless telephony had not then been invented and it was necessary to unwind a weighted aerial while in the air. I failed on my first try because I forgot to wind the aerial in after finishing the test, and dragged it off in a hedge on the perimeter of the field as I approached to land.

Wings and Airscrews

In the early days of the First World War the engineers had no confidence in the theorists because mathematical predictions about the forces on moving bodies never seemed to give experimentally verifiable results. Prandtl's boundary layer theory had not yet been developed or at any rate had not penetrated to Farnborough, and designers could only rely on wind tunnel tests with models to give them design data. Even the applicability of model data to full scale situations was doubted, chiefly because very few measurements which could be compared with calculations had been made on full scale airplanes. For this reason the first thing I did when I returned to Farnborough after passing out from the flying school was to look for something which could be measured both on a model in a wind tunnel and also in flight on a full scale airplane. The weight of the plane was known and this could be correlated with model lift measurements in wind tunnels, but the same method could not be used for measuring drag because nothing was then known about the efficiency of the engine–airscrew combination. I decided to try to measure the distribution of pressure over one section of the wing of the BE2C plane which had been allotted to me for the purpose. I got the workshops to construct a wing with twenty holes spaced round a section and I connected them through copper tubes to a manometer in the cockpit. I was to be alone in the plane, so I had to design a manometer which I could operate while I was flying. Fortunately, the BE2C was a completely stable machine, for I had to fly at a fixed speed for some time to get the manometer steady before pressing a button which photographed the manometer tubes. The results were given in non-dimensional form as $p/\rho v^2$ for the range of speeds at which the BE2C was capable of level flight, namely 50 to 90 m.p.h. When these curves were compared with wind tunnel measurements made at the National Physical Laboratory (NPL) the agreement was not good, but it was found that the NPL had not made a proper correction for the effect of the tunnel walls, and when this was done the agreement was very good. I published the results in 1916 and they may have been the first full scale pressure distribution measurements, though many have been made since.

One of the problems which was tackled at Farnborough in those days was that of setting up a theory of airscrews. Their sections were airfoils and very soon it was found that if the wind tunnel data for each section of the blade were applied as though it were advancing into still air, the calculated result did not agree with measurements made on a test bench. On the other hand, the results could be correlated if it were assumed that the air approaching the airscrew was already in rotation before it reached the plane of the screw. Thus they introduced a fictitious rotational inflow factor which made the calculations agree with observation. This shocked me, because Lord Kelvin had already shown that circulation cannot be produced by the pressure variations that might arise from the motion of the blades and I wrote a note pointing this out. Shortly afterwards, H. Glauert cleared up the difficulty and showed that though the circulation in the approaching ring of air was in fact zero, the direction of the air approaching each blade was changed because of the pressure field produced by all the other blades. Thus the air in the field close to the front of each blade deviates in one direction and the air approaching the spaces between the blades is deviated in the opposite direction, making the total circulation in rings of air approaching the airscrew zero. This example is interesting because it illustrates the condition under which correct conclusions about aeronautical situations can be based on the classical theory of non-viscous fluids. The forces on bodies moving steadily through the air are due to the viscosity of air and the mechanism of the process is described by the viscous boundary layer theory, but when a fluid is moved only by a pressure gradient without the intervention of viscous effects, circulation—that is, the integral of the tangential component of velocity round any circuit of fluid particles—cannot be produced.

Though most of my work in the air was carried out with the stable BE2C, which was controlled laterally by means of ailerons, I flew two machines which had no ailerons and were controlled by warping the whole wing; wings in those days were not the rigid constructions one sees now. This method of control worked satisfactorily when the air was calm but it was exceedingly uncomfortable under bumpy conditions. The reason for this behaviour was that the control wires had to move the whole wing, not just the much smaller aileron. When flying straight in bumpy conditions it was impossible to prevent violent lateral motions of the stick. In spite of this wild waving of the control stick, it only needed a small steady force to make a turn.

Indicating the Vertical

One of the great difficulties in flying at that time was that as soon as one got into a cloud and could no longer see a horizon one had no means of appreciating the vertical direction and pilots often came out of clouds in steep banks thinking they were still horizontal. Some instrument was needed to tell whether one was turning. Gyroscopes had not yet been designed for use in airplanes and some instrument was needed to tell pilots whether they were turning. One was invented by Horace Darwin, a son of Charles Darwin, who had noticed that it would be useless to fit pitot tubes at each wing-tip to measure the difference in velocity during a turn because the pressure difference in the pitots would be balanced by the pressure difference due to centrifugal force in the tubes connecting them with a manometer in the cockpit. On the other hand, if static pressure heads were substituted for pitot tubes, the pressure change in the tubes from the wing-tips to the cockpit would constitute a potential turn indicator. The instrument constructed on this principle made it possible to fly safely through clouds and at night. Of course, people had flown through clouds before turn indicators were invented but few would fly continuously without the visual aid of a horizon. So well was this difficulty publicized that it required quite a group of inverted Micawbers to turn down the ideas of people who invented instruments for indicating the vertical.

The ability to fly at night had not made it possible to undertake night bombing except on very clear nights when land features could be seen all along the route, and on such occasions people did not want to fly over enemy territory. In 1918, Bertram Hopkinson invented a simple method for night bombing at the small distances over which one might assume a constant wind. This entailed the shining of two searchlights vertically from points about a mile apart on the line to the target. On most nights there was enough haze to show up the vertical beams and the pilot manoeuvred his plane into line with them and found the compass bearing on which they kept in line as he approached. He then flew on this bearing through both beams. On passing the first he started a stop-watch which he read on passing the second; then, knowing the ratio of the distance of the objective to the distance between the lights, he flew on the same compass course for a time equal to that ratio multiplied by time between his passages over the two searchlights. The method was not actually used but the required equipment was set up and was to be used for the first time on a certain evening for bombing Coblenz from a point in the American section of the Front near Colombé, but peace broke out on the very day before the exercise was to be performed.

Crossing the Atlantic

At the end of the war the *Daily Mail* offered a prize of £10,000 for the first non-stop flight across the Atlantic. Four firms entered machines which they had designed during the war, adding extra fuel tanks for the competition. I went with the Handley Page party, partly as meteorologist and partly to teach the navigator, Tryggve Gran, some celestial navigation. It was not practical to set out for Newfoundland until the worst of the winter was over because there were no airfields there and the ground would be very soggy. The four firms which competed—Vickers, Handley Page, Martinsyde and Sopwith—sent their expeditions at about the same time. Though I suppose they each expected to gain some commercial advantage, the competition was essentially a sporting event. We each had to make our own airfields and erect our machines in the open. As soon as we landed from the ships which had taken the expeditions to Newfoundland we each chose a piece of land to make into an airfield. Vickers made theirs on high ground behind St Johns. Martinsyde opted for a place called Quidi Vidi Cove, just north of St Johns, and Handley Page chose a field at Harbour Grace about 60 miles from St Johns. Until our move to Harbour Grace all the expeditions lived in the one decent hotel in St Johns and every morning we went off early to the chosen sites of our airfields and worked furiously to try to get going before the other competitors.

The first competitors to try their luck were the Martinsyde group. They had loaded their machine too heavily, I think, and it crashed before it became airborne. The navigator, a man called Morgan, was hurt, and when this news became public, would-be navigators rushed to St Johns from all over the world; one, I remember, came from Argentina. The next to try was the Sopwith, with Hawker as pilot and McKenzie Greave as navigator. I saw them take off and we had no news of them for nearly two weeks. Then they turned up in a Scandinavian freighter which had no radio.

The third to take off was the Vickers, with Alcock as pilot and Brown as navigator. As everyone knows, they got across and crash-landed in a bog in Ireland. Brown pretended to be quite ignorant of navigation, though that must have been a pose. I remember asking him how he would know where he was when he saw Europe beneath him. His reply was, "I will come down and if I see people in Mantillas I will know it is Spain. If I see people eating frogs I will know it is France and if I see them hitting one another over the head I'll know it's Ireland." When the Vickers took off I was standing near the machine when it started. I saw it run the full length of the airfield and disappear below the level of the high ground on which I was standing. We all thought it must have crashed because of excessive loading, but after a few minutes it came back, having climbed about 100 feet, and shot straight out to sea. The Handley Page machine was bigger than the others and took longer to erect; it was just doing its test flights when Brown and Alcock got across, so it very wisely turned south for New York.

The Perennial Dilemma of Science Policy

Pure Chemistry Library
SCIENCE COLLEGE.
92 Upper Circular Road
CALCUTTA

PAUL GARY WERSKEY

Science Studies Unit, University of Edinburgh

". . . Science on the scale it is pursued today is an integral part of the social organization of the State. Wherever he works, the man of science is a part of the machinery of State, and there is no real immunity of science from its political environment." *Nature* had much to say on the organization of science between the two world wars.

THE period between the two world wars was notable in the history of science, because it witnessed not only important discoveries in the laboratory but the first attempts in Britain, Russia and elsewhere to establish national science policies as well. From its vantage point in the United Kingdom, *Nature* kept a hopeful but watchful eye on these new experiments in the organization of research. The journal's editor, Sir Richard Gregory, was of course favourably predisposed to any action which would increase the influence of science and scientists in public affairs[3]. On the other hand, he recognized that the state could encroach too far on the traditional autonomy of "pure" scientists[4]. *Nature* thus demanded of government-science relations a balance between apparently conflicting demands for the scientists' involvement with and detachment from social needs.

How such an equilibrium could be established in practice was a topic then frequently discussed in *Nature*'s leading articles[5]. (Many of these were unsigned, but the authors' names were recorded on one set of volumes of *Nature*, which has been used in the preparation of this article.) Having committed itself to the ideal of "an accepted national scientific policy, definite, continuous and consistent"[6], the journal was forced to construct a workable model for the conduct of research in a socio-political context. Unfortunately, the discursiveness of the leader columns defeated any attempt to outline a complete solution to such a novel and complex problem as "planned" science. What the articles sought instead were answers to more specific questions. How much of scientific activity can be consciously organized? What priorities and which social groups ought to govern the funding and administration of research programmes? Finally, would such a policy conflict with established professional values and/or introduce new ones? *Nature*'s model for a national science policy must therefore be intuited from its views on these subjects.

The Model

Under Gregory's auspices, *Nature*'s commitment to the concept of a national science policy begins with the assumption that science as a social phenomenon is susceptible to some form of planning. Clarity on this point demands a distinction between the planning for science and the planning of science. (Graham uses this distinction in ref. 7.) The former notion suggests that, first, the rate of scientific development can be influenced by the size of the human and capital resources allocated to research, and, second, that specific areas of investigation can be encouraged through preferential treatment. The journal's leaders in this period are sensitive to both possibilities. They argue that the combination of adequate educational opportunities and generous research grants will result in a considerable growth of the scientific community. To make the most effective use of scientists' talents, facilities must be provided for: pure basic research in the universities; applied basic research in government laboratories, independent research institutes, and industrial research associations; and, finally, technological innovations at the industrial level. Sir Richard and his associates further recognize that the knowledge derived from such operations cannot be of use unless it is efficiently disseminated. Some liaison between different kinds of investigators, as well as a rational system of publication and data storage, are therefore required. Once such resources are made available it becomes possible to aid those fields of research which merit special attention. The growing points of important theoretical sciences are obvious candidates on intrinsic grounds alone, as are the borderline areas between well established disciplines, that is biophysics, biochemistry and physical chemistry[8,9]. In the cases of applied basic research and technological innovation, *Nature* concedes that extrinsic factors must predominate. The studies of the properties of dyestuffs, nutrition and aerodynamics ought to be supported primarily on the grounds of their economic, medical or military utility. In short, planning for science can assist in both the growth of knowledge and the solution of social problems.

While editorials assert that the organization of research can be moulded at the macro-level, they are reticent to suggest innovations within the laboratory itself. "The scientific investigator," writes Gregory in 1916, "must have freedom to follow his own course wherever it may lead, whereas technical research can be organized and definite problems presented for which solutions of direct service are sought"[10]. The latter group is therefore counselled that "though they may regret the gradual encroachment of bureaucracy on the freedom of scientific investigation, (they) have to recognize that they are primarily public servants whose first duty is to perform their allotted tasks in the social machine"[11]. Pure scientists, by contrast, are exonerated from attempts to have their work organized for them. "Such a proceeding would be repugnant to the best research workers"[12].

To preserve science's subtlety and sophistication at the micro-level, Gregory and his colleagues reason that the administration of science policy should largely be the responsibility of scientists. They naturally recognize the right of any government to oversee the operations which it finances[19,14]. In practice, though, the leading articles urge that "... scientific men should decide upon the allocation of funds for research ...; and ... they should be responsible for any schemes of organized investigation. Friction and misunderstanding always arise when these functions are performed by official administrators unfamiliar with such a sensitive plant as scientific genius and unable to judge the promise of incipient inquiry"[15].

Nature extends this rationale to applied basic research as well as to theoretical investigations. When it is recalled that funding for the former area ought to be based on socio-economic needs, it is clear that scientists, according to *Nature*, must have more to contribute to policy than just their technical competence. Gregory's writers, especially Rainald Brightman (an ICI librarian), believe that the scientific outlook of certain researchers allows them to view society more objectively than many non-scientists. Therefore, as Sir Richard himself points out, "Many scientific workers ... are no longer content to be regarded as hewers of wood and drawers of water ... They know how science enters into every department of national life or international undertaking, and they claim to be entrusted with a reasonable share of responsible control of the forces created by them"[16]. The political overtones of such a position are clear enough[5]. Scientists themselves should determine not only the scientific criteria but also to some extent the social priorities which govern a nation's science policy.

The professional implications of *Nature*'s "model" for a national science policy are thus of critical importance in at least three areas: international cooperation, freedom of research and social values. (M. King[17] has analysed Gregory's reflexions on professional values from a sociological perspective.) Science as a profession has long prided itself on its ability "to be truly international and capable of transcending national follies"[18]. Once scientists are organized along national lines, however, a potential constraint on such internationalism is introduced. State responsibility for scientific development may also limit another constituent of science's traditional ethos, the operational autonomy of individual researchers. Brightman, for example, grants that "the right of society in a time of emergency to prescribe the directions in which scientific effort shall first be made can scarcely be challenged"[19]. One such crisis would be the mobilization of a country for a war in which science would play a major role. In the absence of a system in which scientists are integrated into the machinery of government, it would be plausible to state that the use of research for destructive purposes "is not a question for science but for the public and its leaders"[20]. The foundation of a national science policy, however, introduces this ethical dilemma into the scientific profession. As Gregory concedes in 1938, science can no longer "be divorced from ethics, or rightly absolve itself from the human responsibilities in the application of its discoveries to destructive purposes in war or economic disturbance in times of peace"[21]. If scientists are the chief architects of policy, then the issue of social responsibility is only further intensified.

Nature therefore advances the theory of a science policy which, if realized, would perhaps modify certain well established values of the scientific profession. In the minds of Gregory and Brightman such a programme would minister to both the needs of science and society far more effectively than the disorganized "system" extant at the end of the First World War. The ICI librarian could accordingly proclaim in an important leader of 1934 that: "The conception of science as a social function intimately linked up with human history and human destiny, moulding and being moulded by social forces, should summon forth from scientific workers something of the energy required to translate into policy and action the knowledge acquired by their work. Such energy will find its expression ... in ... the faith that human reason by using wisely the scientific method can give us the control of our destiny"[22]. On these grounds, how did the journal judge of the uses which Britain and other countries made of science and scientists between the wars?

Science Policies, 1919–1938

Nature was not happy with the organization of research in any country during this period. In Britain successive governments were berated for their failure to mould a number of autonomous research facilities into "a national research organization commensurate with our needs ..."[23]. Brightman went on to complain in 1933 that: "If there has been little attempt to rationalize even the grants made by Government to particular fields of research, there has been no attempt as yet to assess the place of research in the broadest sense in the structure of our national life—to evaluate its contribution in each department of State as well as in industry, to assess the relations of fundamental and applied research on a systematic and thoroughgoing basis and to correlate the contributions from the national exchequer and those from industrial and private sources"[23].

So uncoordinated were the financial procedures of the state's various scientific agencies that "Parliament is not in a position to ascertain with ease the total assistance it is granting to one institution . . ."[24]. There is thus little to wonder at in a Brightman leader of 1937 which states: ". . . we have reached a stage when it is imperative to take stock of the nation's resources and facilities for research, both academic and industrial"[25].

The journal's own stocktaking revealed a number of weaknesses in the national structure of research. For example, *Nature* was convinced that the number of biological and social scientists was grossly inadequate for the needs of either the nation or the British Empire[26]. Several factors were cited as contributing to this deficiency, but the primary one was the neglect of biology and sociology in the curricula of elementary and secondary schools[27]. (*Nature* welcomed the later increase in the number of school certificate examinations in biology[28].) An even more serious problem was the inability of government, industry and the universities to fashion any effective liaison between basic researchers and technologists. "We are still without a real technique of discovery, and important industrial developments as often as not are based on purely fortuitous discoveries"[29]. To remedy such a state of affairs the journal tentatively presented in 1927 a case for the nationalization of industrial research facilities[30], but such a notion was explicitly rejected by Brightman four years later[31]. As for the universities, *Nature* was undecided as to whether they should either spurn the advances of industry[32] or welcome new opportunities for applied research[33]. The journal, unable to formulate a programme for structural change in this area, had to content itself with the plea for an increase in the resources devoted to technological development. In essence, existing administrative techniques would not permit the realization of an important aspect of the science policy model. Whatever hopes Gregory, Brightman and others entertained for the growth of science in national life were therefore ultimately based on the lone fact that "Scientific men are now in real control of scientific policy in Britain, even when it deals with enterprises endowed by the State"[34].

If *Nature* was dissatisfied with the disorganization of research in the United Kingdom, it was horrified by the relatively complete integration of scientists into the differing political systems of Germany and the Soviet Union. Gregory's first concern was that the fervent nationalism which informed the science policy of the two countries was inimical to the maintenance of an international scientific community. The First World War had of course already introduced the first serious frustration of science's traditional internationalism: ". . . for the first time scientific men themselves were brought into the conflict instead of continuing quietly to work in their laboratories, and maintaining correspondence with those of other nationalities, as was formerly the case"[35]. After Germany had become the first nation to utilize laboratory-produced poisonous gases on the battle fields, leaders of research from the allied countries decided to found new international scientific institutions which would exclude German scientists. *Nature*, with an editor who said in a letter to H. G. Wells in 1918 that he was "'bitter against the Germans at having caused the present war'", supported such a policy of estrangement. Sir William Tilden thus demanded in a leader of 1919 that all international associations "must be freed from German membership and influence and to accomplish this . . . new and independent associations must be formed by the Allies, together with such neutral Powers as, after deliberation, choose to dissociate themselves from Teutonic combinations"[60]. Having witnessed and participated in this initial dimming of the international spirit, *Nature* was thereafter extremely sensitive to any further outbreaks of nationalism. Germany was of course brought back into the official life of international science just in time for Hitler to use various science congresses as platforms for Nazi propaganda. From the first, Gregory and his associates were opposed to the Fascists on political grounds alone[37]. When the Nazis attempted "to secure the control of international scientific work", the journal urged that "it is time to call a halt"[38]. Russia was subjected to similar criticism[39], even though it had effected a great part of the science policy model which *Nature* supported (ref. 7, especially Chapter 2).

The science policies of the Fascists and Communists were also rejected for their conscious curtailment of the intellectual freedom of individual scientists. Gregory himself noted of Hitler's Germany that "what seems to men of science most deplorable is the elevation of a national passion into a principle, the acceptance of a policy which teaches that to attempt to find and hold truth is but a secondary and subordinate activity of the human mind to be postponed or slighted for *any* reason whatever"[40]. The specious racial theories used by the Nazis to justify the dismissal of Jews from the universities inevitably incensed the *Nature* coterie[41]. After four years of repeated attacks on Hitler's regime, the journal was, not surprisingly, banned from Germany[42]. The Soviet Union also earned Gregory's displeasure, because of its apparent subordination of science to the demands of Marxist dialecticians. Thus in an article of 1921 an emigré Russian scientist styled the Bolsheviks as "men of simplified views—doctrinaires and politicians—they cannot accept the fact that science must be independent of everybody and everything. They think it quite right and advisable to make scientific men 'obedient' executors of the commands of the Soviet Government"[43]. In the 1930s *Nature*'s views on Russia, although expressed less vehemently, altered very little. To take one example, a leader of 1937 pointed to Lysenko's initial harassment of well-known orthodox geneticists as an illustration of "the atmosphere in which scientific investigators in totalitarian countries have to live and work"[44]. In its equation of the threats posed by Hitler and Stalin to the internationalism and freedom of science, *Nature* was led to support the exclusion of both their respective nations from the councils of world science "on the ground that in these countries at present scientific workers are bound much more closely to their respective Governments than is the case elsewhere"[45].

The difficult relations between various national scientific communities were, however, only one aspect of a progressive deterioration in international relations during the 1930s. As the prospect of war increased, so too did the defence budgets of the European powers, including Britain. *Nature* now feared that even in the United Kingdom "the intensification of preparations for self-defence has tended to strengthen the fetters on freedom of investigation and exposition which dictatorships in many countries have already riveted on industrial and academic workers alike"[46]. If rearmament threatened to tie working scientists too closely to the government, then it stood doubly condemned because of its inherently destructive purpose. Gregory hypothesized in 1938 that "when the object of research is the command of natural forces, without regard to their relation to human life, it can become a social danger and an excuse for scientific barbarity"[47]. Should the scientific worker therefore contribute his talents to an enterprise which might threaten the life of both science and society? *Nature* was not at all certain, at least until 1938. Thus: "Already in the time of the Great War the prostitution of science to warfare was deeply felt by many British men of science, some of whom refused altogether to participate either as soldiers or as scientific workers. The same attitude would again be taken by many, should we be cursed by another war; even in the present time of uneasy peace, the activity in rearmament is renewing in the minds of many men of science the same tension of feeling, the same problems of conscience"[48].

Conflict and Resolution

The perversity of international politics in the 1930s thus elicited in the most unfortunate circumstances the three challenges to professional values implicit in *Nature*'s model for a national science policy. As Gregory's and Brightman's awareness "of the extent to which political organizations can affect the direction of scientific research, and even frustrate its efforts"[49] increased, their interest in the extension of the state's responsibility for science diminished. The journal went so far as to petition its scientist-readers in July 1938 not "to allow any external system of thought or institution to dictate their decisions or activities . . ."[50]. Strict adherence to such an injunction would of course have reduced any conception of national planning for science to a level of theoretical and practical absurdity. For a journal which had been so adamant about the integration of science into society, such a shift in policy could only have been made under the greatest duress. The change was itself primarily occasioned by Gregory's perception of scientists' experience under Hitler. Yet over a longer period of time some alteration in the leaders would have been necessitated had the journal's failure to resolve conflicting social and professional demands persisted.

A resolution of these difficulties was provided, however, two weeks after the signing of the Munich agreement over Czechoslovakia in an anonymous leader entitled "Science and National Service"[51]. It is of great significance that this article was written not by Gregory or Brightman but by the young crystallographer and Marxist philosopher, J. D. Bernal. In the year preceding Munich, Bernal had worked vigorously on both the International Peace Campaign and his forthcoming book, *The Social Function of Science*[52]. He was then persuaded by his friend Solly (now Sir Solly) Zuckerman, in September 1938, that the likelihood of war demanded a careful analysis of the ways in which scientists could best be used by (and/or protected from) the government (S. Zuckerman, personal communication). Gregory, as has already been demonstrated, was vexed by the same situation and therefore welcomed the first fruits of Bernal's work in this area. The latter's first concern was that any mobilization of science for self-defence would not be satisfactory ". . . unless its ultimate aim—the utilization of science for human welfare in times of peace—is kept steadily in view. . . . Consequently, the organization of scientific workers for war must be conceived in such a way that the minimum damage is done to the possible beneficial utilization of science. This involves the safeguarding of fundamental scientific research and of the main lines of its application, so that once the danger of war is definitely removed, science can advance as rapidly as possible in carrying out its true purpose"[51].

Bernal further specified that scientists could not be expected to support either a plan prepared by civil servants or a war directed "against the principles of democracy and civilization"[51]. Having defined the conditions under which researchers would participate, he next attacked the ways in which science was then organized. "Preparation for . . . war requires not only a much more thorough organization of science, but also a much closer integration between scientific research and other activities of the community, particularly those of industrial production, agriculture and health"[51]. In fact, "We need a National Research Council covering the whole of science and relating it to the running of the community *both in peace and in war*". Such a body would be responsible for, first, the determination and, second, the allocation of scientific resources. Bernal envisaged the principal tasks for science in wartime to be: the maintenance of military and civil populations, as well as of war production; defence against aerial attack; assistance in the planning of military operations; and care of casualties. As a means to these ends he recommended an immediate intensification of both fundamental and applied research related to food, non-ferrous metals, rubber, motor fuel, optical instruments and medical supplies. Apart from the utility of their investigations, Bernal finally emphasized the general contributions which the scientists' quantitative and critical outlook could make to the successful conduct of war. "The full utilization of scientific workers requires the use of their ingenuity far more than of their routine service, and this can only be secured by giving them opportunities and liberty of initiative"[52].

The significance of Bernal's leader for the development of *Nature*'s concept of a national science policy cannot be stressed too much[53]. While his article reaffirmed the desirability and practicability of a socially orientated plan for scientific activities, it also attempted to conserve important professional values. Yet the context in which Bernal and *Nature* chose to defend such traditional ideals could not but alter their meaning. For example, the journal had tended up until 1938 to identify the scientific outlook with pacifism. Now it was socially responsible for a researcher to lend his talents to the military defeat of any nation which attempted to abrogate both the freedom of its own scientists and the internationalism of the scientific community. By the same token, scientists who placed the service of an undemocratic state ahead of their devotion to truth were to be ostracized from science's international brotherhood. Finally, unlimited freedom of research might be curtailed when society in a time of economic or military crisis called for the assistance of limited scientific resources. Such values as the internationalism, freedom, and social responsibility of scientists could not, in short, be upheld both universally and simultaneously. Once science was consciously placed in a specific social context, a conflict between such concepts became all the more likely. The following problem therefore emerged: if one value had to be subordinated to another, who would make such a decision and on what grounds? A satisfactory answer to that question has eluded contemporary philosophers of science, no less than it did Sir Richard Gregory's *Nature*.

I am grateful to the present staff of *Nature* for their assistance in this research on their predecessors.

[1] Brightman, R., *Nature*, **136**, 275 (1935).
[2] Jones, T., *Nature*, **124**, 886 (1929).
[3] Armytage, W. H. G., *Sir Richard Gregory, His Life and Work* (Macmillan, London, 1957).
[4] Gregory, R. A., *Discovery or the Spirit and Service of Science*, 235, 257 (Macmillan, London, 1916).
[5] Werskey, P. G., *Nature*, **224**, 462 (1969).
[6] Williamson, J. W., *Nature*, **114**, 707 (1924).
[7] Graham, L. R., *The Soviet Academy of Sciences and the Communist Party*, 1927–32, 64 (Princeton, 1967).
[8] Gregory, R. A., *Nature*, **102**, 254 (1918).
[9] Brightman, R., *Nature*, **137**, 471 (1936).
[10] Gregory, R. A., *Discovery or the Spirit and Service of Science*, 235 (Macmillan, London, 1916).
[11] Jacks, G. V., *Nature*, **135**, 46 (1935).
[12] Gregory, R. A., *Nature*, **133**, 78 (1934); Gregory, R. A., *Nature*, **104**, 259 (1919).
[13] Williamson, J. W., *Nature*, **138**, 53 (1936).
[14] Gregory, R. A., *Nature*, **105**, 221 (1920).
[15] Gregory, R. A., *Nature*, **105**, 223 (1920).
[16] Gregory, R. A., *Nature*, **136**, 885 (1935).
[17] King, M. D., in *Penguin Social Sciences Survey 1968* (edit. by Gould, J.), 51 (Penguin, Harmondsworth, 1968).
[18] Brightman, R., *Nature*, **142**, 50 (1938).
[19] Brightman, R., *Nature*, **136**, 410 (1935).
[20] Gregory, R. A., *Nature*, **112**, 889 (1923).
[21] Gregory, R. A., *Nature*, **142**, 1061 (1938).
[22] Brightman, R., *Nature*, **134**, 119 (1934).
[23] Brightman, R., *Nature*, **132**, 79 (1933).
[24] Stebbing, E. P., *Nature*, **129**, 738 (1932).
[25] Brightman, R., *Nature*, **139**, 527 (1937).
[26] Ritchie, J., *Nature*, **129**, 257 (1932).
[27] Latter, D. H., *Nature*, **130**, 289 (1932).
[28] Brimble, L. J. F., *Nature*, **143**, 259 (1939).
[29] Brightman, R., *Nature*, **130**, 715 (1932).
[30] Church, A. G., *Nature*, **119**, 589 (1927).
[31] Brightman, R., *Nature*, **127**, 393 (1931).
[32] Dawson, W. H., *Nature*, **114**, 921 (1924).
[33] Levy, H., *Nature*, **141**, 388 (1938).
[34] Brightman, R., *Nature*, **133**, 189 (1934).
[35] Evans, J. W., *Nature*, **116**, 1 (1925).
[36] Tilden, W. A., *Nature*, **102**, 341 (1919).
[37] Fallaize, E. N., *Nature*, **131**, 853 (1933).
[38] Stratton, F. J. M., *Nature*, **136**, 928 (1935).
[39] Marvin, F. S., *Nature*, **129**, 330 (1932).
[40] Gregory, R. A., *Nature*, **134**, 28 (1934).
[41] Adams, W., *Nature*, **140**, 170 (1937).
[42] Gregory, R. A., *Nature*, **141**, 151 (1938).
[43] Sokoloff, B., *Nature*, **108**, 20 (1921).
[44] Uvavrov, B. P., *Nature*, **140**, 297 (1937).
[45] Brightman, R., *Nature*, **139**, 689 (1937).
[46] Brightman, R., *Nature*, **138**, 418 (1936).
[47] Gregory, R. A., *Nature*, **137**, 988 (1936).
[48] Chapman, S., *Nature*, **139**, 980 (1937).
[49] Brightman, R., *Nature*, **137**, 963 (1936).
[50] Brightman, R., *Nature*, **142**, 51 (1938).
[51] Bernal, J. D., *Nature*, **142**, 685 (1938).
[52] Bernal, J. D., *The Social Function of Science* (Routledge and Kegan Paul, London, 1939).
[53] Werskey, P. G., *Science Studies*, **1**, 79 (1971).

Proposed Mechanism of Force Generation in Striated Muscle

A. F. HUXLEY & R. M. SIMMONS
Department of Physiology, University College London, Gower Street, London WC1

Recordings of the change in tension in striated muscle after a sudden alteration of the length have made it possible to suggest how the force between the thick and thin muscle filaments may be generated.

ONE approach to the elucidation of the kinetics of movement of the "cross-bridges" which are widely assumed to generate the relative force between the thick and thin filaments during contraction of a striated muscle fibre is to record and analyse the transient response of stimulated muscle to a sudden change either of tension or of length. Considerable progress has been made in this way by Podolsky and his colleagues[1-3] who recorded the time course of shortening after a sudden reduction in load. Similar responses have been recorded repeatedly in this laboratory but have not been published because we did not succeed in making the tension change sharply enough to distinguish the component of length change that is truly synchronous with the tension change from that which lags behind the tension change. We therefore turned[4] to the inverse type of experiment, in which the length of the fibre is suddenly altered (by ±0.1–1.5%) and the time course of the resulting tension change is recorded. The results have led to some fairly definite suggestions about the way in which the cross-bridges may actually produce the force between the thick and thin filaments.

All the experiments were carried out on isolated fibres from the semitendinosus muscle of the frog *Rana temporaria*, at 0°–4° C. Length changes, complete in less than 1 ms, were produced by means of a servo system[5], and tension was recorded with a capacitance gauge[6]. Compliance in the apparatus itself is small enough to be completely disregarded; precautions were taken to reduce the compliance in the tendon attachments to a minimum, and in some of the experiments it was eliminated altogether by using the "spot-follower" device[5], which continuously measures the length of a middle segment of the fibre.

Responses to Stepwise Length Change

The general time course of tension in these experiments is shown in Fig. 1. This article is concerned only with the changes that occur simultaneously with the length change itself and during the first few milliseconds after it. As has already been briefly reported[4], these changes are of the kind shown in Fig. 2: the tension undergoes a relatively large alteration simultaneously with the step change of length, but recovers quickly towards a level closer to that which existed before the step. The final recovery to the original tension which has been seen by many earlier investigators[7-9] takes place on an altogether slower time scale. The early changes seen in Fig. 2 have only come to light through the improved time resolution of present-day apparatus.

The behaviour shown in Fig. 2 suggests the presence of two structural elements in series. One of these would be an elastic element whose length is altered simultaneously with the change of length that is applied to the whole fibre, thus producing the large initial change of tension. The other would be an element with viscous as well as elastic properties, whose length readjusts itself during the period of a few milliseconds immediately after the length change, as a result of the change in tension. As this readjustment proceeds, the imposed length change comes to be shared between the two structures, giving a tension intermediate between the values immediately before and immediately after the length change. At the time of our first note[4] about this quick initial tension recovery, we thought it likely that the recovery took place by sliding, with movement of the cross-bridges, but that the instantaneous elasticity was mostly in the filaments themselves. Since then we have measured these responses in fibres stretched so as to reduce the amount of overlap between thick and thin filaments. We found[10] that the responses to a given absolute length change were altered only by a reduction in the scale of the tension changes, which varied in direct proportion to the amount of overlap and therefore also to the number of cross-bridges capable of contributing to tension. On these grounds we now believe that the instantaneous elasticity (or at least the greater part of it) resides in the cross-bridges themselves, as well as the structure responsible for the tension recovery.

The relations between length change and tension are illustrated in Fig. 3. The curve T_1 shows the extreme tension reached during the step change of length. It is somewhat non-linear, becoming stiffer with increasing tension as is commonly found in biological materials. This curve is the best experimental approach that we have to the instantaneous elasticity of the fibre, but it is clear from records such as viii–x in Fig. 2 that the tension drop in the larger shortening steps is cut down because the quick recovery has progressed

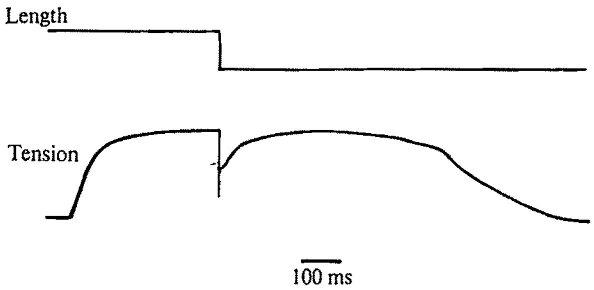


Fig. 1 Isometric tetanus of an isolated muscle fibre (frog, 4° C), with an imposed shortening step. This article is concerned with the tension changes during, and in the first few milliseconds after, the length step; these are shown on a fast time scale in Fig. 2.

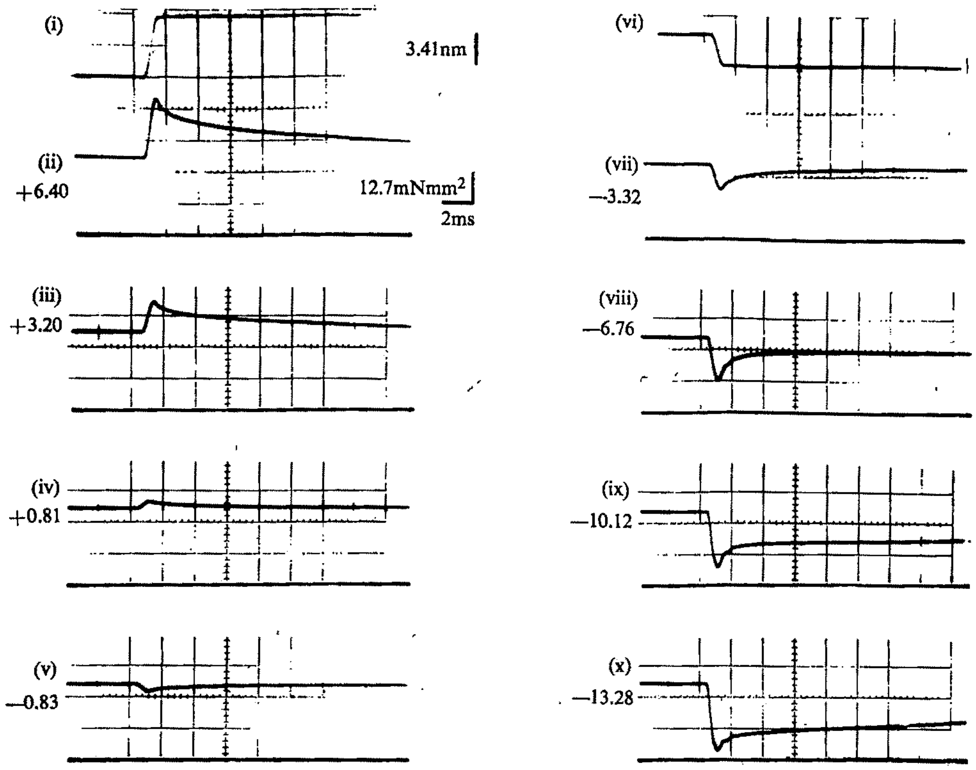


Fig. 2 Transient changes in tension exerted by stimulated muscle fibre when suddenly stretched (ii–iv) or shortened (v, vii–x); same experiment as Fig. 1, which shows the whole of the contraction during which record ix was taken. (i) and (vi): records of length change during tension records (ii) and (vii) respectively. The number by each tension record shows the amount of the length change per half-sarcomere, in nm.

to an appreciable extent before the length change is complete. The true curve of the instantaneous elasticity is therefore less curved on the shortening side than the curve of T_1 in Fig. 3; it is even possible that it is practically straight, as indicated by the broken line.

In contrast to this straightforward behaviour of the instantaneous elasticity, the quick tension recovery is highly nonlinear both in its extent and in its speed. The line T_2 in Fig. 3 shows the level approached at the end of the quick phase of recovery; for moderate amounts of shortening it has the unusual feature of being concave downwards, reflecting the fact that after a small length step the tension returns practically to its previous level (Fig. 2, iv, v). As regards the time course, it is evident from Fig. 2 that the early tension recovery is much more rapid in releases than in stretches, and that its speed varies continuously over a wide range with the size of the length step. The recovery is not exponential, but an estimate of the dominant rate constant can be obtained and is plotted against the size of the stretch or release in Fig. 4; it is roughly fitted by a curve of the form

$$r=\frac{r_0}{2}(1+\exp-\alpha y) \qquad (1)$$

All these features can be given at least a qualitative explanation if we assume that the force on a cross-bridge influences the length changes in that cross-bridge in the way that is assumed by Eyring and others[11,12] in their theory of the visco-elastic behaviour of high molecular weight polymers. The treatment presented in the following paragraphs is meant only to indicate the way in which these features would emerge from such a theory; a more complete treatment will be needed in order to test whether it is fully consistent with the data.

Assumptions

The key assumptions that have to be made are (1) that the movement by which a cross-bridge performs work during the period while it is attached takes place in a small number of steps, from one to the next of a series of stable positions with progressively lower potential energy, and (2) that there is a virtually instantaneous elasticity within each cross-bridge allowing it to shift from one of these stable positions to the next without a simultaneous displacement of the whole thick and thin filaments relative to one another.

These assumptions could be incorporated into some of the mechanisms that have been proposed for the action of the cross-bridges, for example, that of Davies[13], in which each cross-bridge shortens by folding at a number of points, or that of H. E. Huxley[14], in which the head of the myosin molecule (*H*, Fig. 5) rotates relative to the thin filament, acting as a lever which pulls the thick filament along by a link *AB* which is also part of the myosin molecule. Our assumptions fit very conveniently on to H. E. Huxley's proposals, and we shall discuss them on this basis in the way illustrated in Fig. 5. The features shown there which are additional to H. E.

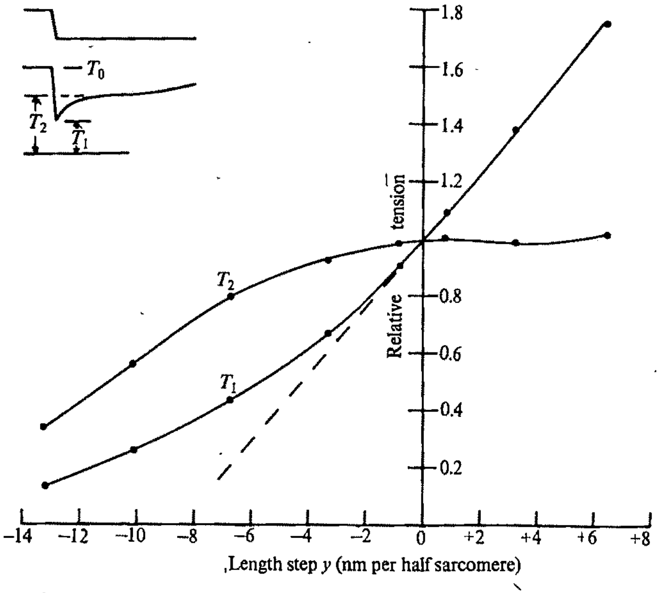


Fig. 3 T_1, Extreme tension reached during step, and T_2, tension approached during quick recovery phase, plotted against amount of sudden stretch (positive) or release (negative). Broken line: extrapolation of the part of the T_1 curve which refers to stretches and small releases. From records in Fig. 2.

Huxley's scheme are as follows. (1) The link *AB* is not inextensible as in H. E. Huxley's proposal but contains the instantaneous elasticity which shows up as curve T_1 (Fig. 3). (2) The head has a small number *s* of combining sites (M_1, M_2 and so on, in Fig. 5), each of which is capable of combining reversibly with a corresponding site (A_1, A_2 and so on) on an actin molecule in the thin filament. A single *M–A* attachment allows variation of θ (rotation in the plane of the diagram of Fig. 5) without hindrance, but no other degree of freedom of the myosin head relative to the actin molecule. (3) The affinity between these myosin and actin sites is smallest for M_1A_1, larger for M_2A_2 and so on. (4) The sites are placed so that the myosin head has $(s-1)$ stable positions, each of which allows two consecutive *M* and *A* sites to be attached simultaneously. (5) When the myosin head is in its $(s-1)$th stable position it can be detached from the thin filament by a process involving the hydrolysis of ATP.

On this basis the quick tension recovery is due to the tendency for the myosin head to rotate to positions of lower potential energy, while the fact that the recovery occurs at a finite speed is a manifestation of the rate constant for movement of the system from one of the stable positions to the next.

Potential Energy Diagram of a Cross-bridge

Curves i–iv in Fig. 6 show the potential energy diagrams for individual attachment sites (M_1A_1, M_2A_2 and so on) on a single cross-bridge. Each contains a flat-bottomed well extending over the range of myosin head positions where that particular attachment can exist. Curve v is the sum of curves i–iv, and therefore gives the total potential energy of the cross-bridge (in the absence of force in the link *AB*); it consists of a series of steps, separated by narrow troughs at the positions where two of the links are attached simultaneously. The depth of each trough will depend on the shapes, and on the exact positions, of the sides of the potential wells that contribute to it; it is assumed in Fig. 6 that these are such that the quantities E_1 and E_2 (v, Fig. 6) are the same for each of the troughs.

The total potential energy of an attached cross-bridge contains also the potential energy of stretching the elastic link *AB*. The latter term is shown in curve vi, and the total in curve vii.

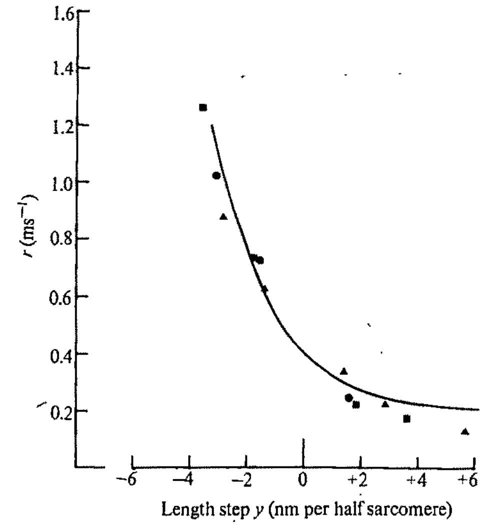


Fig. 4 Rate constant *r* of quick recovery phase following a length step of magnitude *y* (positive for stretch). Estimated as $(\ln 3)/t_{1/3}$ where $t_{1/3}$ is the time for recovery from T_1 to $(2T_2+T_1)/3$ (see Fig. 3). From three experiments using the "spot-follower" device; temperature 4° C. The curve is $r=0.2\ (1+\exp -0.5\ y)$.

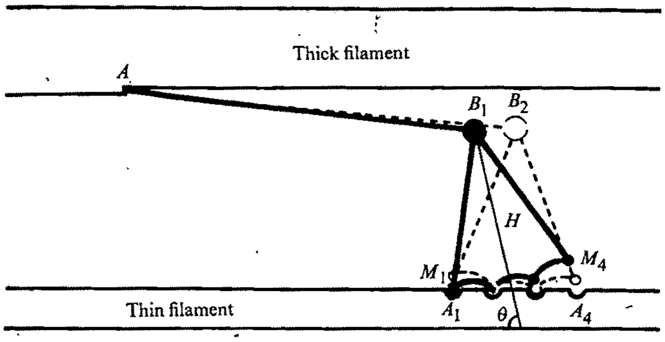


Fig. 5 Diagram showing assumed cross-bridge properties. The myosin head *H* is connected to the thick filament by a link *AB* containing the undamped elasticity which shows up as T_1 (Fig. 3) in the whole fibre. Full line shows head in position where M_1A_1 and M_2A_2 attachments are made; broken lines show position where M_2A_2 and M_3A_3 attachments are made.

Responses Expected Theoretically

In the mathematical section at the end of this article, we derive equations describing the response of a system of this kind to a step change of length. The system treated there is simplified by assuming that only two stable positions are available to each attached cross-bridge. The corresponding potential energy diagram is sketched in Fig. 7. This shows that B_2, the activation energy for transfer of a bridge from position 1 to position 2, contains a term *W* which depends on the force in the link *AB*, being increased by a stretch and reduced by a release. B_1, however, the activation energy for the reverse transfer, contains no such term and is independent of the force. It is this asymmetry which enables the theory to account for the way in which the rate constant of the quick tension recovery varies with the direction and magnitude of the length step: the theoretical result is expressed in equation (12) which is identical in form with equation (1), already shown (Fig. 4) to represent adequately the experimental data. The theory also leads to equation (16) for the tension level approached at the end of the quick recovery phase; this is plotted in Fig. 8 which is seen to reproduce the main features of the experimental T_2 curve in Fig. 3.

The two striking features of the quick tension recovery are thus accounted for by this theory. The numerical values used in obtaining this degree of agreement are: E_1-E_2, the potential energy difference between the stable positions of attachment of a cross-bridge, is equal to 4 kT; *h*, the travel of point *B* between its two stable positions, is 8 nm; *K*, the stiffness of the link *AB*, is 2.5×10^{-4} N m^{-1}.

The following considerations show, however, that the assumption that the cross-bridge movement takes place in a single step is probably not correct.

Isometric force and number of bridges. Equations (4) and (7) show that the isometric force per attached cross-bridge is $(E_1-E_2)/h$; with the values mentioned in the last section this amounts to 2.0×10^{-12} N. To reach a total force of 3×10^5 N m^{-2}, such as real fibres produce, would therefore need 1.5×10^{17} attached bridges m^{-2} in each half-sarcomere. This is about 1.5 times greater than the number of myosin molecules present. The discrepancy would be greater if the number of cross-bridges were equal to the number of projections on the thick filaments detected by low-angle X-ray diffraction since this appears to be about half the number of myosin molecules[15], and greater still if a substantial proportion of the cross-bridges were unattached during isometric contraction. (It is conceivable that each of the two heads on a myosin molecule should be counted separately; this would be appropriate only if the instantaneous elasticity existed within each of the heads, allowing them to shift independently.)

Work per attached cross-bridge. An upper limit to the external work done by the fibre per cross-bridge attachment will be given by the integral in equation (17), between the

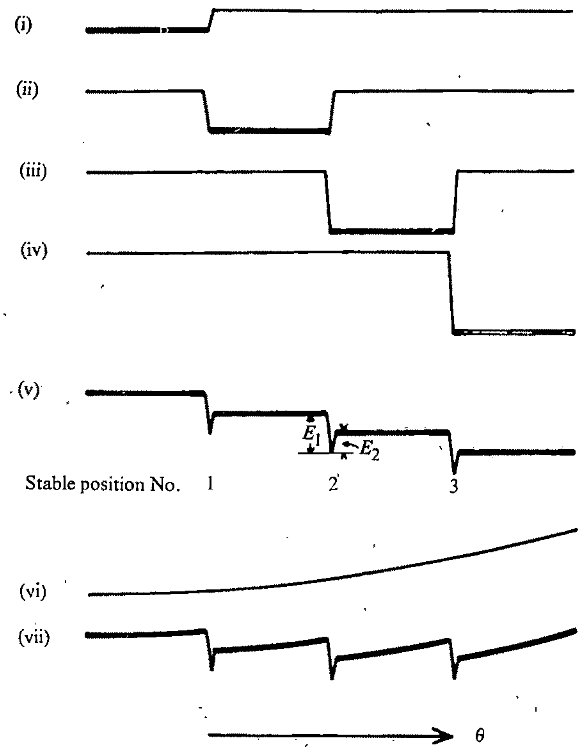


Fig. 6 Potential diagrams relating to the system illustrated in Fig. 5. i–iv, Diagrams for individual attachments M_1A_1, M_2A_2, M_3A_3, M_4A_4 respectively; in each the thick line corresponds to the range of θ within which the corresponding M and A sites are attached; v, sum of i–iv, giving potential energy of a system composed only of a myosin head and a thin filament; vi, potential energy due to stretching the elastic link AB; vii, total potential energy.

isometric point $y=0$ and the point ($y=-12.0$ nm) where $\varphi_2=0$. With the adopted values of α and h, this is 3.8 kT. This result is only about half the value which can be calculated as follows from the actual performance of frog muscle. Work can be as much as 40% of (work+initial heat)[16], and the latter quantity is about 11 kcalories/mol^{-1} of phosphorylcreatine split[17], giving the work term as 4.4 kcalories/mol.$^{-1}$. This is equal to 7.3 kT per molecule, which will represent the work per cycle of attachment and detachment of a cross-bridge if it is assumed that the cycle is coupled (presumably through ATP) to the hydrolysis of one molecule of phosphorylcreatine.

Probable number of stable positions. The quantitative treatment just presented has thus led to low values for the force and work per cross-bridge. It is just possible that revised values for α and for the number of cross-bridges will resolve these discrepancies, but it seems to us more likely that it will be necessary to assume that the cross-bridge movement takes place not in a single step but in two or perhaps more. This would lead to proportional increases in the calculated force and work, but the expected time course and extent of the quick tension recovery, which are already in good agreement with the experimental results, would not be much altered if each step has the same height E_1-E_2 as has already been assumed and the value of h is reduced so as to keep the same total range of travel. Fig. 5 is drawn for the case where the number of steps is 2 (3 stable positions and 4 points of attachment), which at present seems the most probable number.

Relation to Earlier Theories

The idea of applying Eyring's theory of polymers to muscle is not new. A comprehensive theory of muscle was developed by Polissar[18], in which the shortening of links in actomyosin chains was influenced in this way by the load, but this theory lost its relevance when it became clear that major changes of length take place by sliding, not folding, of the filaments.

The proposal that the sliding movement is generated by the tendency of attachments between the filaments to move through a series of a few positions of progressively lower potential energy was made many years ago by H. H. Weber[19], who also pointed out that in this case the rate constants for shifting from one position to another would be affected by the force on the attachment. He discussed this idea purely on the basis of a translational movement of the thick relative to the thin filament, with a site rigidly fixed to one of the filaments transferring itself from one to the next of the sites on the other filament to which it could be attached. It is difficult to visualize a mechanism of this kind operating over the rather large distances—several nanometres—that the transient responses show to be involved, but the difficulty disappears if there is an elastic structure allowing one of the attachment sites to undergo substantial displacements relative to the filament to which it belongs.

On the kinetic side, the scheme discussed here combines the advantages of the proposals made by A. F. Huxley[20] in 1957 and by Podolsky *et al.*[3]. In each of these schemes the production of force was assumed to occur as an immediate consequence of the formation of a myosin–actin link. In A. F. Huxley's scheme, attachment was assumed to be the main rate-limiting factor in steady shortening, while detachment after the performance of work by a cross-bridge was relatively rapid; with these assumptions it is possible to fit A. V. Hill's relations between load, speed and heat production but not the transient responses discussed here. Podolsky *et al.* reversed the assumptions about the rate constants, making attachment rapid and breakage rate-limiting; in this way they were able to fit many aspects of transient responses but, as they recognized, not the thermal data. With appropriate rate constants for the initial attachment and final detachment of each cross-bridge, the present scheme can probably account for the force–velocity and thermal relationships in the same way as A. F. Huxley's 1957 scheme, while the rapid transfer between stable positions of the myosin head produces transient responses not unlike those which, on the theory of Podolsky *et al.*, result from the rapid formation of new attachments. The present proposal is able to combine the successful aspects of both of these theories because it subdivides the force-generating event into distinct stages, one for attachment and others for stretching the cross-bridge, the latter being much more rapid than the former. This separation also removes another difficulty that both of the earlier theories would very likely have met in a fully quantitative treatment. They assumed that tension is already present in the cross-bridge when it attaches to the thin filament; thermal motion would so seldom bring the cross-bridge to such a large deflexion that it might be impossible to account in that way for the rapidity of contraction that some real muscles achieve.

Mathematical Section

Equations will be derived for the extent and time-course of the quick tension recovery to be expected from a system

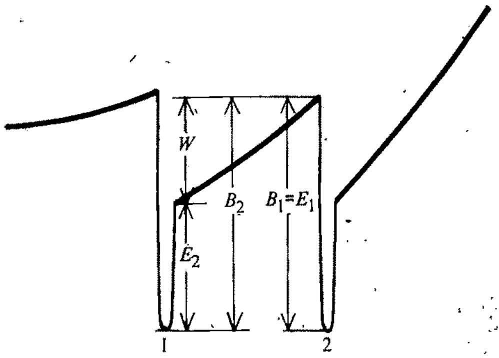


Fig. 7 Potential energy diagram equivalent to Fig. 6 (vii) but referring to the simplified system with only two stable positions.

similar to that shown in Fig. 5 but with only two instead of three stable positions of the myosin head relative to the thin filament. The following additional simplifying assumptions are also made. (1) Actual detachment and re-attachment of cross-bridges are slow enough to be disregarded. (2) Filaments themselves are completely rigid. (3) Filaments undergo no sliding movements except when the total length of the fibre is being altered. (4) The elasticity of the link *AB* obeys Hooke's law. (5) The link *AB* is capable of exerting negative as well as positive tensions. (6) In the isometric steady state, every attached cross-bridge spends equal amounts of time in each of the two available positions (this cannot be strictly true in real muscle because the spacings along the thick and thin filaments are not in any simple ratio, so the relative positions of the myosin and actin molecules must vary from one cross-bridge to another). (7) The time taken in transferring from one to the other of the two positions is negligible.

The following notation will be used; n_1: fraction of attached bridges in position 1; n_2 $(=1-n_1)$: fraction of attached bridges in position 2; y: displacement of thick relative to thin filament when fibre is stretched or shortened (zero in isometric state before the applied length change; positive for stretch); y_0: extension of elastic link *AB* when bridge is midway between positions 1 and 2 (equal to amount of sudden sliding movement needed to bring tension to zero from the isometric state); h: increase in length of *AB* when bridge shifts from position 1 to position 2; K: stiffness of link *AB* (assumed to obey Hooke's law); F_1: tension in *AB* when bridge is in position 1; F_2: tension in *AB* when bridge is in position 2; φ: time average of F_1 and F_2.

From these definitions,

$$F_1=K(y+y_0-h/2) \text{ and } F_2=K(y+y_0+h/2) \qquad (2)$$

and the time average of tension is

$$\begin{aligned}\varphi&=n_1F_1+n_2F_2\\&=K(y+y_0-h/2+hn_2)\end{aligned} \qquad (3)$$

In the isometric state, $y=0$ and $n_2=1/2$, and this equation reduces to the expression for the isometric force per attached cross-bridge

$$\varphi_0=Ky_0 \qquad (4)$$

The rate constants k_+ for transfer from position 1 to position 2, and k_- for transfer from 2 to 1, are governed by the energy barriers B_2 $(=E_2+W)$ and B_1 $(=E_1)$ respectively (Fig. 7). W is the work done in stretching *AB* when the bridge transfers from position 1 to position 2, and is given by

$$\begin{aligned}W&=h\,\frac{F_1+F_2}{2}\\&=Kh(y+y_0)\end{aligned} \qquad (5)$$

from equations (2).

Assuming the ks proportional to $\exp\,-B/kT$, we have

$$\begin{aligned}k_+&=k_-\exp\,(B_1-B_2)/kT\\&=k_-\exp\,(E_1-E_2-W)/kT\\&=k_-\exp\,(E_1-E_2-Kh(y+y_0))/kT\end{aligned} \qquad (6)$$

where k_- is constant since B_1 is a fixed quantity independent of the tension in *AB*.

In the isometric state we have assumed $n_1=n_2$, so $k_+=k_-$; also $y=0$ since y is defined as a length change from the isometric state. It then follows from (6) that

$$E_1-E_2=Khy_0 \qquad (7)$$

and (6) becomes

$$k_+=k_-\exp-y.Kh/kT \qquad (8)$$

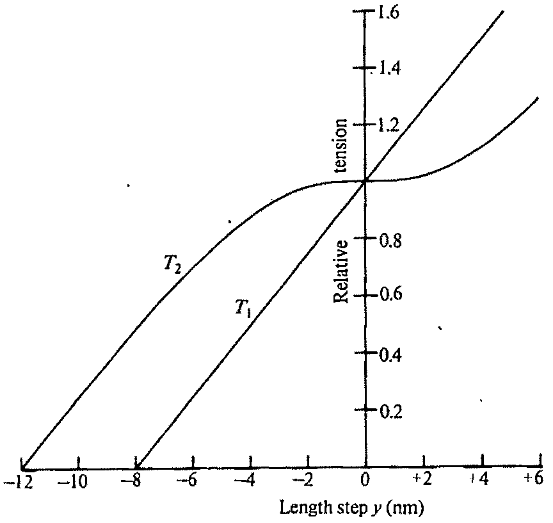


Fig. 8 Curves of T_1 and T_2 calculated from the simplified system, plotted on same scales as the experimental values in Fig. 3. T_2 curve: Equation (15) with $E_1-E_2=4\,kT$, $1/\alpha=2$ nm, $h=8$ nm.

In the experiments we are considering, y is suddenly altered by imposing a length change on the muscle fibre, and is subsequently held constant. The transfer of myosin heads from one position to the other is then governed by the equation

$$\begin{aligned}\mathrm{d}n_2/\mathrm{d}t&=k_+n_1-k_-n_2\\&=k_+-(k_++k_-)n_2\end{aligned} \qquad (9)$$

This equation represents an exponential approach, with rate constant r given by

$$r=k_++k_- \qquad (10)$$

towards an equilibrium where

$$n_2=k_+/(k_++k_-) \qquad (11)$$

Substituting from (8) into (10) we have

$$r=k_-(1+\exp-y.Kh/kT) \qquad (12)$$

This has the same form as equation (1), and the equations become identical, giving approximate agreement with the experimental results in Fig. 4, if we take

$$Kh=\alpha kT \qquad (13)$$

Equation (8) can therefore be written

$$k_+=k_-\exp-\alpha y \qquad (14)$$

and (11) becomes

$$n_2=\frac{1}{2}\left(1+\tanh\frac{\alpha y}{2}\right) \qquad (15)$$

The tension φ_2 at the end of the quick recovery (corresponding to T_2 in the whole fibre) is obtained by combining (3), (13) and (15) to give

$$\varphi_2=\frac{\alpha kT}{h}\left(y_0+y-\frac{h}{2}\tanh\frac{\alpha y}{2}\right) \qquad (16)$$

The work done during shortening at a low enough speed so that n_2 always has its equilibrium value would be obtained by integrating (16):

$$\int\varphi_2\mathrm{d}y=kT\left(\frac{\alpha y}{h}\left(y_0+\frac{y}{2}\right)-\ln\cosh\frac{\alpha y}{2}\right) \qquad (17)$$

To match the points in Fig. 4, α is taken as 5×10^8 m^{-1}, the value used for the curve in that figure. (E_1-E_2) is shown by equations (7) and (13) to be equal to $\alpha y_0 kT$. From Fig. 3 (broken line), y_0 is about 8 nm, giving $E_1-E_2=4\,kT$. h has to be chosen to give the right shape for the curve of T_2 against y (equation 15). A value $4/\alpha$, or 8 nm, is used in Fig. 8; lower values give a less inflected curve and higher values give a curve with a region of negative slope.

Generation of Tension

The tension changes observed in the first few milliseconds after suddenly changing the length of an active muscle fibre suggest the following mechanism for the generation of tension or shortening by the cross-bridges. Each cross-bridge has three stable positions with progressively lower potential energies, in steps of about 4 times kT, separated by about 4 nm of travel. Transfer from one of these positions to the next is made possible, without simultaneous displacement of the whole filaments through an equally large distance, by the presence of elasticity associated with each individual cross-bridge. The tension generated in this way in the elastic element will show up as such if the muscle length is held constant, or will help to make the filaments slide past each other if shortening is permitted.

A simplified theoretical treatment is given; a more complete treatment will be presented later.

Received August 25, 1971.

[1] Podolsky, R. J., *Nature*, **188**, 666 (1960).
[2] Civan, M. M., and Podolsky, R. J., *J. Physiol.*, **184**, 511 (1966).
[3] Podolsky, R. J., Nolan, A. C., and Zaveler, S. A., *Proc. US Nat. Acad. Sci.*, **64**, 504 (1969).
[4] Huxley, A. F., and Simmons, R. M., *J. Physiol.*, **208**, 52*P* (1970).
[5] Gordon, A. M., Huxley, A. F., and Julian, F. J., *J. Physiol.*, **184**, 143 (1966).
[6] Huxley, A. F., and Simmons, R. M., *J. Physiol.*, **197**, 12*P* (1968).
[7] Gasser, H. S., and Hill, A. V., *Proc. Roy. Soc.*, B, **96**, 398 (1924).
[8] Hill, A. V., *Proc. Roy. Soc.*, B, **141**, 104 (1953).
[9] Jewell, B. R., and Wilkie, D. R., *J. Physiol.*, **143**, 515 (1958).
[10] Huxley, A. F., and Simmons, R. M., *J. Physiol.*, **218**, 59*P* (1971).
[11] Eyring, H., *J. Chem. Phys.*, **4**, 283 (1936).
[12] Burte, H., and Halsey, G., *Tex. Res. J.*, **17**, 465 (1947).
[13] Davies, R. E., *Nature*, **199**, 1068 (1963).
[14] Huxley, H. E., *Science*, **164**, 1356 (1969).
[15] Huxley, H. E., *J. Mol. Biol.*, **7**, 281 (1963).
[16] Hill, A. V., *Proc. Roy. Soc.*, B, **127**, 434 (1939).
[17] Wilkie, D. R., *J. Physiol.*, **195**, 157 (1968).
[18] Polissar, M. J., *Amer. J. Physiol.*, **168**, 766, 782, 793 and 805 (1952).
[19] Weber, H. H., *The Motility of Muscle and Cells*, 32 (Harvard University Press, Cambridge, Massachusetts, 1958).
[20] Huxley, A. F., *Prog. Biophys.*, **7**, 255 (1957).

How Dextrous was Neanderthal Man?

JONATHAN H. MUSGRAVE

Department of Anatomy, The Medical School, University Walk, Bristol BS8 1TD

Results of multivariate statistical analyses indicate that the bones of the hand of Neanderthal man were metrically and morphologically unique and that he may not have been as dextrous as living *Homo sapiens*.

THE postcranial skeleton of Neanderthal man has been studied less intensively than his skull. This is to be regretted because any serious attempt to re-assess the phylogenetic status of the Neanderthalers must be based on their total morphological pattern[1] and not solely on evidence of cranial variation. In an attempt to rectify this situation I have made a detailed study of as many as possible of the Neanderthal hand bones listed by Vallois and Movius[2]. Measurements were taken on these bones and on comparative samples of European Upper Palaeolithic, Mesolithic and modern hand bones. These measurements and indices calculated from them were then submitted to discriminant function (canonical variate) analyses and Mahalanobis's D^2 tests[3]. This article presents some of the conclusions drawn from this investigation[4].

Thumb

(1) The metacarpal seems to have been neither relatively nor absolutely short, as earlier workers had suggested[5,6], and it was instead the great transverse width of the head, a feature which had also aroused comment[7,8], which proved to be the most important dimension. This observation may be correlated with a very noticeable anatomical feature, a flange which runs up the distal half of the radial side of the shaft (Fig. 1). Into this was inserted opponens pollicis muscle, clearly large and powerful in Neanderthal man[9], a suggestion which is in keeping with recent theories on the role of this muscle as an abductor of the thumb in a firm grip[10]. Associated with this flange is a marked depression[8] diagonally opposite, from which arose the first metacarpal head of the first dorsal interosseous, another "power" muscle.

Other unusual features of the Neanderthal thumb metacarpal include an asymmetry in the transverse curvature of the head and the presence in several specimens of a condyloid rather than a saddle-shaped proximal articular surface. The asymmetry has been noted in some non-human primate genera[11,12] and may reflect difficulties in opposing the thumb to the other digits. The condyloid articular surface, clearly visible in the specimen from La Chapelle-aux-Saints[5] but only partly developed in those from Kiik-Koba[9] and La Ferrassie

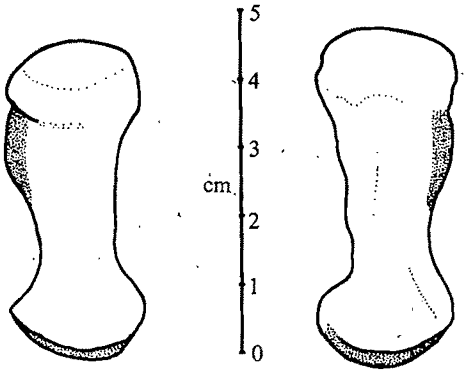


Fig. 1 Palmar aspect of Neanderthal thumb metacarpals. The principal features are: (1) the radially projecting ridge (stippled) into which was inserted opponens pollicis muscle; (2) the asymmetry of the curve of the distal articular surface; and (3) the narrow waist at the proximal end of the shaft, formed on the ulnar side by the impression for the origin of the first metacarpal head of the first dorsal interosseous muscle. Both specimens belonged to male Neanderthalers. Tracings were made from photographs of casts.

(La Ferrassie II, right), is also found in modern specimens[6]. The incidence of thumb metacarpals with atypical bases, however, seems rather high among the Neanderthalers and it will be interesting to watch whether future discoveries reveal a trend towards the development of this feature.

(2) The proximal phalanx is distinguished from its modern counterparts by its robusticity and especially its shortness. Discriminant functions show it to be significantly shorter than modern thumb proximal phalanges both absolutely and proportionately. For example, it is much shorter in proportion to the lengths of the other bones of the thumb than is a modern thumb proximal phalanx.

(3) The shortness of the thumb proximal phalanx is further highlighted by the great length of the distal phalanx. It was confirmed by *t*-tests that the Neanderthal sample was significantly longer, relative to the length of the other bones of the thumb and, of course, the whole thumb, than any of the samples analysed. That so many independent statistical analyses confirmed the shortness of the thumb proximal phalanx and the great length of the distal phalanx indicates that these are important features and ones which may have affected the range for grips used by Neanderthal man.

In addition to being very long the Neanderthal thumb distal phalanx is very broad transversely across its mid-shaft and head; these dimensions are also shown to be important by the discriminant functions (Fig. 2). It is not clear why all Neanderthal thumb distal phalanges, from France to the Crimea, should have broad distal tuberosities, but there are two possible explanations for this trait: first, a broad distal end would support a large distal pulp space, which might be useful in a powerful grip; second, a voluminous pulp space would also presumably be highly vascular, and hence useful in a cold climate.

As well as being broad the whole of the distal end of this bone in most Neanderthal specimens is markedly deflected to the medial side of the hand, a feature which is frequently noticed in modern specimens[13]. It is so noticeable and persistent in the Neanderthal thumbs, however, that it is tempting to postulate that it may be correlated with an imperfect development of efficient opposition. If the distal end is already tilted a millimetre or so to the index finger then such an arrangement, like the asymmetrical curve of the metacarpal head, might help to compensate for any pecularities in the shape of the carpometacarpal joint which might impede efficient opposition.

The shortness of the proximal phalanx and the length of the distal phalanx do not seem to have affected the total length of the only three complete Neanderthal thumbs measured—101.4 mm La Ferrassie I, left); 99.6 mm (La Ferrassie I, right); and 86.8 mm (La Ferrassie II, right). The means and standard deviations of this small sample are 95.9 mm and 8.0 respectively, which compare with scores of 94.2 mm and 7.6 for a sample of thirty-eight modern European thumbs. Furthermore, the thumb apparently was not very short in proportion to the lengths of the index and middle fingers as *t*-tests confirmed.

The chief characteristics of the Neanderthal thumb can be summarized as follows. It was a powerful digit of average length. The metacarpal was not short, but its head was asymmetrically curved and some of its intrinsic muscles, notably opponens pollicis and the first dorsal interosseous, were apparently extremely well developed. The proximal phalanx was exceptionally short and robust. The distal phalanx on the other hand was exceptionally long and wide and its distal end was tilted well over to the medial (or ulnar) side of the hand.

Index Finger

Separate canonical variate analyses of the metrical data on the index metacarpal and proximal phalanx support theories on the functional anatomy of this digit which previously had been based only on macroscopic examination of the bones themselves. Statistical analyses showed the metacarpal to be quite long, narrow transversely at the mid-shaft and extremely wide in the same plane at its head. Indeed, not only the thumb and index metacarpals but also metacarpals III, IV and V were found to have very wide heads. The index proximal phalanx was observed to be significantly shorter and wider across its base and mid-shaft than are the other samples of prehistoric and modern bones analysed.

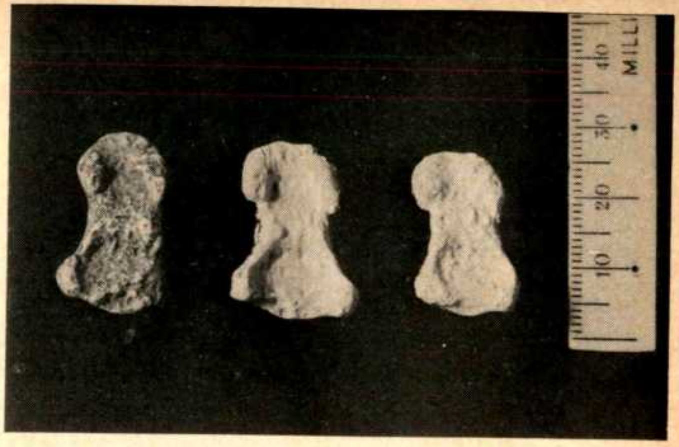


Fig. 2 Palmar aspect of casts of Neanderthal right thumb distal phalanges: (from left to right) La Ferrassie I; Krapina 203.1; Krapina 203.4. Note the stout distal tuberosities and the degree to which they are tilted away from the longitudinal axis of the shaft in an ulnar direction.

It is clear that the intrinsic muscles moving the index finger, especially the first dorsal interosseous, were extremely powerful. The evidence for this statement comes from the origins and insertion of this muscle. Its two bellies arise from the proximal half of the ulnar side of the shaft of the first metacarpal and from the whole of the radial side of the shaft of the second[14]. In the Neanderthal hand these areas of origin are clearly demarcated. First, the area on the first metacarpal, visible as a marked depression, has already been mentioned. Second, a sharp ridge or "crista dorsalis" may be seen running up the proximal one quarter of the shaft on the dorsum of at least four Neanderthal index metacarpals—La Ferrassie I, right; Spy 21A and 22A, right and left (Fig. 3); and Subalyuk, left[16]. This crest, by no means uncommon on modern specimens but frequently not so sharply defined, divides the second metacarpal bellies of the first and second dorsal interosseous muscles and only develops when these two bellies are large enough to warrant a bony separation from each other.

The two bellies of the first dorsal interosseous muscle unite to be inserted by a common tendon chiefly into the dorsal radial tubercle on the base of the index proximal phalanx[14,16–28]. This tubercle may be seen on all examples of this bone and is large enough to make the appearance of the whole of the base asymmetrical[24,29]. It is so large on the Neanderthal specimens that it expands the transverse diameter of the base until it equals almost half the length of the bone (Fig. 4). Moreover, this diameter, when expressed as a percentage of the length, was found to contribute most to the discrimination of the Neanderthal specimens from the others.

The possession of a very powerful first dorsal interosseous muscle presumably had a profound effect on the functional anatomy of the Neanderthal hand as a whole. The function of this muscle has never been determined precisely, but there is little doubt that its major role is that of abducting the index finger towards the thumb[19,20,30] and, by indirect action on the oblique lateral ligaments of the metacarpo-phalangeal joint, pronating the digit so that the distal palmar pad faces away from that of the thumb[24,29].

When a small object is held in a fine pulp-of-thumb to pulp-of-index finger precision grip, the index finger is adducted towards the little finger so that as much as possible of the pulp

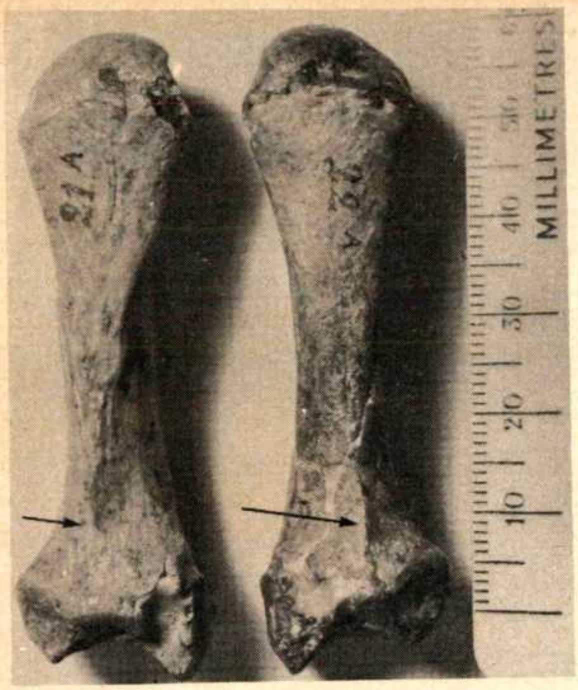


Fig. 3 Dorsal aspect of Neanderthal index metacarpals: Spy 21A (right) and 22A (left). Note the massive heads and bases and the well marked "crista dorsalis" (arrowed) running up the proximal one quarter of the shaft.

of the digit is supinated to oppose the pulp of the thumb. The first dorsal interosseous muscle is only active to prevent the index finger from being pushed too far towards the little finger should too much pressure be applied by the thumb. Precision and fine control may well be lost when this happens. It is suggested that the first dorsal interosseous muscle comes into its own, first, when the fingers are used as the other half of a powerful clamp with the thumb to anchor large spherical or cylindrical objects; and second, in a rather crude precision grip, the so called pinch grip or key grip[22,23,29]. In this grip an object is held between the pulp of the thumb and side of the flexed index finger, at, for example, the distal inter-phalangeal joint or along the side of the middle phalanx. When objects are held thus the contracted bellies of the first dorsal interosseous muscle can be palpated easily. It is also the precision grip habitually adopted by many short-thumbed non-human primates[31,32].

It is also very tempting to suggest that it might have been the kind of precision grip favoured by Neanderthal man. Neanderthal man, however, certainly cannot be called short-thumbed. The ratio of the length of his thumb to his index finger was apparently little different from ours. By using left and right hand bones of La Ferrassie I it was possible to build up a composite index finger and demonstrate that the Opposability Index (length of thumb/length of index finger × 100) was 64.4, a score very close to the figure of 65.4 obtained from a modern sample of thirty-eight (standard deviation 1.94).

It nevertheless may not be the proportion between the total lengths of thumb and index finger that is important but rather the proportions of the individual bones. The proximal phalanges of both these digits in the Neanderthal hand were distinctly shorter than their modern counterparts. This feature alone may well have determined which kind of precision grip he habitually used. It is clear that the proportions of the lengths of the individual bones of a modern human digit are constant, especially in the thumb and index finger, and they presumably affect dexterity. Unfortunately not enough work has been done on the modern hand to allow a precise inference as to what advantages or disadvantages this feature conferred on the Neanderthal hand.

The Rest of the Hand

The other bones of the Neanderthal hand all indicate independently that it was a squat, powerful appendage.

(1) The carpus possesses several interesting features. The tubercle on the trapezium and the hamulus on the hamate are very large and form a deep carpal tunnel for powerful extrinsic flexor tendons (see Fig. 5). The distal articular facet on the trapezium of at least one specimen (Kiik-Koba, left) is strange enough to attract comment[9]. The articular facet for the fifth metacarpal on the hamate of another (La Ferrassie II, right) shows that the little finger could be abducted far enough to increase the span of the hand quite considerably (see Fig. 5).

(2) Metacarpals III to V are relatively long and, like metacarpals I and II, have characteristically wide heads. They are rather narrow at the mid-shaft, however, and this may well have been advantageous; the combination of wide heads and narrow mid-shafts would increase the volume of the inter-metacarpal spaces and hence the volume of the interosseous muscles.

(3) Proximal phalanges III to V, like proximal phalanges I and II, are relatively short. They have thick, wide bases and are very wide at the mid-shaft, presumably to accommodate stout tendons for the extrinsic flexor muscles. The projection formulae of the condyles on the heads of the proximal phalanges of the most complete male Neanderthal hand (La Ferrassie I, left) should also be mentioned. They are so arranged that the ulnar condyles on proximal phalanges II and III project further distally than the radial ones, and the radial condyles project further distally on proximal phalanges IV and V (Fig. 6). This feature may be seen on modern proximal phalanges, but it is so pronounced in this specimen that it is tempting to regard it as additional evidence that the Neanderthal hand possessed a very wide span. From other work[33,34], it seems that the asymmetry of the condyles on proximal phalanges II to V may play quite an important part in divergence at the proximal inter-phalangeal joints of these fingers.

(4) Middle phalanges II to V are very difficult to identify accurately. A comparative analysis of a random sample of Neanderthal specimens showed them nevertheless to resemble most closely middle phalanges II and V of a modern sample. In other words, they are rather short and robust with wide bases and heads, in contrast to the Upper Palaeolithic specimens which are much longer and thinner.

(5) Distal phalanges II to V are among the most interesting bones of the Neanderthal hand. They are relatively long and resemble most closely distal phalanges III and IV of a modern

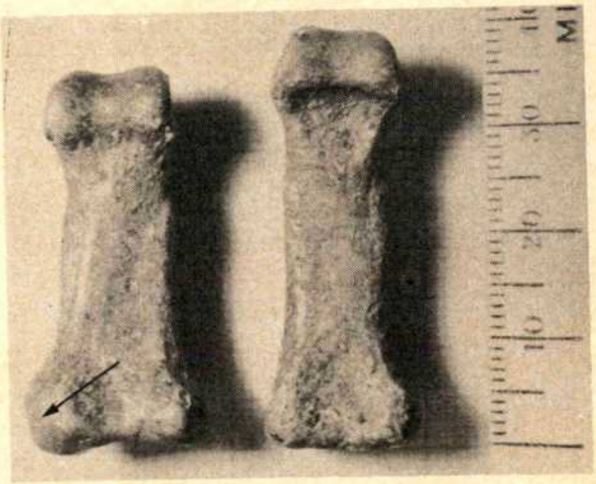


Fig. 4 Palmar aspect of Neanderthal index and ring proximal phalanges: Spy 24C (index; left) and 24A (ring; left). Note the massive development (arrowed) of the radial side of the base of the index proximal phalanx (24C).

sample. The bases and especially the heads are characteristically wide[9]. A possible explanation for this feature, observed on the distal phalanx of the thumb, has been proposed earlier.

This investigation thus seems to show that the Neanderthal hand possessed several distinctive metrical and morphological features. Each feature can be found in modern hands but they rarely occur combined in one individual hand. It seems therefore that the total morphological pattern of the Neanderthal hand was unique, a claim made more plausible by the complete lack of similarity between the hands of Neanderthal man and his immediate successor in time, Upper Palaeolithic *Homo sapiens* (that is, Cro-Magnon man). Whether these features reflect adaptations to his environment or imperfect development of manual skill is still not known. It is not unlikely that both these factors contributed something to the morphology of the Neanderthal hand.

I thank Dr J. R. Napier and Dr M. H. Day, my supervisors; Dr M. R. B. Clarke and his colleagues at the University of London Institute of Computer Science; and all the people who allowed me to study the fossil hand bones in their care. This project was financed by Churchill College, Cambridge; the Wenner-Gren Foundation for Anthropological Research, New York; the Boise Fund, University of Oxford; the Emslie Horniman Scholarship fund, Royal Anthropological Institutes; and the Dowager Countess Eleanor Peel Trust, Lancaster Royal Grammar School.

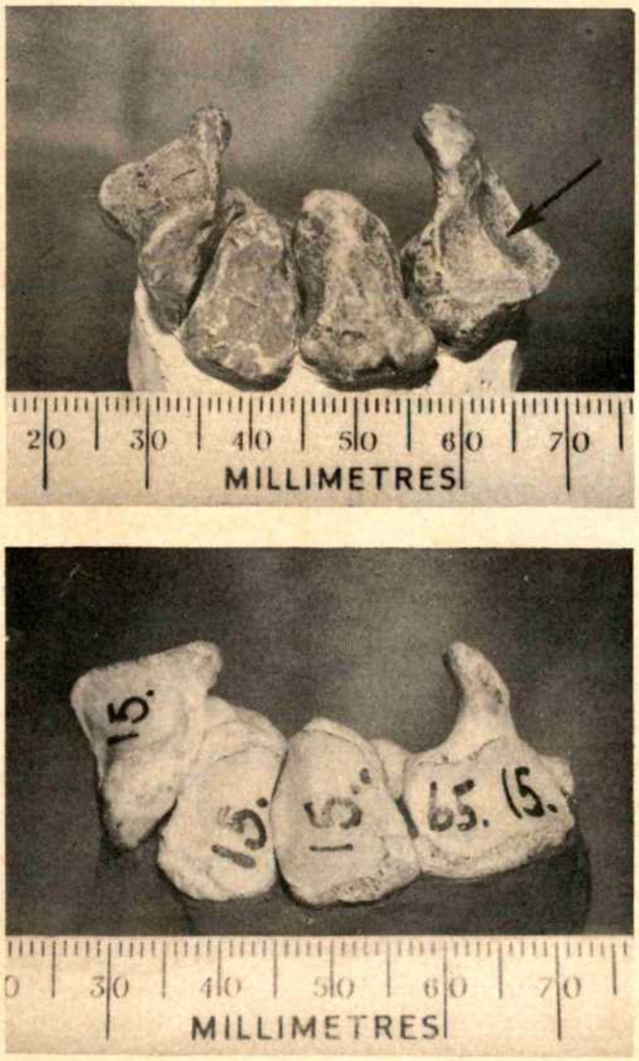


Fig. 5 Cast of the articulated carpus of the right hand of La Ferrassie II (female) compared with that of a modern Western European female (65.15). This view of the distal articular surfaces of the distal row of carpal bones shows the large tubercle on the trapezium and the large hamulus on the hamate features which increased the depth of the carpal tunnel. Note too the discrete and ulnar deviated facet (arrowed) for the fifth metacarpal on the hamate of La Ferrassie II.

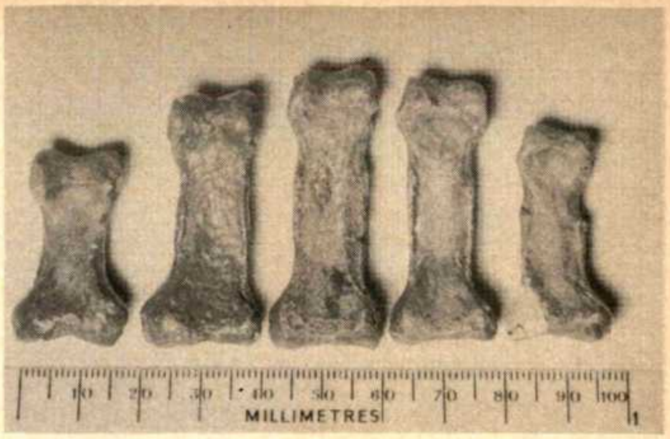


Fig. 6 Palmar aspect of, from left to right, casts of proximal phalanges I to V of the left hand of La Ferrassie I (male). Note the massive bases and heads, and the asymmetrical projection formulae of the condyles.

Received June 24, 1971.

[1] Clark, W. E. Le Gros, *The Fossil Evidence for Human Evolution* (University of Chicago Press, 1955).

[2] Vallois, H. V., and Movius, jun., H. L. (eds.), *Catalogue des Hommes Fossiles* (*CR Cong. Géol. Intern.*, Algiers, 1952).

[3] Clarke, M. R. B., and Maxwell, A. E., *Program Specification PS* 17 *EXCHLF SSP* 1 (Univ. London Inst. Computer Sci., 1966).

[4] Musgrave, J. H., thesis Univ. Cambridge (1970).

[5] Boule, M., *Annls. Paléontol.*, **6**, **7** and **8** (1911–1913).

[6] Sarasin, F., *Z. Morphol. Anthropol.*, **30**, 252 (1931).

[7] Sarasin, F., *Anthropologies der Neu-Caledonier und Loyalty-Insulaner, Nova Caledonia, C: Anthropologie* (Kreidel, Berlin, 1916–1922).

[8] McCown, T. D., and Keith, A., *The Stone Age of Mount Carmel, The Fossil Human Remains from the Levalloiso-Mousterian*, **2** (Clarendon Press, Oxford, 1939).

[9] Bonč-Osmolovskij, G. A., *Kist' iskopaemogo tseloveka iz grota Kiik-Koba*, *Paleolit Kryma*, **2** (Moscow-Leningrad, 1941).

[10] Forrest, W. J., and Basmajian, J. V., *J. Bone Joint Surg.*, **47-A**, 1585 (1965).

[11] Napier, J. R., *Fossil-metacarpals from Swartkrans* (*Fossil Mammals of Africa*, No. 17) (British Museum (Natural History), London, 1959).

[12] Napier, J. R., *Symp. Zool. Soc. London*, No. 5, 115 (1961).

[13] Wilkinson, J. L., *J. Anat.*, **88**, 537 (1954).

[14] Walsh, J. F., *The Anatomy and Functions of the Muscles of the Hand and of the Extensor Tendons of the Thumb* (Walsh, Philadelphia, 1897).

[15] Bartucz, L., *Geologica Hung.*, *Ser. Palaeont.*, **14**, 1 (1940).

[16] Albinus, B. S., *Historia Musculorum Hominis* (Leiden, 1734).

[17] Willan, R., *Anat. Anz.*, **42**, 145 (1912),

[18] Salsbury, C. R., *J. Anat.*, **71**, 395 (1937).

[19] Bunnell, S., *J. Bone Joint Surg.*, **24**, 1 (1942).

[20] Braithwaite, F., Channell, G. D., Moore, F. T., and Whillis, J., *Guy's Hosp. Rep.*, **97**, 185 (1948).

[21] Wood Jones, F., *The Principles of Anatomy as Seen in the Hand* (Baillière, Tindall and Cox, London, 1949).

[22] Littler, J. W., in *A Textbook of Orthopedics* (edit. by Howorth, M. B.), 244 (Saunders, Philadelphia, 1952).

[23] Eyler, D. L., and Markee, J. E., *J. Bone Joint Surg.*, **36-A**, 1 (1954).

[24] Landsmeer, J. M. F., *Acta Anat.*, **25**, *Suppl. 24*, 2 (1955).

[25] Stack, H. G., *Ann. Roy. Coll. Surg.*, **33**, 307 (1963).

[26] Kaplan, E. B., *Functional and Surgical Anatomy of the Hand* (Lippincott, Philadelphia, 1965).

[27] Smith, R. J., and Kaplan, E. B., *J. Bone Joint Surg.*, **49-A**, 31 (1967).

[28] Backhouse, K. M., *Ann. Roy. Coll. Surg.*, **43**, 154 (1968).

[29] Hakstian, R. W., and Tubiana, R., *J. Bone Joint Surg.*, **49-A**, 299 (1967).

[30] Duchenne, G. B., *Physiologie des Mouvements* (Paris, 1867; English translation by Kaplan, E. B., published by Saunders, Philadelphia, 1959).

[31] Napier, J. R., *Proc. Zool. Soc. London*, **134**, 647 (1960).

[32] Krantz, G. S., *Kroeber Anthropol. Soc. Pap.*, No. 23, 114 (1960).

[33] von Bonin, G., *Anthropol. Anz.*, **7**, 241 (1931).

[34] Holcomb, G. R., Irving, jun., T. E., and Smith, R. D., *Amer. J. Phys. Anthrop.*, **16**, 429 (1958).

LETTERS TO NATURE

PHYSICAL SCIENCES

Deposition of Extraterrestrial Nickel in Marine Sediments

THE vertical distribution of nickel components in deep sea sediments was first obtained by the studies of long cores raised from the Swedish Deep Sea Expedition with the Albatros in 1947–1948[1]. The NiO content varied from a maximum of 0.089% by weight to a minimum of 0.041% by weight. Five distinct maxima occurred at various depths, and the average value was 0.056% by weight. Four sources of the nickel content in red clay were considered by Pettersson and Rotschi[1] and the extraterrestrial supply (meteoric dust) was considered by them as the most important. This interpretation of the origin of the nickel component, however, has received several objections[2–4].

Laevastu and Mellis[3] separated magnetic spherules from various depths in the same core, whose vertical nickel distribution has been studied by Pettersson and Rotschi[1]. In the article by Laevastu and Mellis[3] it is emphasized that no correlation between the spherule concentration and nickel content in the core was found.

Smales and Wiseman[2] and Smales, Mapper and Wood[4] have pointed out that the ratio of nickel to cobalt and nickel to copper in red clay is similar to the ratio in igneous rocks but very different from that in meteorites. In reference 3, nickel, cobalt and copper contents in igneous rocks, marine sediments and meteorites were determined using neutron activation analysis. Öpik has also discussed this problem[5] and he stated that comparisons of the ratio of nickel to cobalt are not important and the ratio of nickel to cobalt in solar atmosphere is closer to the ratio in red clay than that in meteorites.

In 1954 Goldberg[6] proposed an idea of the scavenging of various elements into the sediments by hydrated oxides of manganese and iron, and recently one of us proposed a simple model calculation of the nickel component in marine sediments[7].

The nickel component in marine sediments is considered to be supplied in two ways; grain settling and ionic (or colloidal) sedimentation. The former is independent of and the latter is dependent on the sedimentation rate. The expression is as follows:

$$\frac{a + bx}{\rho x} = C$$

where x (cm yr^{-1}) is the sedimentation rate of deep sea deposit, ρ = density of deep sea deposit, C (g of Ni per g of sediment) is the nickel content of deep sea deposit, a (g of Ni cm^{-2} yr^{-1}) assumed to be independent of time is the nickel sedimentation rate through grain settling and bx (g Ni cm^{-2} yr^{-1}) is the nickel sedimentation rate through ionic or colloidal sedimentation, in whose term it is expressed, that the ionic or colloidal sedimentation is related to x. A similar formulation to this was used by Barker and Anders[8] in the study of accretion rate of Ir and Os contents in deep sea sediments, but in that work no concept of grain and ionic supplies of the elements into marine sediments was expressed.

Table 1 Nickel Content in Lamont A 180–76

Zone	Interval (cm)	Duration (yr)	Sedimentation rate (cm per 10^3 yr)	Average nickel content (p.p.m.)
A	0–25	11,000	2.3	13
B	30–260	60,000	3.8	17
C	265–410	80,000	1.8	13

Table 2 Properties of Red Clay Core Lamont, V–20–130

Epoch	Palaeomagnetic event	Sedimentation rate (mm per 10^3 yr)	Average nickel content (p.p.m.)
1 Brunhes	Normal	0.76	104
2 Matsuyama	Reversed	2.47	102
3 Matsuyama	Normal	12.5	98
4 Matsuyama	Reversed	0.81	100
5 Matsuyama	Normal	3.4	84
6 Matsuyama	Reversed	0.47	77
7 Gauss	Normal	7.85	68
8 Gauss	Reversed	0.83	70

The settled grains are brought not only by extraterrestrial sources, but by continental and/or volcanic sources. The ionic and colloidal nickel fractions are of authigenic origin in seawater and they are obtained almost entirely from

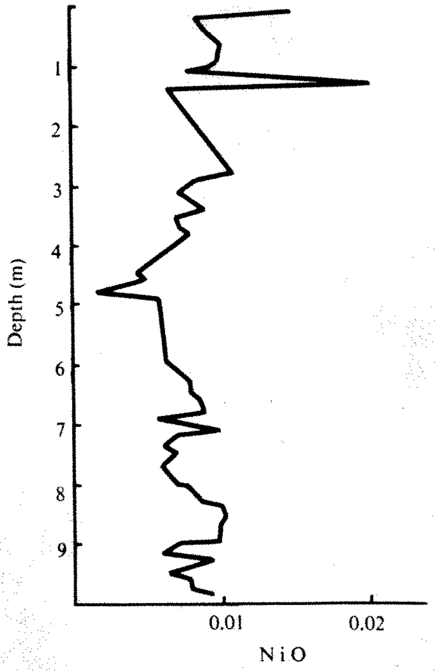


Fig. 1 The vertical distribution of NiO in V–20–130 core.

terrestrial sources. For this calculation the nickel content determination of dated cores is necessary but only two works have been published.

Turekian[9] determined nickel content in a calcareous core, Lamont A 180–76, which was dated using the radiocarbon method in 1958. The results are shown in Table 1.

The nickel concentration profile obtained by Amano, Okada and Shima[10] for a red clay core, Lamont V–20–130, is shown in Fig. 1. The same core was dated by Kobayashi using the palaeomagnetic dating method, which is shown in Fig. 2 (ref. 11).

From Figs. 1 and 2 one deduces the information shown in Table 2. The expression, $a+bx=\rho Cx$, is plotted in Fig. 3 using the data in Tables 1 and 2. The density used is 1.5 for a red clay core and 0.74 for a calcareous core. It can be seen from the figure that the coefficient of a is nearly zero. The parameters a and b are evaluated by a calculation of least squares as follows; $a=0.1\times10^{-6}$ (g Ni 10^3 cm^{-2} yr) and $b=121\times10^{-6}$ (g Ni cm^{-3}) for the red clay sample.

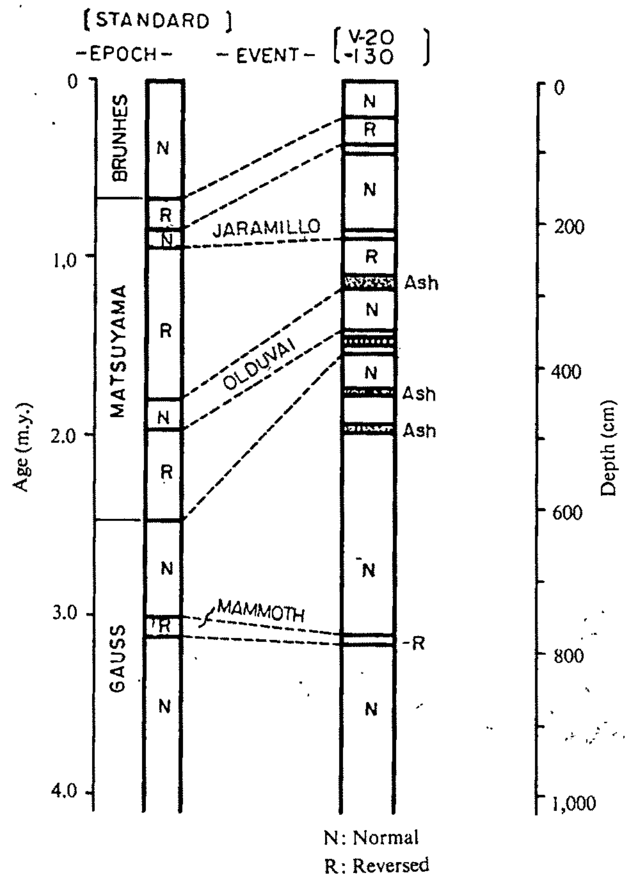


Fig. 2 Age determination of V–20–130 core using palaeomagnetic method.

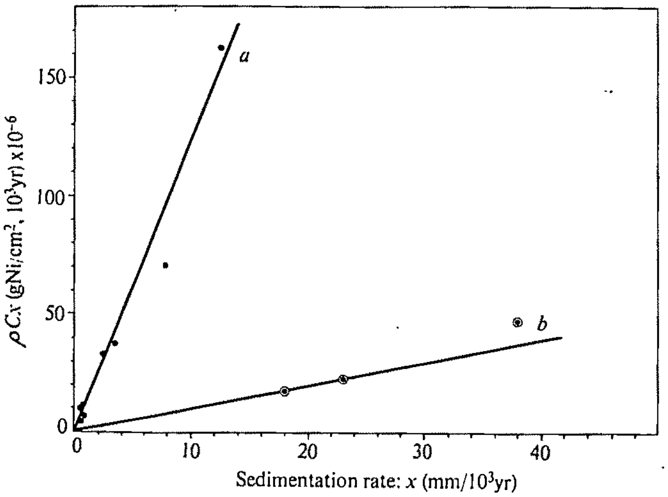


Fig. 3 Estimation of nickel fraction supplied through grain settling. $\rho Cx=a+bx$. a, Amano, Okada and Shima ($\rho=1.5$); b, Turekian ($\rho=0.74$).

Following Pettersson's hypothesis[1], a fairly large amount of nickel fraction is expected to be supplied through grain, that is meteoric dusts, into marine sediments.

We conclude that in marine sediments the nickel fraction supplied through grain settling is negligibly small, and that the extraterrestrial nickel fraction is supplied through magnetic spherules, whose concentration of ~1.1 mg kg^{-1} sediments was obtained by Laevastu and Mellis[3]. This does not reject the possibility of extraterrestrial dust accretion on the Earth, but the amount of the extraterrestrial fraction may be much smaller than Pettersson believed.

We thank Professor H. Hasegawa of the University of Kyoto for valuable advice.

Kazuo Yamakoshi
Yuji Tazawa

Department of Physics,
Faculty of Science,
University of Kyoto,
Kyoto

Received July 1, 1971.

[1] Pettersson, H., and Rotschi, H., *Geochim. Cosmochim. Acta*, **2**, 81 (1952).
[2] Smales, A. A., and Wiseman, J. D. H., *Nature*, **175**, 464 (1955).
[3] Laevastu, T., and Mellis, O., *Trans. Amer. Geophys. Union*, **36**, 385 (1955).
[4] Smales, A. A., Mapper, D., and Wood, A. J., *Analyst*, **82**, 75 (1957).
[5] Öpik, E. J., *Nature*, **176**, 926 (1955).
[6] Goldberg, E. D., *J. Geol.*, **62**, 249 (1954).
[7] Yamakoshi, K., *Memoir of Facul. Sci. Univ. Kyoto* (*Series Phys.*), **33**, 311 (1971).
[8] Barker, jun., J. L., and Anders, E., *Geochim. Cosmochim. Acta*, **32**, 627 (1968).
[9] Turekian, K. K., *Nature*, **182**, 1728 (1958).
[10] Amano, S., Okada, A., and Shima, M., *Bull. Nat. Mus. Tokyo*, **10**, 471 (1967).
[11] Shima, M., Okada, A., and Amano, S., *Bull. Nat. Mus. Tokyo*, **10**, 467 (1967).

Stratospheric Water Vapour Increase due to Human Activities

Great interest has been aroused by the possibility that a fleet of supersonic transports (SST) flying in the stratosphere would deposit sufficient water vapour to increase the average global concentration by about 7%, from 3 p.p.m. to 3.2 p.p.m. (ref. 1). The injection of this water vapour would result in a small decrease in stratospheric temperature according to Manabe and Wetherald[2,3]. Perhaps more significantly, it could decrease the concentration of ozone in the lower stratosphere through a series of photochemical reactions[4–9].

The consequences of this slight ozone reduction have been judged by some to be serious. (McDonald[10], for example, projects about 10,000 additional cases of skin cancer a year in the United States as a result of increased ultraviolet radiation due to a 1% reduction in total ozone content.) It is therefore of interest to see whether other human activities are increasing the water vapour content of the stratosphere. I believe that methane production[11–13] may be an important process today, perhaps more so than the hypothetical SST fleet of the future.

CH_4 is injected into the atmosphere mostly from the decomposition of organic matter at the Earth's surface, from swampy areas, rice paddies, geothermal areas, coal fields, natural gas and petroleum wells, and industrial centres. Koyama[14] has estimated some of the more important sources which contribute to the global atmosphere; the total production rate is about

Table 1 CH_4 Production Rates

	CH_4 g yr^{-1}
Paddy fields	1.4×10^{14}
Enteric fermentation of animals	4.5×10^{13}
Coal fields	2.0×10^{13}
Upland fields, grasslands and so on	1.0×10^{13}
Forests	4.0×10^{11}

2.7×10^{14} g yr^{-1}. They are shown in Table 1 which is by no means complete—for example, the methane production by swamps is probably as important as that of paddy fields[11] and there are no estimates for CH_4 production by various industrial activities and by the anaerobic digestion of sludges, sewage, and especially feedlot wastes. T. C. Byerly (private communication) has estimated the methane production from the metabolism of domesticated ruminants at 7.3×10^{13} g yr^{-1}, with wild ruminants adding another 10% to 50%. The growth rate for domesticated ruminants is estimated to be 1.5% yr^{-1} which seems low. Robinson and Robbins[15] have suggested a total production rate of 1.6×10^{15} g yr^{-1} or about 5 times that of Koyama.

The production of methane on a weight basis is roughly equivalent to that of higher molecular weight volatile organics, for example, terpenes and isoprene, for which the rate is estimated to be from 1.7×10^{8} tons yr^{-1} (ref. 15) to about 4.4×10^{8} tons yr^{-1} (ref. 16). Worldwide hydrocarbon emission is estimated at 0.9×10^{8} tons yr^{-1} and nearly half of this is in the form of gasoline and solvents[15]. The natural background concentration of methane in the atmosphere is estimated to average 0.93 mg m^{-3} (1.39 p.p.m.) over a Southern California mountain area during desert winds[17]; at Point Barrow, Alaska, the concentration is 1.1 mg m^{-3} (1.6 p.p.m., ref. 18) and between Washington, DC, and Puerto Rico the value is 0.83 mg m^{-3} (1.25 p.p.m., ref. 19). The methane content of ocean water is in equilibrium with that of the atmosphere[18,19] and the atmospheric average life of methane has been estimated to be from a few years[15] to twenty years[14].

The most significant fact for our purposes is that a large fraction, perhaps as much as 50%, of the methane production is "cultural", that is, related to human activities and—for want of a better index—related to the population and GNP; it is therefore growing at a rate of about 2% to 3% yr^{-1} (a doubling time of thirty years). The natural contribution in the meantime should remain constant or even decrease somewhat as swampy areas are filled in and as the wild ruminants are reduced in number.

Because of the low value in the atmosphere, approximately 1 p.p.m., the chief methane sink must be at the surface of the Earth, presumably in the form of aerobic bacteria[33–35]. Of special interest, however, is the rate at which methane is oxidized and converted into water vapour in the stratosphere. The data are sparse and are based on a few isolated balloon flights. Ehhalt[11] reports a reasonably constant CH_4 mixing ratio up to a height of 10 km, although the amount may vary on different days in different locations. Bainbridge and Heidt[20] report a constant mixing ratio up to about 17 km above East Texas followed by a rapid fall-off above the tropopause; the mixing ratio falls to 60% of the tropopause value at an altitude of about 23 km. Kyle *et al.* report a fall-off over New Mexico commencing at 12 km and reaching 20% of the earlier value at 22 km. (Care has to be exercised in interpreting this result, however. They refer to methane per air mass and use units of atm-cm; that is, units of reduced thickness. I have established by a personal discussion that their Fig. 3 plots the relative mixing ratio.)

From a rocket experiment Scholz *et al.* conclude that the methane is well mixed in the troposphere over New Mexico, but that it is consumed by chemical reactions within the stratosphere (ref. 22 and D. H. Ehhalt, private communication). They also estimate that methane oxidation in the stratosphere may contribute between 25% and 50% of the water vapour contained in the upper stratosphere. This conclusion is based on the observed methane concentration near the stratopause which is less than 0.05 p.p.m.v.

We can calculate the strength of the sink from the equation of diffusion and continuity

$$\Delta(\chi,\mathbf{v}) = -\delta\chi/\delta\tau + S \qquad (1)$$

where the left side expresses the three-dimensional gradient of the product of mixing ratio of a tracer and vector velocity; and the right side contains the partial derivative of concentration with time plus the source (or sink) S. When $\delta\chi/\delta\tau=0$ we find

$$S = K_{zz}\,[\delta\, n/\delta\, z - (\delta\, n/\delta\, z)_0] \qquad (2)$$

assuming only a vertical gradient in the z-direction K_{zz} is the eddy exchange coefficient ($\sim5\times10^{3}$ cm^2 s^{-1} at 50 mb), averaged over latitude and season (ref. 3 and ref. 23 in which the effects due to a mean vertical motion produced by the general atmospheric circulation are neglected). $\delta\, n/\delta\, z$ is the observed decrease in concentration of CH_4, $(\delta\, n/\delta\, z)_0$ is the expected decrease corresponding to a constant mixing ratio ($\sim2.75+10^{-16}$ g cm^{-4}).

Substituting numerical values from the two balloon experiments, we obtain $\delta\, n/\delta\, z\sim3.35\times10^{-16}$ and a rate of disappearance S of 3×10^{-13} g cm^{-2} s^{-1}. Over the Earth's surface this would be equivalent to about 44 million tons yr^{-1}.

Assuming that 1 g of CH_4 produces 2.25 g of H_2O after oxidation, we find that water vapour is introduced into the stratosphere by methane oxidation at the rate of 3.4×10^{6} g s^{-1} or about twice the rate of introduction by an SST fleet[24,25].

The straightforward interpretation of the observed rapid decrease in the methane mixing ratio, which begins at the tropopause, is that methane moves into the stratosphere from below, and is there chiefly oxidized into water vapour (this agrees with the conclusion of ref. 22 although exactly how much is oxidized, where and by what remains to be settled). A large fraction of the methane appears to be "cultural", so we conclude that the rate of injection of water vapour from methane produced by human-related activities today may be of the same order of magnitude as that injected by a fleet of supersonic transports (see Table 2).

Table 2 Water Vapour Injection into the Stratosphere

	g s^{-1}
By CH_4 oxidation *	3.4×10^{6}
Human-related portion †	1.7×10^{6}
Injected by SST fleet ‡	1.5×10^{6}
Injected by Hadley cell circulation §	5.0×10^{6}
Injected by thunderstorms ‖	0
Injected by tropical storms ‖	0

* Calculated in present paper.

† Estimated as 50% of *.

‡ 400 planes and 4 trips per day[24,25].

§ Calculated by Newell[26], but may be much greater. Newell uses a vertical velocity of 0.02 cm s^{-1} for the tropical tropopause. Higher velocities, up to 0.04 cm s^{-1}, are quoted by Lateef[31] and 0.05 cm s^{-1} is quoted by Reed and Vlcek[32]. A value of 7 to 10×10^{6} g s^{-1} is given by Newell in testimony before the Senate Appropriations Committee on March 10, 1971; his explicit neglect of middle latitude and thunderstorm sources is unacceptable because it is based on a circular argument.

‖ Firm estimates not yet available, but could be of the order of §.

The methane source has a more significant effect on the stratospheric ozone concentration than does the water vapour injected directly from the troposphere or from prospective SSTs. This is because atomic oxygen plays an important role in the oxidation of the methane[27], and is therefore removed and made unavailable for ozone production. These conclusions are not much affected by the detailed chemistry of the methane oxidation; as long as H atoms are conserved, the $H_2O : CH_4$ ratio is fixed. The water vapour produced will, of course, remove some ambient ozone[4–9] in the same way as the directly injected water vapour.

A more careful series of measurements of stratosphere methane concentration with altitude is required, especially at various latitudes, in order to determine the convective transport. It is important to note that nitrogen oxides and a large fraction of carbon monoxide are released into the atmosphere by human activities[28,29]. Leakage of these gases into the stratosphere would diminish ozone there by capture of atomic oxygen, and in the case of NO_x by direct reactions with ozone[30].

S. Fred Singer

Department of Environmental Sciences,
University of Virginia,
Charlottesville, Virginia 22903

Received July 29; revised September 25, 1971.

[1] *Man's Impact on the Global Environment: Assessment and Recommendations for Action*, Report of the Study of Critical Environmental Problems, 73 (MIT Press, Cambridge, 1970).
[2] Manabe, S., and Weatherald, R. T., *J. Atmos. Sci.*, **24**, 241 (1967).
[3] Manabe, S., *Science*, **169**, 534 (1970).
[4] Hunt, B. G., *J. Atmos. Sci.*, **23**, 88 (1966).
[5] Hunt, B. G., *J. Geophys. Res.*, **71**, 1385 (1966).
[6] Leovy, C. B., *J. Geophys. Res.*, **74**, 417 (1969).
[7] Harrison, H., *Science*, **170**, 734 (1970).
[8] McGrath, W., and Norrish, R., *Proc. Roy. Soc., A*, **254**, 839 (1960).
[9] London, J., and Park, J., in *Man's Impact on the Global Environment: Assessment and Recommendations for Action* Report of the Study of Critical Environmental Problems, 73 (MIT Press, Cambridge, 1970).
[10] McDonald, J. E., *Congressional Record*, S3904, March 12, 1971.
[11] Ehhalt, D. H., *J. Air Poll. Control Assoc.*, **17**, 518 (1967).
[12] Junge, C. E., *Air Chemistry and Radioactivity*, International Geophysical Series (edit. by Van Miegham), **4** (Academic Press, New York, 1963).
[13] Reiter, E. R., *Atmospheric Transport Processes*, Part 2, TID 25314 (Atomic Energy Commission, Washington, 1971).
[14] Koyama, T., *J. Geophys. Res.*, **63**, 3971 (1963).
[15] Robinson, E., and Robbins, R. C., *Sources, Abundance and Fate of Gaseous Atmospheric Pollutants* (Stanford Research Institute, 1968) (prepared for American Petroleum Institute, New York).
[16] Rasmussen, R. A., and Went, F. W., *Proc. Nat. Acad. Sci.*, **53**, 215 (1965).
[17] Stephens, D. R., and Burleson, F. R., *62nd Annual Meeting, Air Pollution Control Association* (June 22 to 26, 1969).
[18] Cavanaugh, L. A., Schadt, C. F., and Robinson, E., *Environ. Sci. Technol.*, **3**, 251 (1969).
[19] Swinnerton, J. W., Linnenbom, V. J., and Cheek, C. H., *Environ. Sci. Technol.*, **3**, 836 (1969).
[20] Bainbridge, A. E., and Heidt, L. E., *Tellus*, **18**, 221 (1966).
[21] Kyle, T. G., Murcray, D. G., Murcray, F. H., and Williams, W. J., *J. Geophys. Res.*, **74**, 3421 (1969).
[22] Scholz, T. G., Ehhalt, D. H., Heidt, L. E., and Martell, E. A., *J. Geophys. Res.*, **75**, 3049 (1970).
[23] Reed, R. J., and German, K. E., *Mon. Weather Rev.*, **93**, 316 (1965).
[24] Chatham, G. N., and Huddle, F. P., *The Supersonic Transport*, Library of Congress, Congressional Research Service (71-78 SP) (February 26, 1971).
[25] Daniels, G. M., *Astronautics and Aeronautics*, **8**, 22 (1970).
[26] Newell, R. E., *Nature*, **226**, 70 (1970).
[27] Cadle, R. D., and Powers, J. W., *Tellus*, **18**, 176 (1966).
[28] Robinson, E., and Robbins, R. C., in *Global Effects of Environmental Pollution* (edit. by Singer, S. F.), 50 (Reidel, Dordrecht, and Springer-Verlag, New York, 1970).
[29] Jaffee, L. S., *ibid.*, 34.
[30] Johnston, H., *Science*, August 6, 1971.
[31] Lateef, M. A., *Mon. Weather Rev.*, **96**, 286 (1968).
[32] Reed, R. J., and Vlcek, C. L., *J. Atmos. Sci.*, **26**, 163 (1969).
[33] ZoBell, C. E., *Advan. Enzymol.*, **10**, 443 (1950).
[34] Inman, R. E., Ingersoll, R. B., and Levy, E. A., *Science*, **172**, 1229 (1971).
[35] Abeles, F. B., Craker, L. E., Forrence, L. E., and Leather, G. R., *Science*, **173**, 914 (1971).

Mesozoic Palaeomagnetic Reversal Column

THE frequency of geomagnetic field reversals has not been constant throughout Phanerozoic time and there have been long intervals during which few, if any, reversals occurred; this is in contrast to the Tertiary, for example, when a reversal occurred, on average, every 0.23 m.y. in the interval[1] 0 to 10.6 m.y. The first long interval of fixed (reversed) polarity was discovered by Irving and Parry[2] and named by them the Kiaman Magnetic Interval. It extended from 290 until 230 m.y. ago approximately. More recently, Helsley and Steiner[3] have pointed out that the topmost of the lower and nearly all of the Upper Cretaceous was an interval of predominantly normal polarity (from about 120 until 70 m.y. BP).

McElhinny and Burek[4] have formally divided the Mesozoic period into (i) intervals of mixed polarity during which there were frequent reversals and (ii) intervals of predominantly normal polarity within which there are a number of reversed zones. They decided to name the intervals after people: thus their so called "Graham Interval" lasted from about 203 until 150 m.y. BP and their so called "Mercanton Interval" from about 120 until 70 m.y. ago. They also named the comparatively short "zones" of reversed polarity identified within these longer intervals of normal polarity.

Workers in the USSR have for many years specialized in the application of geomagnetic field reversals to stratigraphy. In particular, many of the zones described and named by McElhinny and Burek have already been named by a Russian palaeomagnetic researcher, D. M. Pecherski[5] in a paper entitled *Palaeomagnetism and Palaeomagnetic Correlation of Mesozoic Formations of North-east USSR*. This is included in volume 37 of the *Scientific Works of the North-East Complex Institute, Magadan*, entitled *Palaeomagnetic and Biostratigraphic Characteristics of some Important Mesozoic and Cenozoic Series from North and Far-East USSR*. At the fifteenth assembly of IUGG held in Moscow last August, Mr Pecherski agreed that a copy of his palaeomagnetic column for the Mesozoic be published in an English journal and Fig. 1 is a copy of Fig. 19 from ref. 5 with names translated into English by Pecherski himself. All (except one) of the reversed zones have been named after the locality in the USSR where they were recognized, although studies from other parts of the world have been taken into account.

The evidence for the division of the Mesozoic, from 203 to 70 m.y. BP, into three intervals by McElhinny and Burek is not so clear in the Pecherski column. If Pecherski's Kuldginskaja zone (Coniacian) is not the same as McElhinny and Burek's Akoh zone (Santonian), the Late Cretaceous normal interval contains at least three, rather than two, reversed zones and suggests that the recognition of three intervals within the Mesozoic may be an illusion. In this respect, it should also be noted that the representation of the so called Serra Geral zones by McElhinny and Burek is only schematic. Having worked on the Serra Geral lavas and dykes myself, I can say that at present we have no idea of how many reversals occurred during their emplacement (between about 145 and 110 m.y. BP). Pecherski recognizes three reversed zones in the time interval 137 to 120 m.y. BP (the Chagilskaja, Shirabadskaja and Severo-Sibirskaja). Between 150 and 137 m.y. the pattern is not clear (see Fig. 1).

On the whole, however, the agreement between Pecherski's column and that of McElhinny and Burek is rather good, but not exact. Thus the Akoh zone of McElhinny and Burek (at 80 m.y.) roughly corresponds to the Kuldginskaja zone of Pecherski (at 90 m.y.). The Hissar zone of McElhinny and Burek and the Gatanskaja zone of Pecherski both occur at 110 m.y. Within McElhinny and Burek's so called Graham interval of normal polarity, the Mateke and Cotswold zones correspond to Pecherski's Pospelovskaja and Startovaja zones and McElhinny and Burek's Nuanetsi zone corresponds to Pecherski's Maseru and Levo-Kedonskaja zones.

Pácherski has not recognized as many reversals within the Triassic as have been identified from studies of sedimentary columns in the Chinle and Chugwater formations from the USA and recorded in McElhinny and Burek's column. In other respects, however, Pecherski's column, based largely on studies of USSR rocks, appears the more complete. Nevertheless many reversals probably await discovery.

The radiometric time scale attached to the palaeontologically defined stratigraphic column used by Pecherski differs somewhat from the Geological Society Phanerozoic time scale[6] used by McElhinny and Burek. Measured K-Ar ages used by Pecherski are listed with their uncertainties in Fig. 1 in the column headed control and the time scale in the left hand column has been constructed using these measured ages.

The occurrence of these reversed zones within the predominantly normal Mesozoic has obvious potential uses in global stratigraphy. But whether the adoption of a new series of stratigraphic names is desirable or necessary is open to doubt. Could we not, for example, refer to the Gatanskaja (Hissar) zone as the reversed zone at 110 m.y. within the Late Cretaceous normal interval; and would it not be more constructive to refer to the Kuldginskaja zone as the reversed zone in the Coniacian division and to the Akoh zone as the reversed zone in the Santonian, and so on?

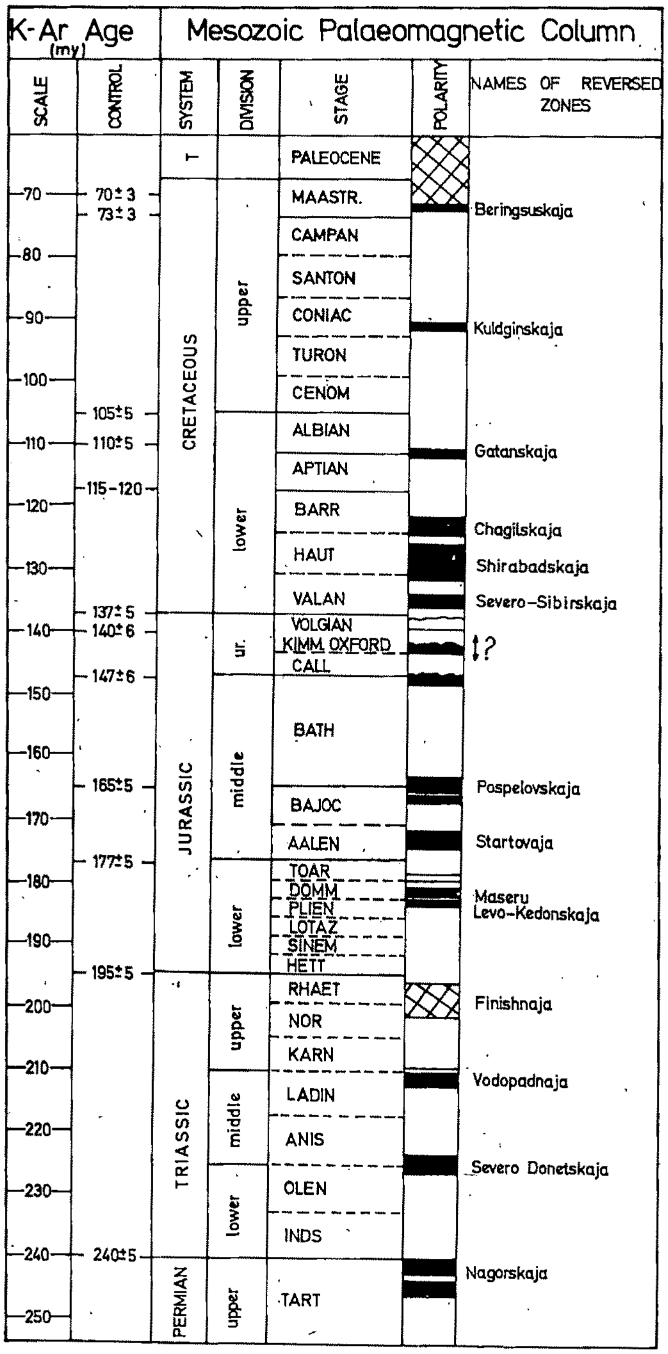


Fig. 1 Pecherski's Palaeomagnetic Polarity Column for the Mesozoic. Measured K-Ar ages are given in the column headed "control". The scale in the left hand column has been adjusted to the measured K-Ar ages. White areas, normal polarity; black areas, reversed polarity; hatched areas, times of frequent change in polarity of the geomagnetic field.

At present, however, we are faced with the problem that two sets of names have been assigned independently to the same set of observations. If we must use a new set of names to describe these zones, surely one set is quite sufficient. McElhinny and Burek describe how, a year or so ago, they each independently assigned different names to the same normal event within the Late Palaeozoic (Kiaman) reversed interval. McElhinny[6] named it the Oak Creek event and shortly afterwards Burek[7] named it the Graham event. These two authors resolved their difference by agreeing to adopt the earlier assigned name, Oak Creek.

Therefore, I venture to suggest that those who find a special set of names useful will recognize Pecherski's precedence by adopting his names to identify these Mesozoic reversed zones.

K. M. Creer

Department of Geophysics and Planetary Physics,
School of Physics,
The University,
Newcastle upon Tyne NE1 7RU

Received August 27, 1971.

[1] Cox, A., *Science*, 163, 237 (1968).
[2] Irving, E., and Parry, L. G., *Geophys. J.*, 7, 395 (1963).
[3] Helsley, C. E., and Steiner, M. B., *Earth Planet. Sci. Lett.*, 7, 325 (1969).
[4] McElhinny, M. W., and Burek, P. J., *Nature*, 232, 98 (1971).
[5] Pecherski, D. M., *USSR Academy of Sciences, Scientific Works of the North East Complex Institute (SVKNEE), Magadan*, 37, 58 (1970).
[6] McElhinny, M. W., *Special Publ. Geol. Soc. Australia*, 2, 61 (1969).
[7] Burek, P. J., *Amer. Assoc. Petrol. Geol. Bull.*, 54, 1389 (1969).

Recognition of Crude Oils by Capillary Gas Chromatography

In serious marine accidents involving oil pollution, the source of the oil is usually obvious, but on other occasions the offender often escapes undetected. In this communication, I propose a method of identifying the origin of spilled crude oil using capillary column gas chromatography.

Preliminary work with twenty-five fresh crude oils demonstrates that oils from different continents yield very different chromatograms, and that there are also significant differences between the chromatograms of oils from neighbouring fields in the Middle East and North Africa.

The three types of persistent oil likely to be spilled at sea are fuel oil, fresh crude oil and crude oil residues from tank cleaning; it is possible to differentiate between them by examining their wax and asphaltenes contents, and their volatility profiles as indicated by gas chromatography[1,2]. The technique described here could probably be applied to each of these oils to help trace its source, but it has so far been confined to crude oils.

Several techniques have been used to establish the origin of crude oils[1,3], of which the most useful are the determination of vanadium and nickel contents, the wax distribution, and "fingerprinting" by gas chromatography. This fingerprinting technique usually involves the use of packed columns to examine the higher boiling fractions, which are resistant to weathering by evaporation, but the fingerprints are limited in detail.

Experience gained in the identification of other petroleum products[4] suggested that average packed columns can provide sufficient compositional detail of materials up to carbon number

13 only, most of which would be lost by evaporation during weathering. It was envisaged that capillary columns would provide extra resolution and thus indicate compositional differences in the higher boiling fractions also.

As it is necessary to programme column temperatures to at least 300° C, the choice of stationary phase is virtually limited to the range of silicone gums such as 'SE-30'. But past experience of silicone gum capillary columns has been discouraging; resolution values of 10 could not be achieved with commercial or homemade 'SE-30' columns, whereas values of up to 40 could be obtained with squalane, for example.

(Resolution is measured between n-hexane and n-heptane and is defined as the distance between the peaks divided by their mean peak width at the baseline. The values quoted refer to columns 150 foot long by 0.01 inch internal diameter operated at 50° C and a nitrogen inlet pressure of about 10 pound inch^{-2}, adjusted to produce a retention time of 10 min for hexane.)

This unsatisfactory resolution is thought to be due to the nature of the gum which forms a skin on the surface of a solution, thus preventing the evaporation of the solvent beneath it; columns coated from solution by the usual techniques will not contain a stable, uniform film of stationary liquid.

Some new silicone stationary phases are viscous liquids rather than gums (which are almost solid at room temperature). We have found them to possess similar thermal stability to 'SE-30', by thermogravimetric analysis. Using 'OV-101' silicone (a non-polar variety) I have made columns providing resolution values of 40.

Table 1 Equipment and Operating Conditions

Chromatograph	Pye 'Model 54', fitted with homemade inlet splitter*.
Detector	Flame ionization.
Column	45 m of 0.25 mm internal diameter stainless steel tube coated with 'OV-101' silicone.
Temperature programme	50 to 310° C at 8° min^{-1}.
Carrier gas	Nitrogen.
Inlet pressure	10 p.s.i.g.
Column flow rate	Approximately 0.6 ml. min^{-1}.
Split ratio	100 : 1.
Injection temperature	300° C.

* This splitter was built to the outline design of Cramers[8] and is uniformly heated from the injection head to the split point to promote good vaporization of the sample.

I have so far examined fresh samples of crude oils from twenty-five different locations, using the equipment and operating conditions set out in Table 1. To prevent fouling the column and inlet system with the involatile portions of the crudes I have used vacuum distillation fractions of the oils, in the range 100° to 430° C, atmospheric equivalent temperature. Fig. 1*A* is a typical chromatogram of a fraction of Brega crude; the n-paraffin peaks have been labelled with their carbon numbers. (Many of these peaks have been allowed to exceed the recorder scale in order to highlight the others.)

The detailed pattern of peaks between the n-paraffins provides excellent fingerprint material. In my comparisons of different oils I have found that the most useful region lies between C_{13} and C_{19}, and the peaks which vary most in size from one oil to the next are those marked *X*. From geochemical work (ref. 5 and A. G. Douglas, private communication) it is clear that these peaks represent saturated terpenoid hydrocarbons including farnesane, pristane and phytane. The peaks immediately following those of the C_{17} and C_{18} normal paraffins explain why, on columns of poorer resolution, the C_{17} and C_{18} n-paraffin peaks appear larger than their nearest neighbours.

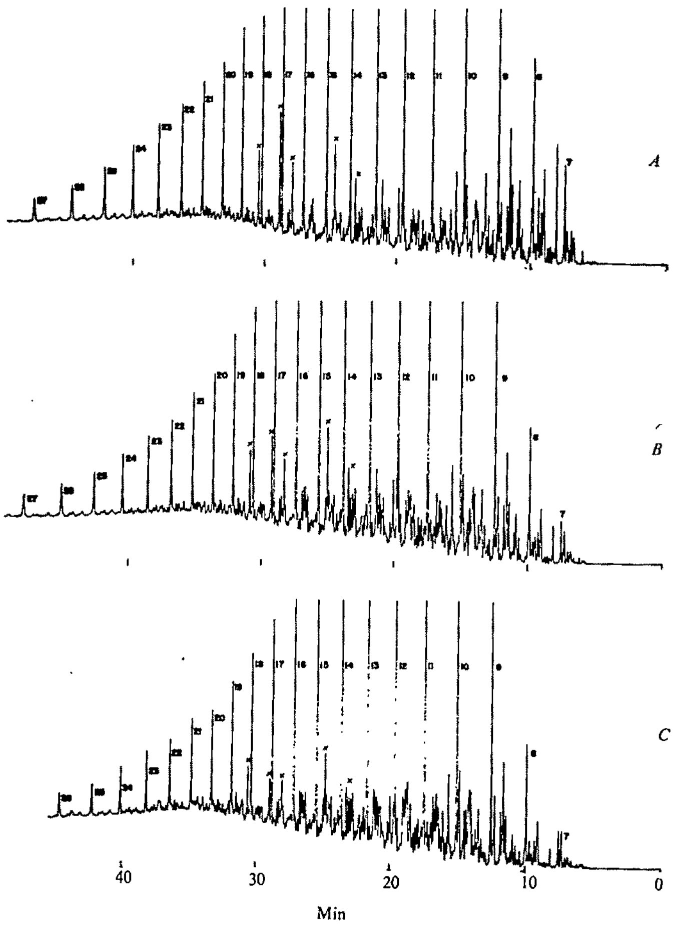


Fig. 1 Fractions of crude oils from *A*, Brega; *B*, Safaniya; *C*, Khursaniyah.

Crude oils from such diverse areas as Venezuela, Australia, Nigeria, Libya and Britain show very different fingerprint patterns, but because most crude oils shipped to Europe originate from the Middle East and North Africa, one must be able to differentiate between the oil fields of these regions if the method of recognition is to be successful.

Libyan crudes can be recognized by their large n-paraffin peaks, larger than average terpenoid peaks, and a unique pattern of the group of five peaks preceding C_{14} (Fig. 1*A*). And although all Libyan crudes which I have examined display this general trend, sufficient minor differences exist to distinguish between oils from Brega, Es Sider, Zuettina and Sarir.

Similarly the oil fields lying along the Persian Gulf between Qatar and Kuwait form a group displaying smaller terpenoid peaks and a different configuration of the five peaks mentioned above. Even so, differences can be seen between the chromatograms of Safaniya and Khursaniyah crudes (Figs. 1*B* and *C*), the locations being only 100 miles apart.

In a few cases, samples have been taken from several shipments of the same nominal crude over 6 months; the fingerprints have been identical.

In most practical situations the sample for identification is likely to be taken from the water surface or from a beach after a period of weathering. So far I have only conducted one very simple weathering test, exposing four crude oils to the elements for 1 week on top of a brine solution in metal trays. The weather during this period was very windy with alternate sunshine and rain. The original layer of oil in each tray was 5 mm thick.

At the end of the week the Brega oil, having a large proportion of light ends, had lost so much by evaporation that the layer of residue had broken up into viscous lumps with clear water between them. The others (Aramco, Kuwait, Sassan) remained as complete layers covering the water.

This artificial weathering period resulted in only minor loss of material above C_{12} and the GC fingerprints were essentially

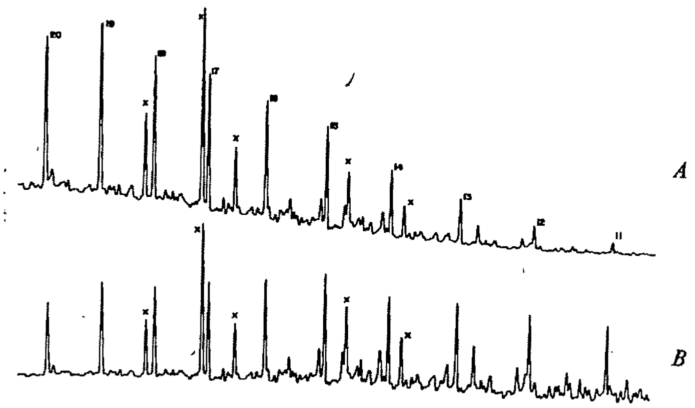


Fig. 2 Sections of chromatograms of (*A*) oil weathered for six days and (*B*) the corresponding fresh oil.

unaffected. At sea, agitation of the surface probably causes further evaporation. There is evidence, however, from the Torrey Canyon incident[3] that oil exposed for weeks or buried in sand for up to 2 yr still retains measurable quantities of material down to C_{13}.

Other processes can occur such as differential solution in water, oxidation and bacterial decay, but I do not have data on the rates of change by these means. It is reported[6] that the more volatile hydrocarbons are most soluble in water (and these will be lost by evaporation anyway) whereas, of the less volatile fractions, the aromatics are most affected. Also bacterial action may be confined almost entirely to the paraffins. The same report quotes an example of diesel oil spilled at sea, which settled into the bottom sediment and oozed out several months later; it displayed a very similar GC fingerprint (on a packed column) to the original oil.

Other views have been expressed[7], including the one that microbial action is usually confined to oil/water and oil/air interfaces so that most of a viscous lump of oil is unlikely to be degraded biologically.

The only way to determine the practical value of this technique is to apply it to spilled oils of known origin. Such samples are not readily available and so far we have been able to investigate one incident only.

After a recent tanker accident off the British coast, oil was collected off beaches up to a week later and up to 100 miles from the incident. Fig. 2 illustrates the significant sections of chromatograms of oil weathered for 6 days (*A*) and fresh oil from the stricken vessel. The principal terpenoid peaks have been marked again, and the n-paraffins numbered according to carbon atom content. The weathered oil was extracted from an aqueous emulsion with chloroform.

More than 90% of the C_{11} portion has been lost by evaporation, and diminishing losses are visible up to C_{20}. The fingerprint pattern has, however, been affected very little, even in the C_{11} to C_{13} region. This first example is very encouraging, and it is hoped to obtain further samples of this type soon. In another context, diesel oils and fuel oils have been examined in a similar way with limited success at distinguishing products from different sources. Extension of the technique to include water pollution by these products could be practicable.

R. D. Cole

Esso Research Centre,
Abingdon, Berkshire

Received January 16; revised April 28, 1971.

[1] Brunnock, J. V., Duckworth, D. F., and Stephens, G. G., *J. Inst. Petrol.*, 54, 310 (1968).

[2] Ramsdale, S. J., and Wilkinson, R. E., *J. Inst. Petrol.*, **54**, 326 (1968).

[3] Duckworth, D. F., in *Water Pollution by Oil*, 165 (Institute of Petroleum, London, 1971).

[4] Cole, R. D., *J. Inst. Petrol.*, **54**, 288 (1968).

[5] Van Hoeven, W., Haug, P., Burlingame, A. L., and Calvin, M., *Nature*, **211**, 1361 (1966).

[6] Blumer, M., *et al.*, *Technical Report 70–74* (Woods Hole Oceanographic Institution, Massachusetts 02543).

[7] Berridge, S. A., Dean, R. A., Fallows, R. G., and Fish, A., *J. Inst. Petrol.*, **54**, 300 (1968).

[8] Cramers, C. A., thesis (Technische Hogeschool, Eindhoven, The Netherlands, 1967).

Evolution of Marginal Basins

The occurrence immediately behind island arcs of small ocean basins such as the Sea of Japan and the Sea of Okhotsk is linked with the evolution of lithospheric features of the Earth, and probably the convection currents in the mantle[1]. More recent oceanographic data together with global tectonic hypotheses[2] have renewed geophysical interest in these basins. We wish to present a model for their formation and evolution.

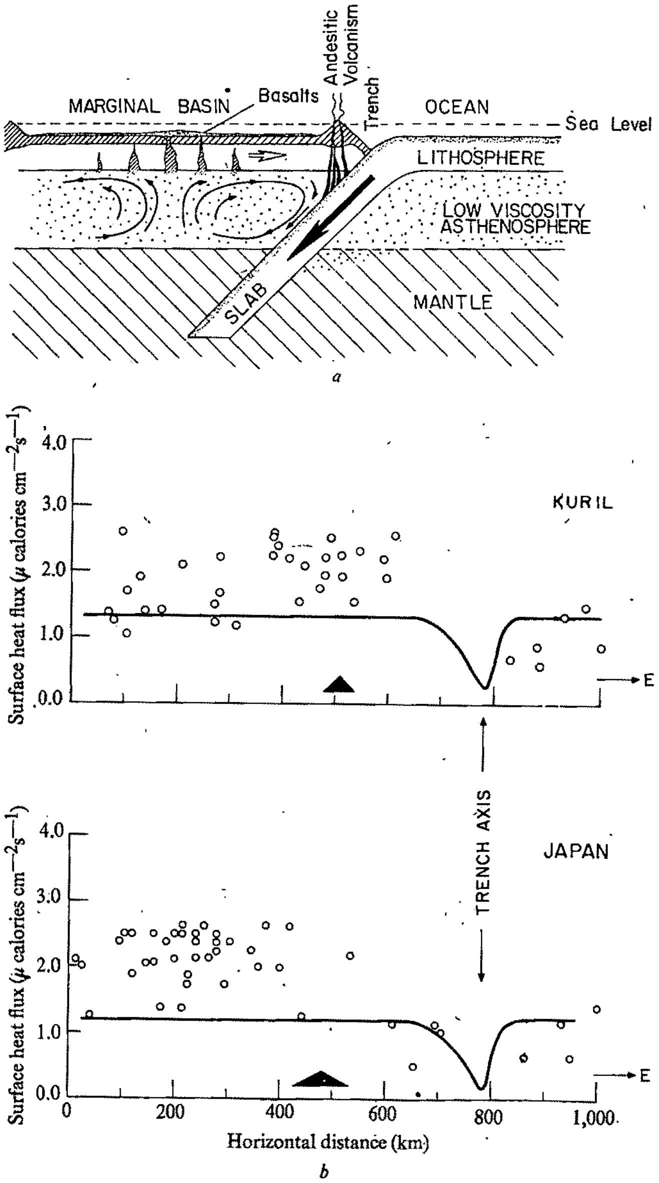


Fig. 1 *a*, Schematic diagram of the proposed mechanism. The descent of the slab causes currents in the asthenosphere which in turn cause the crustal spreading behind the island arc. *b*, Observed heat flow in the Sea of Japan and the Sea of Okhotsk plotted as a function of distance from the ridge axis. Solid line is the theoretical heat flow for a downgoing slab[19,20]. Heat transport by conduction alone cannot explain the high heat flow in the marginal basin.

Anomalously thin sediments, truncation of older geological features and freshly erupted basalts strongly support the idea[3–8] that marginal basins are the result of some type of crustal spreading. Attenuation of S_n waves[9], shallow seismicity[10] and high heat flow provide supporting evidence for relatively hot subcrustal conditions.

While some well studied, active basins such as the Sea of Japan, the Sea of Okhotsk and Lau-Harve basin have most of these characteristics, others (for example, the Bering Sea) seem to be inactive and have nearly normal heat flux and very little seismicity.

Mechanisms for the formation of the basins behind island arcs are of two kinds—trapping and spreading. The trapping hypothesis assumes that the basin behind the island arc consists of oceanic crust predating the arc, but this is inconsistent with the geophysical data. The spreading hypothesis assumes that crustal spreading is caused by the intrusion of asthenospheric material into the lithosphere behind the arc, when spreading may be triggered either by frictional heat generated by the sinking of the slab and convected to the surface[11–13] or—as proposed here—by hydrodynamic forces generated by the sinking of the slab and generating a flow pattern in the asthenosphere which in turn acts on the lithosphere. In this case, the intrusion operates independently of viscous heating and this mechanism could explain the dipping of the slab as well as explaining the above geological and geophysical data.

The model (Fig. 1) consists of a downgoing slab and a relatively low-viscosity layer (asthenosphere) sandwiched in between a rigid lithosphere and a rigid (or high viscosity) mantle. The downgoing slab generates a flow pattern by "dragging" the low viscosity material in the asthenosphere. The convective cell thus driven exerts a stress at the bottom of the lithosphere behind the island arc forcing it in the direction of the trench and causing the lithospheric opening.

This model is consistent with the upper mantle structure obtained from seismic data[14–16], where the low viscosity layer corresponds to low velocity zone, the thickness of which may vary between 100 and 250 km. "High rigidity" mantle starts at a level where the partial melting ceases. Olivine-spinel phase transformation (at a depth of about 300 to 350 km) further increases the rigidity. This rapid increase of viscosity above and below the asthenosphere is also consistent with glacial rebound data[17,18].

The motion, properties and effects of the slab have been discussed in detail[19,20]. The slab without the proposed asthenospheric convection can explain the andesitic volcanism of the island arcs, but it cannot explain the high heat flow and oceanic basalts in the marginal seas (Fig. 1*b*). The heat flow observations in the Sea of Japan and the Sea of Okhotsk are here compared with theoretical models due to the thermal regime of a downgoing slab alone. There is good agreement over the trench and near the arc, but none in the basins.

To compute the theoretical flow pattern in the asthenosphere induced by the slab we numerically solved the equation of viscous flow by a relaxation method[21]:

$$\text{grad}\, p = \eta \Delta^2 \underline{V} \qquad (1)$$

where p = pressure, η = viscosity, V = velocity. After defining a stream function Ψ such that $\Delta^2\Psi = -\text{curl}\ V = -\Omega$ where Ω is vorticity, a convergent solution was obtained by iteration. Shear stress and pressure acting at the bottom of the lithosphere ($y=0$) were found from the vorticity.

The asthenosphere was modelled as a viscous fluid bounded above and below by parallel planes. The slab was modelled as a rigid moving boundary. An artificial rigid boundary was imposed four asthenosphere thicknesses away from the slab. The flow in the region of interest is only weakly affected by this boundary[22].

The result of the calculations (Fig. 2) shows a series of recirculating eddies with only the first divergence zone being of geological interest[23] because the amplitude ratio of successive eddies is 1/350. The recirculating flow zone has two important properties. First, material is trapped in the eddy and can accumulate viscous heat through several cycles. This may be related to the origin of andesites. Second, the divergence point is a preferred place for the lithosphere to fail under tension. These features are different from the broad flow pattern of McKenzie[24].

The observed location of spreading behind island arcs[12] is where one would expect it to occur from this model, in the region near the divergence point where the stress is small. This agreement in predicted location is evidence for, but not proof of, the proposed mechanism.

The crustal spreading as described above fits the overall physical conditions of the upper mantle and the dynamics of lithospheric slab descent into the mantle. At the same time, this dynamically induced spreading explains, at least qualitatively, the geological and geophysical observations associated with active basins. The data which establish the existence of the spreading are not likely to distinguish between the different

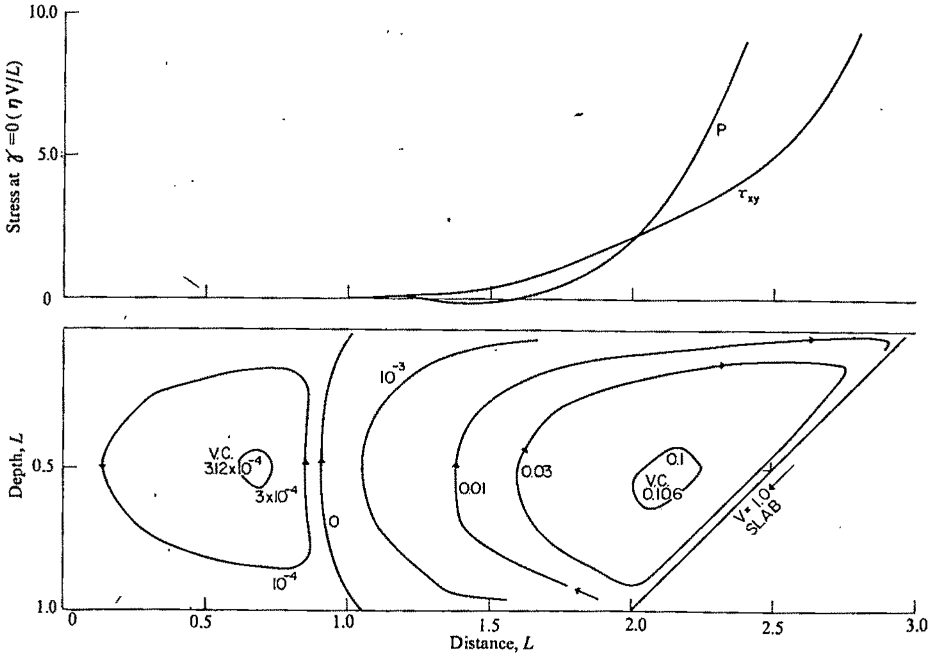


Fig. 2 Theoretical stream lines for flow in the asthenosphere. Shear stress and the vertical component of pressure along the base of the lithosphere are shown in the upper part of the figure.

mechanisms which advocate, for one reason or another, the opening of the lithosphere under the basin. One important consideration is the stress distribution shown in Fig. 2. According to our model, the lithosphere would be under tension near the centre of the basin while gradually giving way to compressive stresses toward the trench. Thus shallow earthquake mechanisms should be associated primarily with normal faulting in the centre and with reverse faulting toward the trench.

We studied the focal mechanism of two earthquakes in the Lau Basin from first-motion data. The earthquake at the centre of the basin was normal faulting (tension in horizontal direction), and the earthquake immediately behind the volcanic arc was thrust faulting (horizontal compression). A systematic study of more shallow earthquake mechanisms in the marginal basins is necessary before a meaningful evaluation of stress patterns can be made. Unfortunately, the earthquakes in the basins are small and the seismicity of this zone resembles that of the mid-ocean ridges in a smaller scale.

As the extension process continues and the basin lithosphere pushes toward the trench, the dip of the slab decreases whatever its initial descent angle. The process, however, becomes self-limiting because as the slab becomes nearly horizontal the resistance to spreading increases until the stress balance becomes compressive and the spreading ceases. A long time may be required for the slab to acquire a steeper dip under gravitational pull. Spreading is then likely to restart nearer the arc abandoning the older spreading centre.

This work was supported by the US Air Force Office of Scientific Research.

NORMAN SLEEP
M. NAFI TOKSÖZ

Department of Earth and Planetary Sciences,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received April 27, 1971.

[1] Vening Meinesz, F. A., *Koninkl. Ned. Akad. Wetenschap Proc.*, *B* **54**, 432 (1951).
[2] Isacks, B., Oliver, J., and Sykes, L., *J. Geophys. Res.*, **73**, 5855 (1968).
[3] Belousou, V., and Ruditch, E., *J. Geol.*, **69**, 3 (1961).
[4] Rudolfo, K., *Geol. Soc. Amer. Bull.*, **80**, 1203 (1969).
[5] Karig, D., *J. Geophys. Res.*, **75**, 239 (1970).
[6] Karig, D., *New Zealand J. Geol. Geophys.*, **13**, 21 (1970).
[7] Karig, D., and Glassley, W., *Geol. Soc. Amer. Bull.*, **81**, 2143 (1970).
[8] Hawkins, J. J., Sclater, J., and Hohnhaus, G., *Geol. Soc. Amer.*, 571 (1970).
[9] Molnar, P., and Oliver, J., *J. Geophys. Res.*, **74**, 2648 (1969).
[10] Hatherton, T., *New Zealand J. Geol. Geophys.*, **13**, 126 (1970).
[11] Hasebe, K., Fujii, N., and Uyeda, S., *Tectonophysics*, **10**, 335 (1970).
[12] Karig, D., *Geol. Soc. Amer. Bull.*, **82**, 323 (1971).
[13] Oxburgh, E., and Turcotte, D., *J. Geophys. Res.*, **76**, 1315 (1971).
[14] Toksöz, M. N., Chinnery, M., and Anderson, D., *Geophys. J. Roy. Astron. Soc.*, **13**, 31 (1967).
[15] Press, F., *Phys. Earth Planet. Interiors*, **3**, 3 (1969).
[16] Anderson, D., and Sammis, C., *Phys. Earth Planet. Interiors*, **3**, 41 (1969).
[17] Artyushkov, E., *J. Geophys. Res.*, **76**, 1376 (1917).
[18] McConnell, R., *J. Geophys. Res.*, **73**, 7089 (1968).
[19] Minear, J., and Toksöz, M. N., *J. Geophys. Res.*, **75**, 1397 (1970).
[20] Toksöz, M. N., Minear, J., and Julian, B., *J. Geophys. Res.*, **76**, 1113 (1971).
[21] Burggraf, O., *J. Fluid Mech.*, **24**, 113 (1966).
[22] Pan, F., and Acrivos, A., *J. Fluid Mech.*, **28**, 643 (1961).
[23] Moffatt, H., *J. Fluid Mech.*, **18**, 1 (1964).
[24] McKenzie, D., *Geophys. J. Roy. Astron. Soc.*, **18**, 1 (1969).

Theoretical Evidence Against the Existence of Polywater

WE present here new results that disprove a widely discussed, and seemingly successful, structural model for polywater[1], which was derived using semi-empirical calculations, and obtained its utility by consistently correlating more than twenty experimental observations. Defeat of this model thus casts serious doubt on the existence of a new water allotrope. It is important to distinguish clearly between the essentially empirical interrelation of experiments achieved by the original model and the current attack on it, because it is obvious from ref. 1 that we have placed ourselves in the unusual and all too easy to discredit position of being authors both of the original model and of the new results against it.

The original structure model which provided a scheme for rationalizing the many diverse properties of polywater consisted of stacked layers built from hexagonal units, composed of symmetrical hydrogen bonds as proposed on qualitative grounds by Lippincott *et al.*[2]. The resulting three-dimensional polywater lattice is to ice I as graphite is to diamond, and it was found to be metastable with respect to the normal liquid. This model was derived by comparing total energies computed with a semi-empirical molecular orbital model for a very large number of possible geometrical arrangements of oxygen and hydrogen atoms. The resulting structure gave a lower internal energy than other proposed arrangements, and it made plausible the experimental measurements of infrared and Raman absorption frequencies, proton magnetic resonance frequency shift, optical excitation, viscosity, density, index of refraction, vapour pressure, high and low temperature behaviour, X-ray diffraction line spacings, nuclear magnetic resonance line shape versus temperature, electrical conductivity, optical anisotropy, deuterium exchange rate with normal water, high frequency dielectric constant and absorption, surface tension, oxygen inner-shell ionization potential shift relative to the normal liquid, and the predominance of ionic over neutral impurities.

To test whether or not this model could exist as a metastable state, the smallest representative group of atoms—a single hexagonal unit $(H_2O)_6$ with hydrogen atoms symmetrically placed around the ring between the oxygen atoms—was studied with *ab initio* molecular orbital calculations augmented by zero point and correlation energy corrections[3]. The quantitative validity of these wavefunctions for determining binding energies was verified by calculations on well established asymmetrically bonded polymers of water and hydrogen fluoride. Our computations yielded values within the range of experimental error for each of the known polymers (H_2O and HF dimers, cyclic $(HF)_4$ and $(HF)_6$). Thus we have confidence in the results showing symmetrically bonded hydrogen fluoride and water to be 1 or 2 kcalories/H-bond more stable than six monomers, but approximately 5 kcalories/H-bond less stable than the corresponding asymmetric structures. The semi-empirical calculations in the original model[1] show the same relative ordering of energies, but the symmetric structures are placed only very slightly above the asymmetric ones. Analysis of the errors inherent to the semi-empirical method reveals that its tendency to produce too short bond distances, in such cases as the $O \cdots O$ separation in the water dimer, leads to an unrealistic lowering of the total energy for symmetrical hydrogen bonds.

The wall attachment process was a central feature of the original model, and because of the large energy separation between symmetric and asymmetric hydrogen bonds noted above, we have extended the original semi-empirical surface model calculations to greater distances from the wall. Going out from the surface, the oxygen atoms carry charges of 8.37, 8.46 and 8.49. When compared with an isolated cyclic symmetric hexamer (oxygen charge 8.47), it is apparent that the surface is inducing a very small oscillatory charge redistribution that completely damps out in a few layers. Charges on the symmetrical hydrogen atoms show a parallel behaviour. Charged ions (for example, Na^+) may also play a role in the surface effects, and we have made semi-empirical calculations for the close approach of Na^+ to both oxygen and hydrogen in a water molecule. Here again the charge redistribution is relatively small, and a long-range stabilization is precluded. To span the inner diameter of 10–100 μm capillaries with anomalous material requires a wall effect extending out more than 100,000 layers, and this is completely ruled out.

In addition to the two theoretical results discussed in the above paragraphs, a recent molecular weight measurement in Derjaguin's laboratory[4,5] cannot be accommodated by our original model. Not only is their value of 180 considerably less than implied by the original model, but the one or two-ring structure required by Derjaguin's measurements raises an even more serious objection. The higher energy of the symmetric bonds permits a one-ring or two-ring structure to rearrange to the lower energy conventional asymmetric bonds. This prevents realization of the kinetic barrier postulated for the more complex, three dimensional original model structure.

From the results presented above we conclude that a new allotrope of water does not exist. Our argument is based on disproof of the model which appeared to offer a consistent and compelling rationalization of the formation and twenty-odd properties of polywater. Finally, the position we take in this letter is clearly opposite to that in our original work.

LELAND C. ALLEN
PETER A. KOLLMAN

Department of Chemistry,
Princeton University,
Princeton, New Jersey 08540

Received August 3, 1971.

[1] Allen, L. C., and Kollman, P. A., *Science*, **167**, 1443 (1970).
[2] Lippincott, E. R., Stromberg, R. R., Grant, W. H., and Cessac, G. L., *Science*, **164**, 1482 (1969).
[3] Allen, L. C., and Kollman, P. A., *J. Amer. Chem. Soc.*, **92**, 4108 (1970).
[4] Derjaguin, B. V., Zhelezny, B. V., Rabinovich, Ya. I., Simonova, V. Kh., Talayev, M. V., and Churayev, N. V., *Dokl. Akad. Nauk SSSR*, **190**, 372 (1970).
[5] Derjaguin, B. V., Zhelezny, B. V., Zakhavayeva, N. N., Kiseleva, O. A., Konovalov, A. I., Lychnikov, D. S., Rabinovich, Ya. I., Talayev, M. F., and Churayev, N. V., *Dokl. Akad. Nauk SSSR*, **189**, 1282 (1969).

BIOLOGICAL SCIENCES

New Approach to the Synthesis of Polyribonucleotides of Defined Sequence

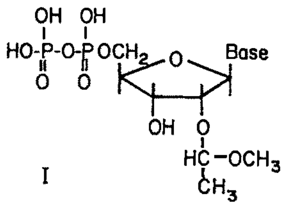
SOME of the problems associated with the chemical synthesis of polynucleotides are the lower yields obtained in the synthesis of larger oligonucleotides and the considerable labour involved in their preparation and purification because of the necessity for chemical protection of the bases and the occurrence of side reactions during synthesis[1]. We have developed a new method of synthesis of polynucleotides containing well defined sequences which promises to avoid some of these difficulties. The basis of the method lies in the use of a nucleotide polymerizing enzyme with substrates containing chemical modifications such that only one nucleotide at a time would be permitted to add to a growing polynucleotide strand.

We have studied nucleoside 5′-diphosphates and the enzyme, polynucleotide phosphorylase, which catalyses the successive transfer of the nucleoside 5′-phosphoryl moiety from the substrate to the 3′-hydroxyl group of a polyribonucleotide chain. A number of nucleotide 5′-diphosphates containing chemical blocks at their 2′ and 3′-positions have been synthesized with expectation that, if the modified nucleotide were transferred to the 3′-terminus of a polynucleotide, the presence of the blocking group would prevent any further enzymatic addition. Subsequently, the blocking group could be removed and a second single addition effected and so on. The choice of blocking group is subject to several considerations: (i) it should be chemically stable in the conditions of the enzyme reaction, (ii) the removal of the blocking group should be possible in chemical conditions which would not affect the structural integrity of the synthesized polymer, (iii) the group should be sufficiently small for the modified diphosphates to be accepted as substrates by the enzyme, (iv) it should be of such size and configurations that a polynucleotide chain containing the group at its 3′-terminus would be totally resistant to further enzymatic addition.

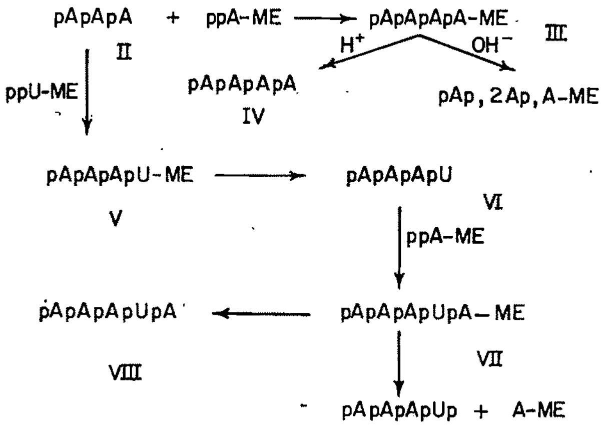
Nucleoside 5′-diphosphates containing the following blocking groups have been synthesized: 2′(3′)-0-(α-methoxyethyl), 2′,3′-cyclic carbonate, 2′,3′-0-methoxymethylidene, 2′(3′)-0-acetyl, and 2′,3′-cyclic phosphate. 2′(3′)-0-(α-Methoxyethyl) nucleoside 5′-diphosphates (I) were prepared by the direct acid catalysed reaction of each nucleoside diphosphate with methyl vinyl ether.

(Other α,β-unsaturated ethers, Δ^2-dihydropyran and ethyl vinyl ether, have already been exploited for the blocking of hydroxylic functions in nucleotides[2–4].) The nucleoside 5′-diphosphate (0.5 mmol) and toluene-sulphonic acid (2.5 mmol) are dissolved in dimethylsulphoxide (4 ml.) and frozen by immersion in an ice bath. Methyl vinyl ether (8 ml.) at 0° C is added and the two components mixed thoroughly. In the case of guanosine 5′-diphosphate it is advisable to use a slightly higher ratio of dimethylsulphoxide to methyl vinyl ether to avoid precipitation of the nucleotide. The liquid mixture is kept at 0° C for 10 min and the reaction is then stopped by the addition of concentrated ammonia (1 ml.). The solution is slowly warmed to room temperature to remove the excess methyl vinyl ether and the product streaked on Whatman 3 MM chromatographic paper (80 cm). Chromatographic separation with the solvent system, n-propyl alcohol–concentrated ammonia–water (55 : 10 : 35), gives three nucleotide bands which contain, in order of decreasing R_F, 2′,3′-di-0-(α-methoxyethyl)nucleoside 5′-diphosphate, 2′(3′)-0-(α-methoxyethyl)nucleoside 5′-diphosphate and nucleoside 5′-diphosphate. The monosubstituted product, which is obtained in yields of 25–50%, is cut out, eluted with water and concentrated. The stability of the blocking group was studied using 2′(3′)-0-(α-methoxyethyl)adenosine 5′-phosphate prepared in a similar way. The α-methoxyethyl group in this compound was found to be quite stable at *p*H 9 and was completely removed by treatment at *p*H 2 for 15 min at 37° C or at *p*H 3 for 90 min at 37° C.

Pure Chemistry Library
SCIENCE COLLEGE
92, Upper Circular Road
CALCUTTA



I



A typical reaction mixture consists of the primer or acceptor molecule (0.4 μmol), 2′(3′)-0-(α-methoxyethyl)nucleoside 5′-diphosphate (1.2 μmol), Tris-chloride buffer, *p*H 9.0 (20 μmol), $MnCl_2$ (2 μmol) and the enzyme (2 units) in a total volume of 0.2 ml. The *E. coli* polynucleotide phosphorylase was obtained from General Biochemicals, Chagrin Falls, Ohio, and the unit is defined as that amount of enzyme which catalyses the incorporation of 1 μmol of adenylic acid into polymer/h at 37° C. The reaction mixture is incubated at 37° C for 7 h and then applied to a DEAE-'Sephadex A-25' column (0.5 × 20 cm) and separation is carried out with a triethylammonium bicarbonate gradient (0.1 M to 1.0 M in 300 ml.). The product is collected and concentrated to dryness to remove the volatile eluting salt. To remove the terminal 2′(3′)-blocking group a solution of the nucleotide is brought to *p*H 2 or 3 with dilute hydrochloric acid and kept at 37° C for 15 min or 90 min respectively. After this treatment the polynucleotide is ready for a further enzymatic reaction.

We used as the primer or acceptor molecule adenosine trinucleotide (II) prepared by the degradation of polyadenylic acid with pork liver nuclease[5] followed by fractionation of the products by ion-exchange chromatography. In the presence of the enzyme and 2′(3′)-0-(α-methoxyethyl)adenosine 5′-diphosphate (ppA-ME) the trinucleotide was completely converted into the blocked tetranucleotide, III. The structure of this product was proved by the analysis of its elution position on ion exchange chromatography, by chromatography on a dihydroxyboryl-substituted cellulose derivative, and by chemical degradation to its components. The product was not retained by a column of N-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose indicating that the molecule did not possess a free 2′,3′-*cis* glycol group at its 3′-terminus[6,7]. But after the polynucleotide had been treated with mild acid (to produce the adenosine tetranucleotide, IV) it was strongly bound to a column of the substituted cellulose. On degradation with alkali in standard conditions oligonucleotide, III, gave a mixture of components which were separated by 'Dowex' 1 × 2 ion exchange chromatography. The products, adenosine 2′(3′),5′-diphosphate, adenosine 2′(3′)-phosphate, and 2′(3′)-0-(α-methoxyethyl)adenosine were obtained in a ratio of 1.0 : 2.0 : 1.0. The identity of the nucleoside obtained in this hydrolysis was confirmed by comparison of its paper chromatographic R_F values with those of an authentic derivative obtained from 2′(3′)-0-(α-methoxyethyl)adenosine 5′-phosphate by dephosphorylation with bacterial alkaline phosphatase. The two solvent systems used for the comparison were n-propyl alcohol–concentrated ammonia–water (55 : 10 : 35), and 0.1 M sodium phosphate, *p*H 7 (100 ml.) + ammonium sulphate (40 g). In the former system the substituted adenosine has R_F 0.80, while in the latter system the derivative splits into two spots with R_F values 0.17 and 0.10, presumably the 2′- and 3′-isomers. The predominant nucleoside obtained from the terminus of III was identical to that isomer which has R_F 0.17 in the ammonium sulphate solvent system. Thus, although both 2′- and 3′-substituted adenosine diphosphates are available in the addition reaction mixture, the enzyme incorporates one of these isomers preferentially. Preliminary mass spectral data suggest that the 2′-0-(α-methoxyethyl)-adenosine 5′-diphosphate is the preferred substrate, and unambiguous synthesis of both 2′- and 3′-(α-methoxyethyl)-adenosine are now being carried out to confirm the structure of the blocked terminal nucleoside present in the tetranucleotide, III.

To demonstrate the technique of successive addition a pentanucleotide has been prepared. The trinucleotide, II, was reacted with 2′(3′)-0-(α-methoxyethyl)uridine 5′-diphosphate (ppU-ME) to quantitatively yield the blocked tetranucleotide, V. Fig. 1 shows the elution patterns from the analysis of this reaction. Elution pattern B exhibits the typical shift in the elution position of the polynucleotide on increasing its chain length by one nucleotide unit. No trace of the acceptor molecule, II, remains after the enzyme reaction. The small peak preceding the nucleoside diphosphate peak in each case is due to small amounts of substituted nucleoside 5′-phosphate which is a contaminant of the substituted nucleoside 5′-diphosphate. These substances do not, however, affect the enzyme reaction and it is not necessary to remove them from the nucleoside diphosphate substrate beforehand. The blocked tetranucleotide, V, was converted to its unblocked form, VI, by mild acid treatment and used as the acceptor molecule for a second addition. On treatment with enzyme and the blocked adenosine diphosphate it was converted quantitatively into the blocked pentanucleotide, VII (Fig. 1). The molecule was then hydrolysed to the unblocked product, VIII, by mild acid treatment. The structure of the blocked pentanucleotide, VII, was confirmed by hydrolysis with pancreatic ribonuclease. The release of 2′(3′)-0-(α-methoxyethyl)adenosine by this enzyme showed that a uridine moiety was located at the penultimate position of the pentanucleotide and that the 3′-terminal nucleoside was a blocked adenosine.

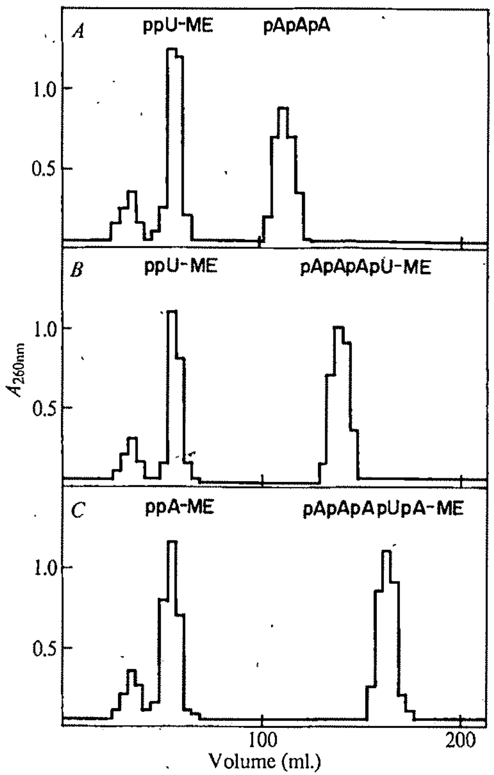


Fig. 1 Elution patterns from the DEAE-'Sephadex A-25' column chromatography of enzyme reaction mixtures. The columns (0.5 × 20 cm) were eluted at 20° C with a gradient of triethylammonium bicarbonate buffer (0.1 M to 1.0 M in 300 ml.) at a flow rate of 15 ml./h. *A*, Elution pattern for a reaction mixture containing the adenosine trinucleotide, II, and 2′(3′)-0-(α-methoxyethyl)uridine 5′-diphosphate but no enzyme. *B*, Elution pattern for the products of the reaction of the adenosine trinucleotide, II, and 2′(3′)-0-(α-methoxyethyl)uridine 5′-diphosphate with polynucleotide phosphorylase. *C*, Elution pattern for the products of the reaction of the tetranucleotide, VI, and 2′(3′)-0-(α-methoxyethyl)adenosine 5′-diphosphate with polynucleotide phosphorylase.

Preliminary experiments with the α-methoxyethyl derivatives of cytidine and guanosine diphosphates have shown that it is also possible to add these nucleotides enzymatically to an acceptor molecule in a way similar to that we have described for the adenosine and uridine derivatives.

To determine the generality of our new synthetic method, experiments are now in progress to measure the efficiency of the addition reaction with acceptor molecules containing various chain lengths and nucleotide sequences. Further work has begun on the development of a solid support system to be used in an automated synthetic machine. The problems under investigation are the method of attachment to the solid support of the initial acceptor molecule and the incorporation of a chemically induced release mechanism for the separation of the polynucleotide from the solid support after synthesis.

J. K. MACKEY
P. T. GILHAM

Biochemistry Division,
Department of Biological Sciences,
Purdue University,
Lafayette, Indiana

Received May 26, 1971.

[1] Khorana, H. G., *Pure Appl. Chem.*, **17**, 349 (1968).
[2] Smrt, J., and Sorm, F., *Coll. Czech. Chem. Commun.*, **27**, 73 (1962).
[3] Chládek, S., and Smrt, J., *Chem. Industry* (in the press).
[4] Smrt, J., and Chládek, S., *Coll. Czech. Chem. Commun.*, **31**, 2978 (1966).
[5] Heppel, L. A., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, D. R.), 31 (Harper and Row, New York, 1966).
[6] Weith, H. L., Wiebers, J. L., and Gilham, P. T., *Biochemistry*, **9**, 4396 (1970).
[7] Rosenberg, M., Wiebers, J. L., and Gilham, P. T., *160th National Meeting Amer. Chem. Soc.* (Chicago, 1970).

Application of Computer Experimentation to the Cornea

BECAUSE of the variability of tissue culture samples, it is often impossible or impractical to measure precisely all the relevant dependent variables under a selected set of conditions. Computer modelling is an attractive technique for studying complicated structures in complex environments, because of the perfect reproducibility of the results and the precision with which all the characteristics (fluxes, internal compositions, potentials) of the tissue are self-consistently determined. The accuracy is of course no better than those input tissue properties to which the answers are most sensitive.

To illustrate the capabilities of this approach, a computer model of the cornea has been used to examine the implications of Green's[1] measurements of the variation of the short-circuit current (SCC) of the isolated rabbit cornea as the sodium content of the media bathing both the epithelial and endothelial surfaces is replaced in part by potassium and as the sodium bathing the epithelial surface is further replaced by choline. It is generally accepted[2–4] that the current is generated by a sodium pump in the epithelium; pumping is from the tear film towards the stroma. Tracer studies[3] on isolated endothelium have revealed no difference between the unidirectional fluxes of sodium or chloride across this tissue; accordingly, the epithelial sodium pump was assumed to be the sole operative active transport mechanism.

The model properties of the cornea are given in Table 1. Table 2 gives the composition of the solutions bounding the cornea and the values of SCC measured by Green[1]. Green's measurements were made on a series membrane system (in the antero-posterior direction, epithelium–stroma–endothelium) across which there were, except for cases 1 and 2, concentration gradients. Thus, the usual identification of the epithelial pump rate, *P*, with SCC is inappropriate[13,14].

Although the identity *P*=SCC is in error here, the pump rate can still be found from the flux equations, but the solution is not explicit. In setting up the problem, the corneal current density, I, and the compositions of the bounding solutions $\{c_j\}_1$ (epithelial bath) and $\{c_j\}_3$ (endothelial bath) are known, and a value of *P* is assumed. In the steady state the gross flux X_j of the *j*-th ion is the same in each layer. The gross fluxes corresponding to the given *I*, $\{c_j\}_1$, $\{c_j\}_3$ and *P* were obtained by starting with the given values of $\{c_j\}_1$ at the epithelial surface and finding the values of $\{X_j\}$ such that the calculated current density equalled *I*, and the endothelial composition was $\{c_j\}_3$. The intracorneal compositions and membrane potentials are also found as a matter of course. To carry out the calculation, two classes of solutions were developed. The description of multiionic passive transport[15] needed to "cross" each layer and the general Donnan condition[16] used at the stromal interfaces have been presented elsewhere. The flux equations[15] are based on the frictional formulation[17] of irreversible thermodynamics, include direct interactions between oppositely-charged ions, and may be regarded as generalized from the Nernst-Planck form. The passive fluxes

Table 1 Properties of Model Rabbit Cornea

Corneal temperature=298 K

Membrane:	Epithelium	Stroma	Endothelium
$\bar{R}^{(a)}$, *j*-s cm mol^{-2}	$-1.91\times10^{13(b)}$	$-2.65\times10^{11(c,d)}$	$-3.37\times10^{12(b)}$
$f_{\mathrm{Na}}^{(e)}$, *j*-s mol. cm^{-2}	$7.94\times10^{11(f)}$	$3.78\times10^{8(c,d)}$	$4.06\times10^{11(c,d)}$
f_{Cl}	$5.22\times10^{11(g)}$	$3.08\times10^{8(c,d)}$	$2.68\times10^{11(c,d)}$
f_{K}	$5.41\times10^{11(g)}$	$2.58\times10^{8(g)}$	$2.79\times10^{11(g)}$
$f_{\mathrm{HCO_3}}$	$8.95\times10^{11(g)}$	$5.28\times10^{8(h)}$	$4.59\times10^{11(h)}$
$f_{\mathrm{C^-}}$	$1.38\times10^{13(i)}$	$5.28\times10^{8(j)}$	$1.15\times10^{12(i)}$
Thickness, cm	$4\times10^{-3(k,l)}$	$3.55\times10^{-2(k,l,m)}$	$5\times10^{-4(k)}$
Charge density, mequiv. l.$^{-1}$ fluid	0	$-38^{(n)}$	0

(*a*) Phenomenological resistance measuring direct interactions between oppositely-charged ions. $\bar{R}$ and f_j (*e* below) are defined by $-\mathrm{d}\tilde{\mu}_j/\mathrm{d}x = -\sum_{i(z_i=-z_j)} \bar{R}(J_jc_i/c_j - J_i) + f_jJ_j/c_j$ where *i* and *j* index the ions, $\tilde{\mu}$ is electrochemical potential, *x* is distance through the membrane, *z* is ionic charge, *J* is passive flux, and *c* is concentration. See ref. 7 for detailed discussion. (*b*) Donn *et al.*[5] measured the self-diffusion of HTO through the corneal membranes. Their data were used to estimate the effective membrane tortuosity, which was then used to obtain the needed *R* from the pure saline data in (*c*). The value of $\bar{R}$ given here is likely to be an overestimate: in any event the influence of ion–ion interactions is small in the epithelium and endothelium, where the solute–membrane interaction is large. (*c*) Katchalsky and Curran[6]: the value of $\bar{R}$ in the stroma is found from that in saline. (*d*) Maurice[7] gives the obstruction of stroma and endothelium to diffusion; this obstruction applied to known friction coefficients (*c*) in saline. (*e*) Phenomenological friction coefficient[8] measuring solvent and membrane drag. See (*a*) for defining equation. (*f*) Green[9]: tracer measurement. (*g*) Based on friction coefficient of sodium, assuming *f* inversely proportional to limiting mobility at infinite dilution; mobility values from Edsall and Wyman[10]. (*h*) Similar to (*g*); based on friction coefficient of chloride. (*i*) K. Green, private communication; tracer measurement. (*j*) Friction coefficient of choline equated to that of bicarbonate. (*k*) Maurice[11]. (*l*) Trenberth and Mishima[12]. (*m*) The stromal thickness used throughout is the normal value, though in some of the experiments *in vitro* to be modelled here, the cornea was swollen. The stroma is so permeable that the electrical properties of the entire cornea are essentially independent of stromal thickness over the range of interest. (*n*) M. H. Friedman and K. Green, unpublished work. This is a preliminary experimental value. The results presented in the text are insensitive to the stromal charge density between zero and the value used here.

Table 2 Bathing Solutions and Short-circuit Current (SCC)

Solution I: 143 Na, 123 Cl, 5 K, 25 $\mathrm{HCO_3}$
Solution $\mathrm{II_x}$: (118-x) Na, 123 Cl, 30 K, 25 $\mathrm{HCO_3}$, x choline.

Case	Epithelial bath	Endothelial bath	SCC, equiv. cm^{-2} s*
1	I	I	1.111×10^{-10}
2	$\mathrm{II_0}$	$\mathrm{II_0}$	9.73×10^{-11}
3	$\mathrm{II_{18}}$	$\mathrm{II_0}$	6.95×10^{-11}
4	$\mathrm{II_{58}}$	$\mathrm{II_0}$	5.00×10^{-11}
5	$\mathrm{II_{98}}$	$\mathrm{II_0}$	2.78×10^{-11}
6	$\mathrm{II_{113}}$	$\mathrm{II_0}$	1.111×10^{-11}

All concentrations are in mequiv. l.$^{-1}$.

* Positive ion flux, tears to aqueous. These values do not reflect Green's[1] experimental precision; the "insignificant" digits are a consequence of converting his results to c.g.s. units.

on which these equations operate are given by $J_{jk}=X_j-P_{jk}$ where J_{jk} (or P_{jk}) is the passive (or pump) flux of j through the k-th corneal layer. In the present study, the only non-zero P_{jk} is P_{Na}, epithelium$=P$.

To interpret Green's short-circuit data, I was equated to SCC, and the value of P was found for which the transcorneal potential ψ was zero. These values and the SCC are plotted in Fig. 1 against the sodium content of the epithelial bath. It can be seen that there is no evidence that the epithelial pump rate is sensitive to the sodium content of the epithelial bath over the range of compositions studied. The average value of P, based on the computer results for cases 1–4 and 6, is $(10.3\pm0.6)\times10^{-11}$ equiv. cm^{-2} s.

To test the conclusions of the short-circuit simulations, cases 1–6 were re-run under open circuit conditions ($I=0$) with $P=10.3\times10^{-11}$ equiv. cm^{-2} s, to give ψ. The calculated transcorneal potentials are shown in Fig. 2 with the transcorneal potentials measured *in vitro* (Green, private communication) for each case. Recognizing that the simulator calculation of ψ is based on the measured properties of the corneal membranes and on a value of P calculated from other experimental data, the agreement in Fig. 2 is really quite good.

Green found[1] that as the sodium concentration in the epithelial bath was reduced, the cornea swelled to larger equilibrium thicknesses. This might most readily be explained as the consequence of a concomitant increase in the osmolarity of the stromal fluid, a hypothesis which is unfortunately contradicted by the results of the computer experiments. The replacement of 25 mequiv. Na by K on both corneal surfaces (case 2 *vs* case 1) has a minor effect on the stromal osmolarity when $I=0$; it is 306.3 (posterior stroma)—306.8 (anterior stroma) mOsm l.$^{-1}$ in both cases. Continued replacement of sodium by choline on the epithelial side, however, results in a drop in stromal osmolarity, even though no decrease in pump current is assumed. In case 6 the stroma contains 300.2–300.4 mOsm l.$^{-1}$. The free sodium content of the stroma drops correspondingly from 139.0–139.3 mequiv. l.$^{-1}$ in case 2 to 133.8 mequiv. l.$^{-2}$ in case 6. The decrease in stromal tonicity as sodium is removed from the epithelial bath represents a considerable decrease in stromal osmotic pressure—a change opposite to that required to explain Green's results on osmotic grounds.

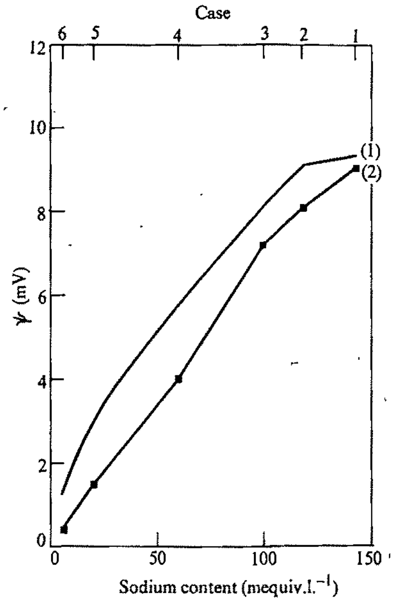


Fig. 2 Open circuit corneal potential (ψ) *vs* sodium content of the epithelial bath. (1) Computed transcorneal potential, $P=10.3\times10^{-11}$ equiv. cm^{-2} s; (2) experimental transcorneal potential.

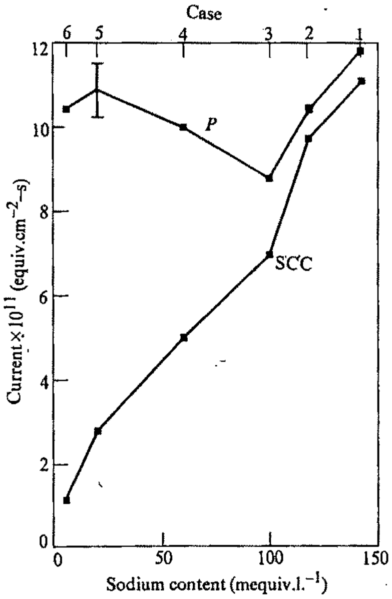


Fig. 1 Measured short-circuit current (SCC) and computed epithelial pump current (P) *vs* sodium content of the epithelial bath. Convergence difficulties permitted only a range of values of P to be found for case 5.

Pusa Cha sity Library
SCIENCE COLLEGE
Upper Circular Road
CALCUTTA

A principal role of model building is the testing of hypotheses, in the present case the hypothesis[1] that the epithelial sodium pump is implicated in the control of corneal hydration. This view has been contested by other corneal physiologists[18-20], who are supported by the results of the present work, which shows that the pump rate is not changed in the experiments in which a decrease in epithelial sodium was shown to induce swelling. If the pump rate were to fall as $c_{\mathrm{Na},1}$ was lowered, the stromal tonicity would decrease even more rapidly than found here. It follows that the corneal swelling produced by the replacement of sodium by choline in the epithelial bath is not pump-related and indeed does not appear to be osmotic in origin. The possibility of trauma cannot be discounted in such nonphysiologic environments; measurements of epithelial permeability in choline-containing solutions would surely be of interest.

The interaction between computer and physical experiments is symbiotic and the combined technique can be used to study a wide variety of biological systems; the computer program used here to investigate the cornea is directly applicable to other tissues, such as frog skin, which can be represented as series plane–parallel membrane structures. Geometric variations from the plane–parallel case should offer no difficulty, and more structurally complex systems should also be susceptible to this approach. The computer experiments rely on the results of physical experimentation in that the latter yield the property data (membrane permeabilities) used by the former, and because the physical experiments provide the essential phenomenological data for analysis (variation of SCC with $c_{\mathrm{Na},1}$) and which serve to check the results of the modelling (ψ). The computer supports the physical experimentation by allowing the study of complex systems in realistically complex environments, reducing questions of artefact and trauma. In addition, the computer experiments can generate data (stromal tonicity variations) to whose values tissue behaviour (swelling) is highly sensitive but which cannot be found well enough experimentally. Finally, the results of the computer experiments can identify critical physical experiments (variation of epithelial permeability with ambient composition), whose results can further illuminate the problem at hand. This synergism makes the combination of physical and computer experiments a powerful combined approach to the study of biological systems.

I thank K. Green for supplying choline permeabilities and corneal potential data *in vitro*. The computer programming and execution services were provided by Mary E. Lynam.

This investigation was supported by the National Institute of Neurological Diseases and Stroke, National Institutes of Health, US Public Health Service.

M. H. FRIEDMAN

Applied Physics Laboratory,
The Johns Hopkins University,
Silver Spring, Maryland 20910

Received December 16, 1970.

[1] Green, K., *Nature*, **217**, 1074 (1968).
[2] Donn, A., Maurice, D. M., and Mills, N. L., *Arch. Ophthal.*, **62**, 748 (1959).
[3] Green, K., *Exp. Eye Res.*, **6**, 79 (1967).
[4] Ehlers, N., and Ehlers, D., *Acta Ophthal.*, **46**, 767 (1968).
[5] Donn, A., Miller, S. L., and Mallett, N. M., *Arch. Ophthal.*, **70**, 515 (1963).
[6] Katchalsky, A., and Curran, P. F., *Nonequilibrium Thermodynamics in Biophysics* (Harvard University Press, Cambridge, 1965).
[7] Maurice, D. M., in *Structure of the Eye* (edit. by Smelser, G.) (Academic Press, New York, 1961).
[8] Kedem, O., and Katchalsky, A., *J. Gen. Physiol.*, **45**, 143 (1961).
[9] Green, K., *Exp. Eye Res.*, **6**, 79 (1967).
[10] Edsall, J. T., and Wyman, J., *Biophysical Chemistry*, **1** (Academic Press, New York, 1958).
[11] Maurice, D. M., in *The Eye* (edit. by Davson, H.), **1** (Academic Press, New York, 1962).
[12] Trenberth, S. M., and Mishima, S., *Invest. Ophthal.*, **7**, 44 (1968).
[13] Rehm, W. S., *J. Theoret. Biol.*, **20**, 341 (1968).
[14] Ussing, H. H., *Quart. Rev. Biophys.*, **1**, 365 (1969).
[15] Friedman, M. H., *Biophys. J.*, **10**, 1013 (1970).
[16] Friedman, M. H., *Proc. Austral. Chem. Eng. Conf.* (in the press).
[17] Kedem, O., and Katchalsky, A., *J. Gen. Physiol.*, **45**, 143 (1961).
[18] Ehlers, N., *Danish Med. Bull.*, **13**, 133 (1966).
[19] Maurice, D. M., *Exp. Eye Res.*, **6**, 138 (1967).
[20] Trenberth, S. M., and Mishima, S., *Invest. Ophthal.*, **7**, 44 (1968).

Non-Identity of Apparently Similar Chromosome Aberrations in Human Lymphoblastoid Cell Lines

A FEW chromosome aberrations seem to recur with significant frequency among the many aneuploid human lymphoblastoid lines established from various sources. (For discussion and references see ref. 1.) These include trisomy *C*, a marker chromosome ("m_B") larger than the chromosomes of group 4/5 but with a similar arm ratio and an acrocentric marker ("m_D") similar to an enlarged *D* group chromosome.

Since clones of cells bearing one or more of these abnormalities seem to have a selective advantage for growth *in vitro* it is important to know whether these apparently morphologically identical abnormalities consist of the same genetic material in each case. By using quinacrine fluorescence[2,3] to identify features specific to particular chromosomes in the human complement we can answer this question. Chromosomes in cells from fourteen lymphoblastoid lines, which originated in this laboratory or had been maintained here for more than 1 yr (Table 1), were examined by a technique described before[3].

From each line karyotypes from photographs of four to twelve metaphase spreads, showing good fluorescent banding patterns, have been studied in detail. In most of the aneuploid lines, the amount of chromosomal rearrangement revealed by quinacrine staining is greater than could be anticipated from orcein preparations, and some lines are composed of several distinct clones. In these cases, many more cells require to be examined before the definitive karyotype can be established. From the preliminary studies, however, the following results have been obtained.

Trisomy *C* was detected, on orcein staining, in lines JIYOYE and F137. In each of the seven JIYOYE cells examined with fluorescence there was trisomy of the number 9 chromosome and an additional metacentric, about the size of a number 16 which appeared to be composed of a number 7 chromosome with partial deletion of the long arm (partial trisomy 7) (Fig. 1*a*).

Twelve F137 cells have been photographed. In eight of these there was an extra chromosome morphologically belonging to group *C*. In five cases this was due to trisomy 7 (Fig. 1*b*) but in the other three the extra chromosome seemed to have been derived from a number 3 by partial deletion of one arm (Fig. 1*c*). In all five cells with trisomy 7, two of the number 7 chromosomes had a prominent subterminal secondary constriction on the long arm. This was not present in the other lines examined. An abnormality of this type has been reported in many cell lines, its frequency apparently depending on the technique used to establish each culture (see refs. 1 and 5 for further refs.).

Table 1 Cell Lines Examined

Line	Source	Modal chromosome number	Diploid or aneuploid on orcein staining
JIYOYE*	Burkitt's lymphoma	48–50	Aneuploid
RAJI*	Burkitt's lymphoma	46	Aneuploid
F137†‡	Chronic lymphatic leukaemia	47	Aneuploid
F89†‡	Subacute lymphatic leukaemia	46	Aneuploid
GOL_1‡§	Infectious mononucleosis	46	Aneuploid
$G\text{-}S_1$‡§	Chronic lymphatic leukaemia	47	Aneuploid
Bla_1¶	Acute leukaemia (adult)	45	Aneuploid
COA_1‡§	Myelofibrosis	46–48	Aneuploid
FLE_1‡§	Infectious mononucleosis	46	Diploid
DUN_1‡§	Infectious mononucleosis	46	Diploid
SAD_1‡§	Infectious mononucleosis	46	Diploid
HUN_1‡§	Acute leukaemia (child)	46	Diploid
MIT_1¶	Acute leukaemia (adult)	46	Diploid
ORI_1¶	Healthy adult	46	Diploid

* Unpublished results of Pulvertaft.
† Ref. 4.
‡ Ref. 5.
§ Ref. 6.
¶ My unpublished results.

Trisomy 7 was also present in all five RAJI cells examined. This could not have been predicted from the orcein preparations since another *C* group chromosome (number 8) was missing from every cell (Fig. 1*d*).

Three examples of marker m_B occurred among the eight aneuploid lines. Two were present in every $G\text{-}S_1$ cell and one in each RAJI cell. The fluorescent banding patterns showed that each had been formed in a different way.

One of the pair in $G\text{-}S_1$ appeared to match, in the centromere and proximal part of the long arm, with a number 3 chromosome and the distal part of the long arm was probably derived from a number 7. This line is monosomic for chromosomes 3 and 7. The other marker in $G\text{-}S_1$ corresponded to a number 4 with additional, brightly fluorescent, material on the end of the long arm. In RAJI the marker also seemed to be derived from a number 4 but the extra material at the end of the long arm appeared very dark on quinacrine staining (Fig. 1*e*–*f*).

An enlarged *D* chromosome was a feature of every cell of lines JIYOYE, F137, F89 and GOL_1. In JIYOYE marker m_D was derived from a number 14 (Fig. 1*g*), and in F89 and GOL_1 from a number 13. The latter two, however, had quite different banding patterns (Fig. 1*h* and *j*). Ten of the twelve F137 cells had a large number 13 (Fig. 1*k*) but in four cells there was one member of the 14 pair which was almost as large (Fig. 1*l*). In one cell from this line a number 14 was clearly the largest and there was a partial deletion of a number 13, possibly representing a 13/14 translocation (Fig. 1*m*).

In this relatively small sample of aneuploid lines, the apparently similar recurrent aberrations Trisomy *C*, m_B and m_D have each evolved in at least three different ways. It seems likely therefore that aneuploidy arises in lymphoblastoid cells by a wide variety of non-disjunctional, breakage and

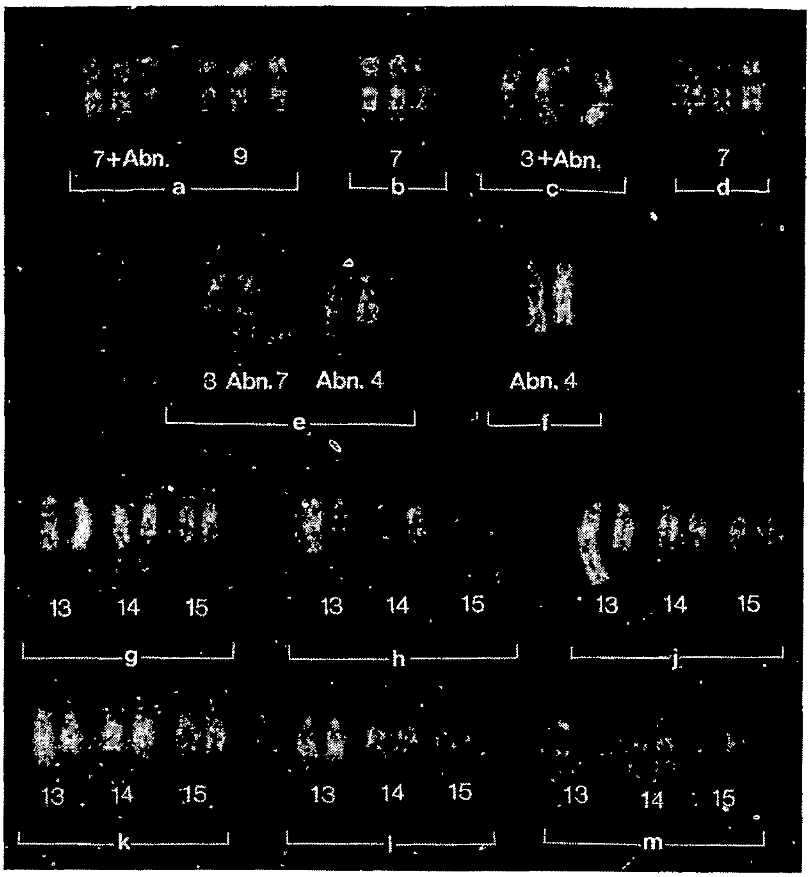


Fig. 1 The chromosomes in each group, *a*–*m*, are from a single cell. *a*, JIYOYE: trisomy 9 and partial trisomy 7. *b*, F137: trisomy 7. Two of the chromosomes show a prominent subterminal secondary constriction of the long arm. *c*, F137: abnormal chromosome, morphologically belonging to group *C* but, on banding pattern, probably derived from a number 3 as shown. *d*, RAJI: trisomy 7. *e*, G-S$_1$: two abnormal chromosomes of type "m_B", one probably derived from a number 3 and a number 7, the other from a number 4. *f*, RAJI: an abnormal chromosome of type "m_B" probably derived from a number 4 with additional non-fluorescent material on the long arm. *g*, JIYOYE: the chromosomes of group *D*, showing enlargement of a number 14. *h*, F89: the chromosomes of group *D*, showing enlargement of a number 13. *j*, GOL$_1$: the chromosomes of group *D*, showing enlargement of a number 13. (Probably duplication of the long arm.) *k*, F137: the chromosomes of group *D*, showing enlargement of a number 13. *l*, F137: the chromosomes of group *D*, showing enlargement of a number 13 and of a number 14. *m*, F137: the chromosomes of group *D*, showing probable 13/14 translocation.

recombination events, so that the apparent recurrence of distinctive markers in several lines reflects chiefly the limitations of standard staining techniques for the identification of individual chromosomes. These limitations apply not only to obviously aneuploid lines since it is theoretically possible for major rearrangements of genetic material to be concealed in an apparently diploid karyotype. No abnormality has yet been detected, however, on constructing quinacrine fluorescence karyotypes of the six diploid lines listed in Table 1.

In permanent human cell lines, all chromosomes may not be equally susceptible to loss, reduplication or breakage while it is likely that certain aberrations have a more profound effect than others on the viability of the involved cell and its progeny. Thus, for example, an abnormality involving chromosome number 7 was present in four of the eight aneuploid lines in this series. Since up to twelve separate aberrations could be recognized in each cell of any one line, however, there may be more significance in the fact that a normal pair of numbers 1, 10, 15, 17, 18, 20 and 22 was present in every line and no reduplication of these chromosomes could be detected. More definite information on the frequency (and importance to the cell) of aberrations affecting the different chromosomes of the human complement should prove to be one of the most interesting outcomes of further studies along these lines.

C. M. STEEL

*MRC Clinical and Population Cytogenetics Unit,
Western General Hospital, Edinburgh*

Received June 4, 1971.

[1] Macek, M., Seidel, E. H., Lewis, R. T., Brunschwig, J. P., Wimberly, I., and Benyesh-Melnick, M., *Cancer Res.*, **31**, 308 (1971).

[2] Caspersson, T., Zech, L., and Johansson, C., *Exp. Cell Res.*, **62**, 490 (1970).

[3] O'Riordan, M. L., Robinson, J. A., Buckton, K. E., and Evans, H. J., *Nature*, **230**, 167 (1971).

[4] Jensen, E. M., Korol, W., and Dittman, S. L., *J. Nat. Cancer Inst.*, **39**, 745 (1967).

[5] Steel, C. M., McBeath, S., and O'Riordan, M. L., *J. Nat. Cancer Res.* (in the press).

[6] Steel, C. M., and Edmond, E., *J. Nat. Cancer Res.* (in the press).

Detection of Human Leukaemia Associated Antigens in Leukaemic Serum and Normal Embryos

THE occurrence of tumours or leukaemia in experimental animals is frequently associated with the appearance of "new" antigens perhaps indicative of gene activation or mutation, or infection with oncogenic virus. The reliable detection of such antigens in man would provide an immunological signal of incipient tumour formation and might permit early or more precise diagnosis. The study of human tumour antigens might also be expected to yield information about the causes and natural history of human cancer as well as suggest improved means of treatment. This communication is concerned with the detection of one group of "new" antigens, those associated with human leukaemia.

We have previously shown that leukaemic cells will induce transformation of autologous lymphocytes *in vitro* and that a reaction of increased intensity can be obtained by immunizing a patient during remission with his own leukaemic cells taken during relapse and stored in liquid nitrogen[1]. We concluded that "new" antigens occurring in leukaemia were not recognized as "self" by autologous lymphocytes and this view has been supported by Powles *et al.*[2] and other workers[3,4] using similar methods. We have identified, solubilized and partially purified leukaemia associated antigens (LAA) obtained from human leukaemic cells[5] and, using xenogeneic antisera, have demonstrated that LAA may also be detected in the serum of patients with leukaemia and in embryos[6].

Full details of the techniques of antigen extraction and solubilization and of rabbit immunization and serum preparation have been described elsewhere[5-9]. We used the double diffusion technique (DD) as used previously[5,6] and in addition cross over electrophoresis (COE)[10-12] for the rapid detection of LAA in human serum. In this latter technique rabbit antiserum was placed in the anodal well and human test serum in the cathodal well. The agar plates were then subjected to a potential difference of 9.5 V cm^{-2} for 15 min. After washing and overnight drying, slides were stained by a very brief immersion in amido-black.

Table 1 Double Diffusion (DD) and Cross Over Electrophoresis (COE) Positive Reactions obtained using Absorbed Rabbit Antiserum FXP and Serum Concentrated Three Times from Patients

Diagnosis	No. of patients tested	No. of positive reactions	
		DD	COE
AML	14	1	5
CML	6	0	3
ALL	3	0	2
CLL	13	1	2
Chronic monocytic leukaemia	1	0	0
Hodgkin's	11	0	3
Myelofibrosis	1	0	0
Primary hepatoma	1	0	0
Total	50	2	15

AML, Acute myeloblastic leukaemia; CML, chronic myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia.

Using a suitably absorbed xenogeneic antiserum, FXP prepared against material extracted from acute myeloblastic leukaemia cells, about one-third of patients with leukaemia could be shown to have circulating LAA in their serum (Table 1); LAA do not necessarily disappear in haematological remission. When DD was used, positive reactions were associated with two or more precipitation lines[5,6]. Positive reactions were also obtained with sera of three of eleven patients with Hodgkin's disease, with five of the six foetal sera tested and with the four solubilized extracts of leukaemic cells that were tested. Negative results were obtained with twenty-two sera from healthy volunteers, with solubilized extracts of normal spleens and buffy coat and with haemolysed group A red cells, inactivated papain and prednisolone (Table 2). We have shown that solubilized extracts of rapidly growing established lymphoid cell lines failed to react with xenogeneic antisera raised against LAA and in addition whole cells of these lines did not absorb out leukaemic specificities (Viza, Phillips and Harris, unpublished). It is unlikely therefore that LAA are artefacts or that they are simply antigens common to all rapidly growing tissues. More sensitive techniques and stronger antisera, now being investigated may, however, detect more positive reactions and it is not yet clear to what extent quantitative and qualitative phenomena are involved.

Table 2 Reactivity of Absorbed Rabbit Antiserum (FXP) and Various Antigens

Antigen	No. of specimens tested		No. of positive reactions	
	DD	COE	DD	COE
Serum from patients	50	50	2	15
Soluble semi-purified leukaemic antigen (2 mg/ml.)	4	4	4	4
Foetal serum	6	6	5	5
Normal human serum	22	22	0	0
Sol. HL-A extracts from normal spleens (2.5 mg/ml.)	16	NT	0	NT
Soluble extract of normal buffy coat (2.5 mg/ml.)	1	NT	0	NT
Haemolysed group A red cells	NT	2	NT	0
Inactivated papain (10 mg/ml.)	4	2	0	0
Prednisolone (20 mg/ml.)	NT	1	NT	0

NT, Not tested; DD, double diffusion; COE, cross over electrophoresis.

The reactivity of xenogeneic antisera, prepared against solubilized extracts of AML cells, with sera obtained from some patients with other forms of leukaemia or with Hodgkin's disease is perhaps surprising. Common oncogenic viruses may be involved in producing LAA and it is possible that hitherto unsuspected aetiological heterogeneity may exist within the framework of current histological classification. Alternatively different agencies might open up similar biochemical pathways leading to "new" antigen formation. Thus our earlier results suggested that antigens common to leukaemia and embryonic extracts occur[5,6] and the results described here show that these may be found also in embryonic serum. Such leukaemo-embryonic antigens may be an expression, following malignant transformation, of genes which are partially or completely repressed in the normal adult genome and our observations provide an interesting analogy with those of Gold[13] and von Kleist and Burtin[14] who have described a carcino-embryonic antigen in human colonic tumours.

R. HARRIS

University Department of Medical Genetics,
The Royal Infirmary,
Manchester M13 9WL

D. VIZA
R. TODD
J. PHILLIPS
R. SUGAR

Searle Research Laboratories,
High Wycombe,
Buckinghamshire

R. F. JENNISON
GRETA MARRIOTT
M. H. GLEESON

St Mary's Hospital,
Manchester M13 0JH

Received May 12; revised June 28, 1971.

[1] Viza, D., Bernard-Degani, Ora, Bernard, Cl., and Harris, R. *Lancet*, ii, 439 (1969).
[2] Powles, R., Balchin, L., Hamilton Fairley, G., and Alexander, P., *Brit. Med. J.*, **1**, 486 (1971).
[3] Bach, Marylin L., Bach, F. H., and Joo, Patricia, *Science*, **166**, 1520 (1969).
[4] Fridman, W. H., and Kourilsky, F. M., *Nature*, **224**, 277 (1969).
[5] Viza, D., Davies, D. A. L., and Harris, R., *Nature*, **227**, 1249 (1970).
[6] Viza, D., Davies, D. A. L., Todd, R., Bernard-Degani, Ora, Bernard, Cl., and Harris, R., *La Presse Médicale*, **78**, 2259 (1970).
[7] Davies, D. A. L., Manstone, A. J., Viza, D., Colombani, J., and Dausset, J., *Transplantation*, **6**, 571 (1968).
[8] Davies, D. A. L., *Immunology*, **11**, 115 (1966).
[9] Davies, D. A. L., Alkins, B. J., Boyse, E. A., Old, L. J., and Stockert, Elizabeth, *Immunology*, **16**, 669 (1969).
[10] Lous, P., Olesen, H., and Skimohoj, P., *Lancet*, ii, 119 (1970).
[11] White, G. B., Lashen, R. M., and Turner, G. C., *Lancet*, ii, 368 (1970).
[12] Culliford, B. J., *Nature*, **201**, 1092 (1964).
[13] Gold, P., *Cancer*, **20**, 1663 (1967).
[14] von Kleist, Sabine, and Burtin, P., *Cancer Res.*, **29**, 1961 (1969).

Modification of Hyperacute Rejection of Sheep Kidney Heterografts in the Dog using a Trypsin Inhibitor

WHEN the species barrier is crossed in organ transplantation, rejection is often hyperacute and immediate. Thus when a sheep kidney is perfused from the dog circulation, the blood flow through the kidney ceases in about 10 min and the transplanted organ increases in weight considerably. Platelet levels

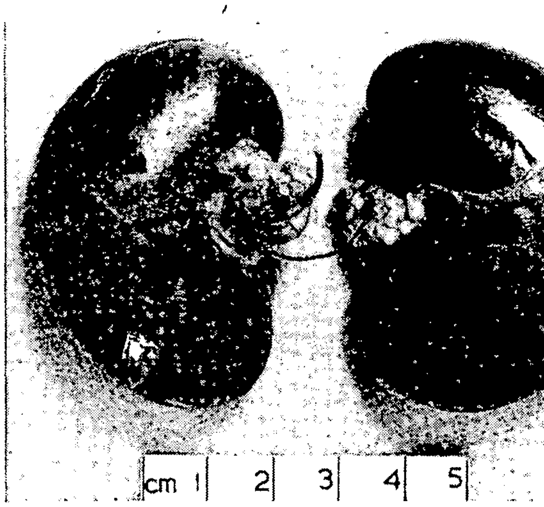


Fig. 1 Sheep kidney heterograft (right) after hyperacute rejection by the dog and its pair (left) showing less severe changes on rejection when trypsin inhibitor was present in the circulation.

and platelet stickiness measured in the sheep renal vein effluent and in the dog circulation reveal marked platelet trapping by the rejected organ. Those platelets that do emerge are not very sticky. The second of a pair of sheep kidneys connected to the same dog circulation after rejection of the first follows a similar pattern of rejection (Tables 1 and 2).

Table 1 Peak Flow, Duration of Perfusion and Weight Gain of Each of a Pair of Sheep Kidneys Perfused Sequentially from the same Dog Circulation

	Experiment	Peak flow (ml./min)	Duration of perfusion (min)	Weight gain (g)
(1)	First kidney	20	12	+5
	Second kidney	26	13	+7
(2)	First kidney	240	11	+5
	Second kidney	250	12	+5
(3)	First kidney	260	17	+5
	Second kidney	200	16	+5
(4)	First kidney	110	10	+5
	Second kidney	130	11	+5

Table 2 Platelet Levels and Stickiness from Each of a Pair of Sheep Kidneys Perfused Sequentially from the same Dog Circulation

Experiment	Dog systemic blood Platelets/mm³	Dog systemic blood Stickiness (%)	First kidney effluent Platelets/mm³	First kidney effluent Stickiness (%)	Second kidney effluent Platelets/mm³	Second kidney effluent Stickiness (%)
1	240,000	55	51,500	5.5		
	223,000	65			46,000	2.6
2	272,000	65	60,000	3.8		
	287,000	44			58,000	4.7
3	266,000	37	76,000	2.8		
	270,000	31			47,000	3.7
4	190,000	45	44,000	2.3		
	194,000	58			48,000	2.6

Because of the reproducible pattern of rejection of pairs of sequentially transplanted sheep kidneys into the same dog circulation, the system can be used as a model to study the effect on hyperacute rejection of an agent present during perfusion of the second kidney and not during the first. Dogs (20 kg) were injected intravenously with 25,000 U of a trypsin inhibitor, 'Trasylol', to prevent the release of the vasoactive local tissue hormone bradykinin from the α_2 globulins of the dog blood; and the role that this hormone plays in the processes of hyperacute rejection was studied. The injection of a trypsin-inhibitor into the dog circulation had no effect by itself on platelet levels or stickiness (Table 3).

The recipient dogs were anaesthetized with intravenous thiopentone and maintained with halothane, nitrous oxide and oxygen through an endotracheal tube. The kidneys were removed quickly from sheep anaesthetized with thiopentone; heparinized isotonic saline was injected into the renal artery to prevent blood clotting and the kidneys were weighed and cooled on ice. Connexion was made by short siliconized 'Portex' tubes from dog common carotid artery into sheep renal artery and the renal vein effluent blood flow was measured by drop count. The time taken for perfusion to cease was recorded and the kidney re-weighed after disconnexion. Serial platelet counts were done in the dog circulating blood and in the sheep renal vein effluent, and platelet stickiness was measured by the method of Hellem[1].

The results show a marked effect on the pattern of rejection of the second kidney after introduction of trypsin inhibitor. The time before perfusion ceased was prolonged, the peak flow increased, the weight gain of the transplanted kidney was not so prominent and the appearance of the organ was less engorged (Fig. 1). Platelet counts in the emergent renal blood flow did not drop so much as from the first kidney, revealing that the second kidney under the influence of trypsin inhibitor was not trapping platelets as powerfully as the untreated first kidney. The platelets that emerged after perfusing the second kidney

Table 3 Effect of Intravenous Trypsin Inhibitor on Platelet Levels and Stickiness in Dogs

	Platelets/mm³	Stickiness (%)
Dog 1	210,200	29
Dog 1+'Trasylol'	221,600	34
Dog 2	196,800	33
Dog 2+'Trasylol'	187,800	37

Table 4 Effect of a Trypsin Inhibitor on Peak Flow, Duration of Perfusion and Weight Gain on Sheep Kidney Heterografts in the Dog

Experiment	Peak flows (ml./min)	Duration of perfusion (min)	Weight gain (g)
1			
First kidney (untreated)	92	7	+15
Second kidney (treated)	160	13	+5
2			
First kidney (untreated)	60	6	+15
Second kidney (treated)	300	12	+5
3			
First kidney (untreated)	104	9	+5
Second kidney (treated)	250	14	+5
4			
First kidney (untreated)	100	11	+2
Second kidney (treated)	170	16	+0
5			
First kidney (untreated)	72	11	+10
Second kidney (treated)	122	16	+0

Table 5 Effect of Trypsin Inhibitor on Platelet Levels and Stickiness from Sheep Kidney Heterografts in the Dog

Experiment	Dog systemic blood Platelets/mm³	Dog systemic blood Stickiness (%)	Control kidney effluent Platelets/mm³	Control kidney effluent Stickiness (%)	Trypsin inhibitor treated kidney effluent Platelets/mm³	Trypsin inhibitor treated kidney effluent Stickiness (%)
1	231,600	58	61,000	4		
	212,400	66			100,800	26
2	288,000	67	80,000	2		
	289,000	45			113,600	20
3	304,800	60	60,400	3		
	318,400	46			96,500	20
4	313,200	46	83,400	2		
	293,000	63			92,200	21
5	313,200	59	56,000	6		
	311,200	62			80,200	32

were of undiminished stickiness compared with the dog circulation (Tables 4 and 5).

The finding that the introduction of 'Trasylol', which prevents the release of bradykinin, delays the rejection of sheep kidney heterografts by the dog suggests that this vasoactive hormone is one mediator in the changes that take place in the florid and immediate hyperacute rejection seen when species barriers are crossed in transplantation.

We thank Dr H. B. Allen and Mr J. Dixon of Bayer Laboratories for 'Trasylol'.

MARGARET W. GHILCHIK
A. S. MORRIS

Surgical Unit,
St Mary's Hospital Medical School,
London W2

Received June 23, 1971.

[1] Hellem, A. J., *Scand. J. Clin. Lab. Invest.*, **12**, Suppl. 51 (1960).

Presence of EBV Antibodies in Sera from Wild Chimpanzees

THE marked geographical distribution of Burkitt's lymphoma in Africa has suggested that the aetiological agent is infectious and arthropod-borne[1,2]. A herpes-like virus, first described by Epstein *et al.*[3], has been associated consistently with lymphoid cells cultured from cases of Burkitt's lymphoma[4]. Furthermore, immunofluorescent tests, using Burkitt lymphoma cells, have demonstrated antibodies against this Epstein-Barr virus (EBV) in all sera from patients with Burkitt's lymphoma and in sera from many healthy African controls living in the endemic regions[5-6]. These observations indicate that EBV infection is widespread, and suggest that the virus in some individuals might be involved in the development of Burkitt's tumour.

In spite of the several epidemiological studies of the disease[7-9], the manner by which an infectious agent might pass from man to man has not been determined. Because many Africans are affected each year, Williams[10] proposed that some animal reservoir might account for the continual presence of the disease and that it could be passed to man by insects. The areas of Africa in which the lymphoma is common overlap those in which malaria is prevalent[11-12] but no EBV has been isolated from mosquitoes or other insects[13]. Banfield[14] has reported that whole cells from reticulocyte tumours of hamsters can be carried by mosquitoes, but no evidence of this kind of transmission, such as the presence of female cells in male patients, has been found in cases of Burkitt's lymphoma.

Gerber and Birch[15] found that chimpanzees had the highest frequency and titres of antibodies to EBV, but that sera from Old World primates (baboons and monkeys) also reacted with EBV antigen. In contrast, none of the sera from nine species of lower mammals, including horse, cow and pig, had antibodies to the antigen. Landon *et al.*[16] have found a herpes-like virus similar to EBV in cultivated leucocytes from chimpanzees. The animals had antibodies which reacted with this agent in their cells and with the EBV present in cultured human Burkitt lymphoma cells. These data strongly suggest that wild primates, particularly the chimpanzee, might be the reservoir for the spread of EBV infection by insects to man.

All the primates studied so far had been exposed to man for sufficient periods of time to become infected with the virus. It was important therefore to investigate animals that had not been in contact with humans. Rhesus monkeys tested within 4 days after capture had been found to have EBV antibody[17], but these animals are known to have contact with man. The chimpanzee, on the other hand, was ideal for this purpose since it fears man, has no natural contact with him and seems to be highly susceptible to the herpes-like virus.

A preliminary study was conducted in the Budango Forest in Western Uganda, an area which is situated within the "lymphoma belt" and which contains a large chimpanzee population. The chimpanzees were captured by immobilizing them with darts containing an anaesthetic agent. Blood samples were taken immediately, and the animals on awakening were allowed to return to the jungle.

Ketamine hydrochloride ('Ketalar'), a relatively new anaesthetic agent (provided in powder form by Parke, Davis and Company, Chicago), was dissolved in water (*p*H adjusted to 6.8 with sodium hydroxide) at a concentration of 200 mg/ml. This drug has been shown to induce muscle immobilization without adverse effects on the autonomic or cardiopulmonary systems[18]. Moreover, it was chosen because at relatively low doses (10 mg/ml.), the onset of action in monkeys occurs within 5 min and recovery from anaesthesia takes only 30 min[19]. And because it is without side effects in other primates, it was considered superior to the more commonly used immobilizing agent, phenylcyclidine hydrochloride ('Sernylan'), in spite of the lack of a trial with chimpanzees. The drug was placed in darts of varying sizes which were fired by the Cap Shur gun designed by Palmer Associates, Georgia. In the Budango Forest the chimpanzees feed at the top of fig trees at a distance of 80–100 feet. They were approached by foot with care but their keen senses made them extremely difficult to catch. In a week, three chimpanzees, each from a different chimpanzee colony, were successfully bled; the anaesthetic agent was as effective as it is in monkeys and no change in respiration or heart rate was noted during immobilization.

The P3 (Jijoye) Burkitt lymphoma cell line[20] and the LE-7 chimpanzee lymphocyte line[16] were used. Both lines contain a herpes-like virus in 3–5% of the cells. Fluorescent antibody (FA) testing was conducted as previously described[5,21] using fluorescent-labelled goat anti-human globulin and anti-monkey globulin. Both react well with chimpanzee globulin; the anti-human globulin is somewhat more efficient.

Table 1 Wild Chimpanzee Sera Anti-HLV Titres

			Anti-globulin titres	
Sera	Age	Sex	Goat anti-human	Goat anti-monkey
(*A*) Antigen: human HLV (Jijoye cell line)				
4419	A	M	1/160	1/40
4420	I	M	1/40	1/10
4421	A	F	Negative	Negative
(*B*) Antigen: chimpanzee HLV (LE-7 cell line)				
4419			1/160	1/40
4420			1/5	Negative
4421			Negative	Negative

Chimpanzee sera were incubated with either human or chimpanzee cell lines. After 1/ h the sera were removed by washing twice with phosphate-buffered saline and the cells were allowed to react for 1 h with fluorescent-labelled goat anti-human or anti-monkey antisera (both are known to react with chimpanzee globulin). A, Adult; I, 4 month old infant; HLV, Herpes-like virus.

As noted in Table 1, two of three sera obtained from the wild chimpanzees reacted with both human and chimpanzee lymphocyte cells. The FA staining resembled that seen with human anti-EBV sera[4,5,21] and involved 3–5% of the cells. Sera 4419 obtained from a 200 pound male chimpanzee gave comparable titres in both chimpanzee and human cells. Serum 4420, on the other hand, reacted with a somewhat greater titre against the herpes-like virus in the Jijoye line than against the herpes-like virus in the LE-7 cells. This result contrasts with reports[22] of a preferential staining of the LE-7 chimpanzee cell line by chimpanzee sera.

These data on blood specimens from three different chimpanzee groups which had not been in contact with humans demonstrate that the wild chimpanzee has been infected by EBV or a very similar herpes-like virus. Whether these antibodies are specific for the EBV associated with Burkitt's lymphoma, as implied by the serum of 4420, or whether they represent a cross-reaction with related chimpanzee virus(es) is not yet known. The presence of these antibodies suggests that wild chimpanzees, and perhaps other primates in the forest within the lymphoma belt, could be the reservoir for an arthropod-spread of EB virus infection to man.

These studies were supported by a grant in aid from the Kresge and Strasburger Foundations and by a grant from the US National Institutes of Health.

JAY A. LEVY

Department of Medicine,
University of California,
San Francisco, California

STUART B. LEVY

Blood Research Laboratory,
New England Medical Center Hospitals,
Boston, Massachusetts

YASHAR HIRSHAUT

Sloan-Kettering Memorial Hospital,
New York

GEORGE KAFUKO

East African Viral Institute,
Entebbe

ALFRED PRINCE

New York Blood Center,
New York

Received March 8, 1971.

[1] Burkitt, D., *Brit. J. Surg.*, **46**, 218 (1958).
[2] Burkitt, D., and Davies, J. N. P., *Med. Press*, **245**, 367 (1961).
[3] Epstein, M. A., Achong, B. G., and Barr, Y. M., *Lancet*, ii, 702 (1964).
[4] Henle, G., and Henle, W., *Trans. NY Acad. Sci.*, **29**, 71 (1966).
[5] Henle, G., and Henle, W., *J. Bact.*, **91**, 1248 (1966).
[6] Levy, J. A., and Henle, G., *J. Bact.*, **92**, 275 (1966).
[7] Burkitt, D. P., *E. African Med. J.*, **38**, 511 (1961).
[8] Haddow, A. T., *E. African Med. J.*, **40**, 429 (1963).
[9] Pike, M. C., Williams, E. H., and Wright, B., *Brit. Med. J.*, **2**, 395 (1967).
[10] Williams, M. C., *UICC Monog.*, **8**, 42 (1967).
[11] Dalldorf, G., Linsell, C. A., Bernhart, F. F., and Martyn, R., *Perspect. Biol. Med.*, **7**, 435 (1964).
[12] Kafuko, G. W., and Burkitt, D. P., *Intern. J. Cancer*, **6**, 1 (1970).
[13] Goma, L. K. H., *East African Virus Res. Inst. Rep.*, p. 34 (1962-63).
[14] Banfield, W. G., Woke, P. A., MacKay, C. M., and Cooper, H. L., *Science*, **148**, 1239 (1965).
[15] Gerber, P., and Birch, S., *Proc. US Nat. Acad. Sci.*, **58**, 478 (1967).
[16] Landon, J. C., Ellis, L. B., Zeve, V. H., and Fabrizio, D. P. A., *J. Nat. Cancer Inst.*, **40**, 181 (1968).
[17] Gerber, P., and Rosenblum, E., *Proc. Soc. Exp. Biol. Med.*, **128**, 541 (1968).
[18] Bree, M. M., *Lab. Animal Care*, **17**, 547 (1967).
[19] Chen, G., Glazko, A. J., and Kaunp, D. H., *CI-581, Revised Lab. Summary* (Exp. Therap. Dept. Res. Div., Parke, Davis and Co., Jan. 15, 1968).
[20] Pulvertaft, R. J. V., *Lancet*, i, 238 (1964).
[21] Hirshaut, Y., Glade, P., Vieira, L. O. B. D., Aurhender, E., Dvorak, B., and Siltzbach, L. E., *New Engl. J. Med.*, **283**, 502 (1970).
[22] Goldman, M., Landon, J. C., and Reischer, J. I., *Cancer Res.*, **28**, 2489 (1968).

Possible Structural and Functional Differences of the Two Conformers of Myoglobin-like Molecules with Respect to the Haem-Haem Interaction

IN spite of the significance of myoglobin (Mb)[1] the details of its functional mechanism remain unknown. This is probably due, first, to the lack of physical models for the oxygenation mechanism[2] and, second, to the scant knowledge of the properties of the different conformations of Mb in the native state.

The temperature dependence of a number of molecular parameters in the range 0°–50° C is anomalous, and can be explained by postulating the existence of two conformational isomeres in a temperature-dependent equilibrium[3]. Such a consideration of the data on the nuclear magnetic relaxation (NMR: the rate of spin-lattice relaxation, $1/T_1$, of water protons) indicates the displacement of the first hydration shell Mb neighbouring or of the haem iron atom in the high-temperature conformer Mb(B) and its withdrawal from the haem by about 1.0–1.6 Å in the low temperature form Mb(A)[4,5]. It follows from the protein structure[6] that this must arise from a shift in the *F*-helical chain. Fig. 1 shows the relative change of $1/T_1$ with temperature for dolphin met-Mb in aqueous solution (black squares). The (A)⇄(B) transition elicits (or is perhaps provoked by) a change in the nature of the hydration shell. The conformers differ in stability by not more than 1–2 kcalories/mol[3].

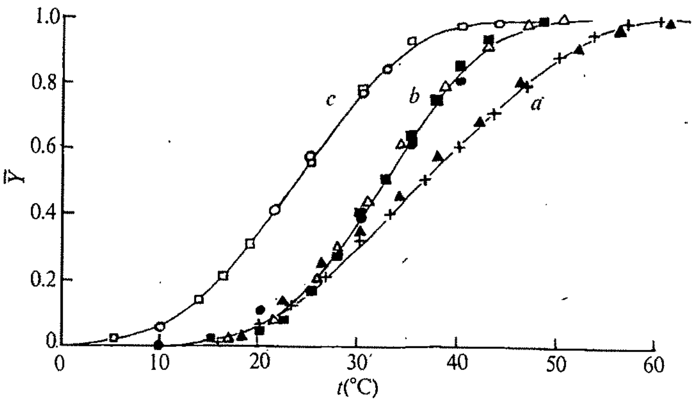


Fig. 1 Temperature-dependence of fractional changes ($\bar{Y}$) $\bar{Y}=\frac{Yt-Y_{min}}{Y_{max}-Y_{min}}$ for some physico-chemical parameters ($Y=1/T_1$, $E_{a,r}$, ε_{245}, C_p and $p/_{\frac{1}{2}O_2}$) of ferri-MbCN (curve *a*), met- and oxy-Mb (curve *b*) from some homoeotherms and of ferri(met)-Hb-s from some poikilotherms (curve *c*).

Our recent investigations of sperm whale met-Mb modified by iminoxyl spin-labels (Yu, D., Akhmedover, B. P. A., and Lichtenstein, G. I., unpublished) (three iminoxyl spins per molecule of protein, probably attached to *his 48*, *his 81* and *his 119*) showed a change in activation energy ($E_{a,r} \sim d(\ln\upsilon)/d(1/T)$) of the label rotation on heating. This parameter follows the same curve as the NMR (Fig. 1, open triangles, $Y=E_{a,r}$). The label rotation in Mb(A) is more restricted than in Mb(B). The conformational transition (A)⇄(B) probably involves *CD*, *EF* and *GH* regions of Mb[6].

On heating the sperm-whale ferri-MbCN solution (*p*H 11.0–11.5), the absorbance changes at 245 nm in a biphasic manner, with $\Delta\varepsilon_m$ of 10^4 and 2×10^4 for the two steps. The change reflects ionization of tyrosine on heating. The absence of the first step when dolphin ferri-MbCN is heated (*try HC3→phe*[7]) indicates that during the transition to Mb(B), changes also occur in the *C*-terminal region of the molecule. The concurrent change in ε_{280} reflects the true heat denaturation of the protein. The relative change with temperature of ε_{245} is shown in Fig. 1 (crosses, $Y=\varepsilon_{245}$).

Microcalorimetric studies of sperm whale ferri-MbCN have demonstrated a change in heat capacity (C_p) in the range 35° ± 18° C (ref. 8). This is effectively a phase transition of the second type and also indicates changes in the hydration shell of the protein (change of molar volume as a result of changes of the free volume of the solvent[9], and the increase in freedom of solvent at the surface of Mb(B)). Apparently, the transition (A)⇄(B) encompasses all the surface of the molecule (Fig. 1, black triangles, $Y=C_p$).

Partial oxygen pressure at half-saturation[10], $P_{\frac{1}{2}O_2}$, that is the affinity of Mb for oxygen, shows a rise with temperature, the course of which coincides with the conformational equilibrium (A)⇄(B) (Fig. 1, black circles, $Y=p_{\frac{1}{2}O_2}$). A complete description of the process demands, first, that the conformational isomers differ in oxygen affinity; second, that of Mb(B) being less than that of Mb(A); and third, that the predenaturation transition is related to the oxygenation function.

Analogous parameters for some poikilotherms (Fig. 1, open squares, $Y=1/T_1$ for lamprey haemoglobin (Hb)[11]; open circles, $Y=p_{\frac{1}{2}O_2}$ for frog Hb[12]) have similar profiles shifted, however, to a lower temperature. There is probably a deeper causal connexion between the physiological temperature of each animal species and the temperature of the conformational transition ($T=T_{tr}$ when $\bar{Y}=0.5$) with which it coincides. The relation between the optimum of conformational stability for the proteins of a given organism and the physiological temperature has been the subject of many investigations[13].

A shift of the F-helical chain towards the haem is equivalent to a "push in" of proximal *his F8* (the fifth ligand of the iron atom). This should lead to a decrease in binding strength of the sixth (distal) ligand, particularly if the latter, like oxygen, is principally bound by a π-dative bond—an effect not realizable in small molecules and denoted here a "conformational trans-effect" (see also Lumry[14,15]). A shift of the bonding nitrogen atom of *his F8* by as little as 0.2 Å results in such a strong tetragonal distortion of the ligand field (a change in the electronic population of the higher orbitals, mainly those with ε_g-symmetry) that the binding strength of the sixth ligand may decrease by 5–8 kcalories/ml. Naturally, the inverse should also apply, the binding of the sixth ligand leading to a displacement of the fifth with a corresponding displacement of the (A)$\rightleftarrows$(B) equilibrium. The shift of the F-helix might cause a small concurrent separation of the *E*(*D*) and *C*(*B*)-helical chains, resulting from the absence of cavities in that part of the molecule. Thus one can expect the conformers to differ in structurally small but functionally important details[3].

The (A)$\rightleftarrows$(B) equilibrium may thus function as a control mechanism for the oxygen affinity of Mb (a simple analogue for the conformational control of the affinity of enzyme for substrate). The position of the equilibrium, and still more the activation parameters of the transition, must be affected by the presence or absence of the ligand molecule, as well as its nature, and by intermolecular protein interactions. The latter are probably the cause of "freezing" of four Mb-like subunits of deoxy-Hb in the (B) conformation. If the O_2 bond to the first subunit leads to a change into form (A) and a decrease in interaction energy between other subunits[17], the structure "melts" and the remaining subunits enter the more reactive state (A). The concept of such a conformation-dependent haem-haem interaction in Hb is supported by a number of lines of evidence.

These studies were carried out in collaboration with the laboratories of P. L. Privalov (Institute of Protein Research, Academy of Sciences, USSR), G. I. Lichtenstein (Institute of Chemical Physics, Academy of Sciences, USSR) and A. Derzhanski (Institute of Physics, Bulgarian Academy of Sciences).

BORIS P. ATANASOV

Institute of Organic Chemistry,
Bulgarian Academy of Sciences,
Sofia 13, *and*
Institute of Protein Research,
Academy of Sciences, USSR,
Poustchino

Received June 25; revised October 29, 1970.

[1] Perutz, M. F., *Proc. Roy. Soc.*, B, **167**, 448 (1967).
[2] Wyman, J., *Quart. Rev. Biophys.*, **1**, 35 (1968).
[3] Atanasov, B. P., *Molek. Biol.*, **4**, 348 (1970).
[4] Atanasov, B. P., Derzhanski, A., and Georgieva, A., *Biochim. Biophys. Acta*, **160**, 225 (1968).
[5] Derzhanski, A., Georgieva, A., Kotev, K., and Atanasov, B. P., *Biochim. Biophys. Acta*, **214**, 83 (1970).
[6] Kendrew, J. C., *Brookhaven Symp. Biol.*, **15**, 216 (1962).
[7] Rakadjieva, A., and Meloun, B., *Coll. Czech. Chem. Commun.*, **34**, 2151 (1969).
[8] Privalov, P. L., Khechinaschvili, N. N., and Atanasov, B. P., *Biopolymers* (in the press).
[9] Lumry, R., and Rajender, S., *Enthalpy-Entropy Compensation Phenomena in Water Solutions of Protein and Small Molecules* (Wiley, London, in the press).
[10] Rossi-Fanelli, A., and Antonini, E., *Arch. Biochem. Biophys.*, **77**, 478 (1963).
[11] Maričič, S., and Rumen, N. M., *Biochim. Biophys. Acta*, **154**, 496 (1968).
[12] Leggio, T., and Morpurgo, G., **212**, 495 (1968).
[13] Alexandrov, V. Ya., *Prog. Mod. Biol.*, **60**, 28 (1965).
[14] Lumry, R., *Biophysics*, **1**, 2 (1961).
[15] Lumry, R., Solbakken, A., Sullivan, J., and Reverson, L. H., *J. Amer. Chem. Soc.*, **84**, 142 (1962).
[16] Noble, R. W., *J. Mol. Biol.*, **39**, 479 (1969).

Non-coordinated Accumulation and Synthesis of 5S Ribonucleic Acid by Ovaries of *Xenopus laevis*

ALTHOUGH the primary structure of 5S RNA from a number of organisms[1–3] is known and although it is clear that the attachment of a 5S RNA molecule is necessary if ribosomes are to be effective in protein synthesis, the precise role of these molecules is unknown. Molecules of 5S RNA are non-covalently attached to the large ribosomal subunit[4–7] and can be removed only by procedures which change the conformation of the subunit[8] and, in bacterial systems at least, they can be reunited to give particles which are effective in protein synthesis[9]. Studies of 5S RNA in HeLa cells have shown that, in the cytoplasm, 5S RNA molecules are found only in association with ribosomes, but that as much as a fifth of all the 5S RNA in a cell is to be found in the nucleus, much of it in the nucleolus[10] in association with particles which seem to be precursors of ribosomes[7]. In the circumstances, it would be natural to expect that the three RNA molecules found in ribosomes—5S RNA, 18S RNA and 28S RNA—would be synthesized in equal numbers, and there is indeed some evidence of coordinated synthesis from the development of *Xenopus laevis*[6–11]. During the early stages of oogenesis in the same organism, however, we have now found that 5S RNA molecules are formed in great excess.

Table 1 Molar Ratio of 5S rRNA, 18 and 28S rRNA and 4S RNA in *Xenopus* Liver and Ovary

Ovary	5S/18 and 28S	4S/18 and 28S	4S/5S
Late vitellogenesis	7.5	5.4	0.72
Late vitellogenesis	10.4	6.1	0.59
Late previtellogenesis	110.0	254.0	2.30
Early previtellogenesis	74.0	92.0	1.24
Liver	0.88–1.21	19.5–26.3	20.0–30.0

Total RNA from ovaries of different ages was prepared and analysed on 'Sephadex G100' columns as described in Fig. 2. The total absorbance at 260 nm was calculated for each fraction. It is assumed that the absorbance of the void fraction is due entirely to 18 and 28S rRNA. The molar ratios were calculated assuming a combined molecular weight of 2.2×10^6 for 18 and 28S RNA, and of 40,000 for 5S rRNA and 30,000 for 4S RNA.

RNA was isolated from *Xenopus laevis* ovaries at various stages of development and separated on 'Sephadex G100' columns into three fractions, the void fraction containing the 18S and 28S rRNA, the 5S and the 4S fractions. (For comparison, RNA from *Xenopus* liver has been separated in the same way.) The results (Table 1) suggest that previtellogenic ovaries accumulate 5S and 4S RNA in great excess, but that 18S and 28S rRNA is accumulated during vitellogenesis. From the molar ratios obtained in several experiments, it seems that enough 5S RNA is accumulated in previtellogenesis to allow a hundred-fold increase in ribosome content during vitellogenesis without the need for further 5S synthesis.

Two interpretations of these results are possible—either 5S RNA is synthesized in excess of 18S and 28S rRNA or that

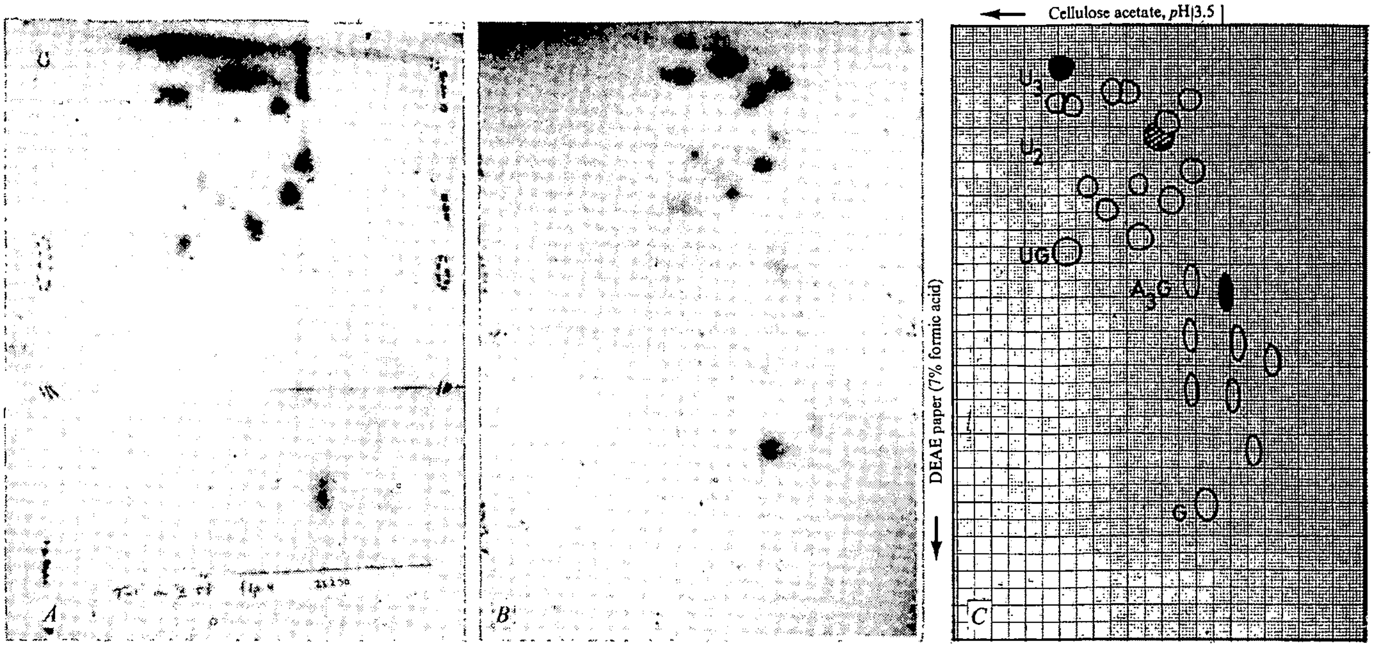


Fig. 1 10 μg 5S RNA (30,000 d.p.m. ^{32}P/μg) isolated[19] from *Xenopus* kidney cells grown in continuous cell culture, and 20 μg of *Xenopus* previtellogenic ovary 5S RNA (10,000 d.p.m. ^{32}P/μg) prepared by extraction of total RNA from ovaries incubated in 2/3 Eagle's medium plus 10% calf serum with 2.5 mCi/ml. ^{32}P for 2 days and separation by G100 chromatography as described in the legend to Fig. 2, were hydrolysed with T_1 ribonuclease[20] (Sankyo). The oligonucleotide fragments were separated by a two dimensional electrophoresis method[14]. *A, Xenopus* kidney cell ribosome 5S RNA; *B, Xenopus* previtellogenic ovary 5S RNA. The diagram (*C*) indicates (filled circles) oligonucleotides present in *A* but absent in *B*, and (hatched circle) oligonucleotides present in *B* but absent in *A*. Open circles are oligonucleotides common to both *A* and *B*.

synthesis is coordinated but that the 5S RNA species is more stable than the other two. We have therefore carried out an experiment to test whether the synthesis of the two species is coordinated. Ovaries at various stages of development were labelled with ^3H-uridine in culture for one day and total RNA from a post-mitochondrial supernatant was isolated and mixed with ^{14}C-labelled RNA prepared from 80S ribosomes isolated from late vitellogenic ovaries incubated with ^{14}C-uridine for six days. After separation of RNA on a sucrose gradient and by G100 chromatography, the $^3H/^{14}C$ ratios were computed and normalized with respect to 28S rRNA in sucrose gradients and to the void fraction in the 'Sephadex G100' columns. Table 2 shows that, as expected, 18S rRNA is synthesized coordinately with 28S rRNA, but that 5S RNA is synthesized in fifteen to twenty-fold molar excess of 18S and 28S rRNA in early previtellogenic stages and that this ratio decreases to a three to four-fold molar excess at later stages. These results are compatible with the non-coordinate synthesis hypothesis, but incompatible with the non-coordinate degradation hypothesis unless the half life of the 18S and 28S rRNA is less than 1–2 h. They do not exclude the possibility that 5S RNA is synthesized coordinately with the 40S rRNA precursor[13] which is degraded without maturing into 18 and 28S RNA.

To show that the 5S RNA accumulated in previtellogenic oocytes is similar if not identical to the 5S RNA from *Xenopus* kidney cell ribosomes, T_1 ribonuclease digests were obtained from each molecule and analysed by two dimensional electrophoresis[14]. Fig. 1 shows that the two oligonucleotide maps are

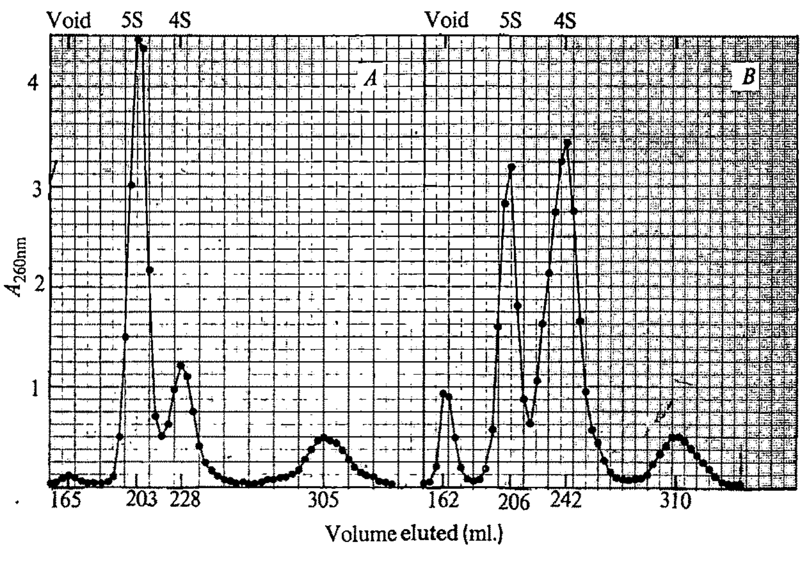


Fig. 2 Pooled previtellogenic ovaries were homogenized in 0.25 M sucrose, 50 mM Tris HCl (*p*H 7.6), 35 mM KCl and 1.5 mM $MgCl_2$ and centrifuged at 11,000 r.p.m. for 15 min at 0° C in a Sorvall RC2° centrifuge. The supernatant was layered over 50 ml. 10–50% linear sucrose gradients in 50 mM Tris HCl (*p*H 7.6), 35 mM KCl and 1.5 mM $MgCl_2$ and centrifuged at 25,000 r.p.m. for 18 h at 2° C in a Beckman SW 25.2 rotor. The optical density was monitored at 254 nm using an Isco density gradient fractionator and the fractions corresponding to the peaks at the top and 42S region were collected, precipitated with one volume of ethanol and recovered by centrifugation. The RNA was extracted from the pellets by the method of Kirby[18]. After recovery by alcohol precipitation the RNA was dissolved in 2 ml. 10 mM sodium acetate (*p*H 5.0) and 0.1 mM EDTA and loaded on 200×1.9 cm G100 'Sephadex' columns in the same buffer. The columns, at room temperature, were eluted at 6.5–7.5 ml./h and fractions were collected every 30 min. The absorbance at 260 nm was read for each fraction in a 10 mm light path cell using a Unicam 'SP500' spectrophotometer. *A*, RNA from the top of the gradient, 104 A_{260nm} units added, recovery 84%; *B*, RNA from the 42S particle, 168 A_{260nm} units added, 95% recovery. The elution buffer contained 0.01 mM sodium azide and was treated with 0.1% diethylpyrocarbonate at 60° C for 4 h before use.

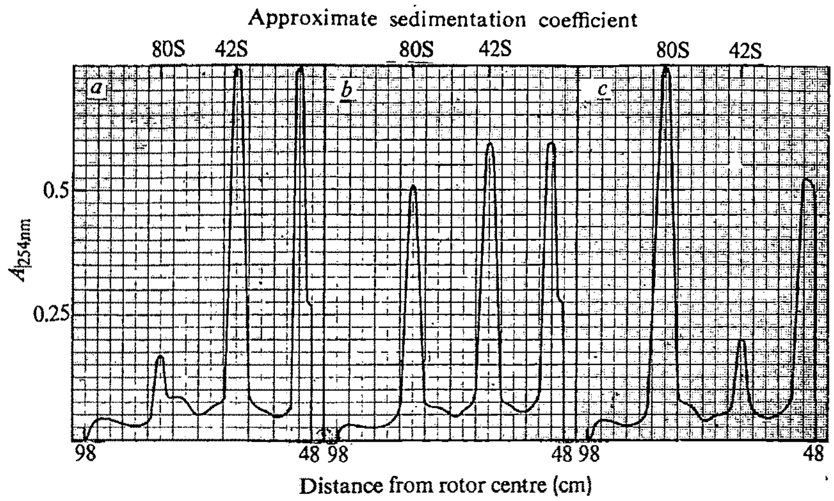


Fig. 3 Ovaries at three stages of development, (*a*) previtellogenic, (*b*) beginning of vitellogenesis and (*c*) mid vitellogenesis, were homogenized in 0.25 M sucrose, 50 mM Tris HCl (*p*H 7.6), 50 mM KCl and 1.5 mM $MgCl_2$ and centrifuged at 20,000 r.p.m. for 15 min at 0° C in a Sorvall RC2B. Aliquots of the supernatant fraction were layered on 5 ml. 10–30% sucrose gradients in the same buffer and centrifuged for 4 h at 39,000 r.p.m. at 10° C in a Spinco 'SW39' rotor. The absorbance at 254 nm was monitored continuously by an Isco density gradient fractionator.

very similar and the nucleotide compositions determined in sixteen of the twenty-three spots after elution and alkaline hydrolysis were identical. In most cases the position of a spot and its nucleotide composition give an unequivocal identification of the oligonucleotide sequence. The relative amounts of the oligonucleotides of the two RNA species were also found to be similar.

In the ovary 5S RNA, two spots in the kidney cell ribosomal 5S RNA map, one in the U_3 isoplith and the other near A_3G, are present in very small amounts and there may be an extra spot in the U_2 isoplith. Although these results are tentative, it is possible that secondary structure effects will render regions of the molecules less accessible to the nuclease, thus altering the relative proportions of particular spots. It is also possible that the base sequence of the oocyte 5S RNA is modified when it enters the ribosome.

The exact sequence relationship between the oocyte 5S RNA and 5S rRNA cannot be determined until the complete sequence is known, but it is sufficient at present to note that the oligonucleotide maps are extremely similar, and therefore that the ovary 5S RNA is very closely related to kidney cell 5S rRNA.

Although it has been shown[15] that the cytoplasm of previtellogenic oocytes is full of RNA it is not possible to observe typical ribosomes by electron microscopy of thin sections. Furthermore, it has been possible to demonstrate (Fig. 3) the existence of a ribonucleoprotein (RNP) particle sedimenting at 42S in the post-mitochondrial supernatant from an immature ovary. The 42S RNP is not a small ribosome subunit since in EDTA it does not cosediment with the small ribosome subunit, and in 0.5 M KCl it is converted into material sedimenting at the top of the gradient. The RNA isolated from the 42S material and from the top fraction of such gradients contains both 5S and 4S components (Fig. 2) but the relative amounts of each are different. Since labelled 5S and 4S RNA added before homogenization do not enter the 42S particle, one might infer that it is not an unspecific artefact of homogenization.

Table 2 Normalized Ratios of the Test for Coordinate Synthesis of Ribosomal RNAs by Immature *Xenopus* Ovaries

Normalized ratios					
Sucrose gradients			'Sephadex G100' columns		
28S	18S	4+5S	Void	5S	4S
1.00	0.90	12.05	1.00	16.4	38.20
1.00	1.12	15.60	1.00	21.95	51.80
1.00	0.96	5.41	1.00	4.55	22.30
1.00	0.91	4.60	1.00	5.04	15.30
1.00	0.84	2.19	1.00	4.44	11.45
1.00	0.96	2.58	1.00	3.19	13.87

After incubation in 2/3 Eagle's medium plus 10% calf serum with 200 µCi/ml. 5^3H-uridine for 1 day at 20° C, RNA was prepared from the post-mitochondrial supernatants of ovaries at different stages of growth. 1 and 2 represent pooled previtellogenic ovaries, 3, 4, 5 and 6 are duplicates from different early vitellogenic ovaries. The ^3H-labelled RNA was mixed with ^{14}C-RNA prepared from 80S ribosomes derived from late vitellogenic ovaries incubated in $2^{14}C$ uridine (2 µCi/ml.) for 6 days. The mixed RNA preparations were separated on sucrose gradients and G100 columns, fractions collected, precipitated with TCA, washed and counted in a toluene-PPO-POPOP mixture using a Beckman LS 200 liquid scintillation counter. The $^3H/^{14}C$ ratios were computed by the ^{14}C channels ratio method. The average $^3H/^{14}C$ ratios for the peak fractions were taken and normalized in the case of the sucrose gradients to the 28S RNA fraction and in the case of the 'Sephadex G100' columns to the void volume fraction, assumed to be entirely 18 and 28S RNA.

It would seem that a substantial proportion (60%) of the low molecular weight RNA of the immature oocyte is present in a unique form of cytoplasmic ribonucleoprotein particle. It is unique for several reasons: first, it is not usual for 4S RNA to enter such large RNP complexes; second, there is no previous report of a cytoplasmic particle other than a ribosomal large subunit containing 5S rRNA; third, it is shown to contain two major proteins by acrylamide gel electrophoresis in 6 M urea at *p*H 4.5, and proteins with similar mobilities are present in the top fraction of a gradient in only small amounts. Finally, knowing that the amounts of 18S and 28S rRNA and 5S rRNA change during oogenesis (Table 1), it is predicted that the relative amounts of the 42S material and 80S ribosomes also change during oogenesis (Fig. 3).

In the normal diploid *Xenopus* cell 5S rRNA genes are in twenty-five to thirty-fold excess of 18S and 28S rRNA genes[11], while in the oocyte 18S and 28S rRNA genes are in forty-fold excess of 5S rRNA genes, since, unlike the 5S genes[12], the 18S and 28S genes are amplified[16]. There is evidence[17], in the case of the *Xenopus* oocyte, that the 18S and 28S cistrons are maximally packed with polymerase, and if one assumes the same is true for the 5S rRNA cistrons, and that the nucleotide step time is the same for both sets of genes, then the oocyte is capable of producing forty times more 18S and 28S rRNA than 5S rRNA in a given time, which clearly makes 5S rRNA limiting for ribosome assembly. It would seem therefore that before the maximum rates of 18S and 28S rRNA synthesis are attained a large store of 5S rRNA molecules is required.

I thank the Medical Research Council for support. Most of this work was performed at the National Institute for Medical Research, Mill Hill, London. I thank Dr Robert Williamson and Mrs T. McShane for preparing oligonucleotide maps and Dr Max Birnstiel for help and criticism.

P. J. Ford

MRC Epigenetics Research Group,
Department of Genetics,
West Mains Road,
Edinburgh EH9 3JN

Received June 22, 1970; revised May 25, 1971.

[1] Forget, B. G., and Weissman, S. M., *J. Biol. Chem.*, **244**, 3148 (1969).
[2] Forget, B. G., and Jordan, B., *Science*, **167**, 382 (1970).
[3] Williamson, R., and Brownlee, G., *FEBS Lett.*, **3**, 306 (1969).
[4] Rosset, R., Monier, R., and Julian, J., *Bull. Soc. Chim. Biol.*, **46**, 87 (1964).
[5] Galibert, F., Larsen, C. L., Lelong, J. C., and Boiron, M., *Nature*, **207**, 1039 (1965).
[6] Brown, D. D., and Littna, E., *J. Mol. Biol.*, **29**, 95 (1966).
[7] Knight, E., and Darnell, J. E., *J. Mol. Biol.*, **28**, 491 (1967).
[8] Sarkar, N., and Comb, D. G., *J. Mol. Biol.*, **39**, 31 (1969).
[9] Nomura, M., and Erdmann, V. A., *Nature*, **228**, 744 (1970).
[10] Warner, J. R., and Soeiro, H. C., *Proc. US Nat. Acad. Sci.*, **58**, 1984 (1967).
[11] Brown, D. D., and Weber, C. S., *J. Mol. Biol.*, **34**, 661 (1968).
[12] Brown, D. D., and Dawid, I., *Science*, **160**, 272 (1968).
[13] Loening, U. E., Jones, K., and Birnstiel, M. L., *J. Mol. Biol.*, **45**, 353 (1969).
[14] Sanger, F., Brownlee, G. G., and Barrell, B. G., *J. Mol. Biol.*, **13**, 373 (1965).
[15] Thomas, C., *Arch. Biol. (Liège)*, **78**, 347 (1967).
[16] Gall, J. G., *Proc. US Nat. Acad. Sci.*, **60**, 553 (1968).
[17] Miller, jun., O. L., and Beatty, B. R., *Science*, **164**, 955 (1969).
[18] Kirby, K. S., *Biochem. J.*, **96**, 266 (1965).
[19] Reynier, M., Aubert, M., and Monier, R., *Bull. Soc. Chim. Biol.*, **49**, 1205 (1967).
[20] Brownlee, G. G., and Sanger, F., *J. Mol. Biol.*, **23**, 337 (1967).

Lead Absorption from the Intestine in Newborn Rats

THE tendency of the young of a given species to be more prone to lead poisoning than the adult has been observed by several authors[1], and we have investigated whether one of the reasons for this could be an increased absorption of lead from the intestine during an early stage of life.

We used 5–7 day old rats which were artificially fed for 8 h with cow's milk to which $CaCl_2$ and KH_2PO_4 were added to adjust the calcium and phosphate content to the normal level of rat's milk[2]. The technique was essentially that used before[3,4]. Carrier-free lead-203 was added to the milk and each animal received about 2 μCi. At the end of the artificial feeding period the young were returned to their mothers where they stayed until they were killed 40 and 80 h after the beginning of the experiments. The radioactivity of lead-203 was determined in the whole body before and after removal of the gastrointestinal tract in a two sodium iodine crystal assembly. Retention was expressed as percentage of the ingested dose.

Table 1 reveals a high retention of about 55% of lead-203 at both intervals. The lower value in the carcass at 40 h indicates that some radioactive lead was still present in the gastrointestinal tract at the earlier time interval. This fraction becomes absorbed after 80 h, as indicated by the higher value in the carcass and by the same retention figure obtained in the whole body and carcass at the later interval.

Table 1 Absorption of Radioactive Lead from the Intestine in Artificially Fed 5–7 Day Old Rats

		Lead-203 in the body % ingested dose	
No. of rats	Time of death (h)	With intestinal tract	Without intestinal tract
12	40	55.80±2.3	41.86±1.85
17	80	57.41±1.71	53.37±1.77

Lead-203, $CaCl_2$ and KH_2PO_4 were added to cow's milk which was fed to rats by means of a dropper during 8 h; each rat received an average of seventeen drops (0.45 ml.); each figure represents the arithmetic means and the standard error of the mean.

The principal finding, however, remains the very high absorption of lead from the intestine of newborn rats. Adult rats (4 month old females) retain within the same time interval only about 1% of the oral dose of radioactive lead[5]. If we compare these results with those obtained in our previous experiments on absorption of calcium and strontium in newborn rats with almost the same experimental technique (70–75% absorption for ^{47}Ca and ^{85}Sr after 40 h), it is seen that the absorption of lead is only 25% lower than the very high absorption of calcium and strontium characteristic for this age group. If we assume that the percentage of intestinal lead absorption is not influenced by dose[6], we could expect much more lead to be absorbed from the intestine in the young than generally supposed. If these values are expressed per kilogram body weight, the difference in the amount of lead absorbed per unit weight in young and adults would be even greater. We therefore consider that this factor should be taken into consideration when interpreting the well known differences in the symptoms of lead poisoning in adults and young children. Our results could also be important for determining criteria for human safety from contamination of the ambient atmosphere with lead, which might have to be corrected for young children when compared with adult values.

This work was supported by a research grant from the US Environmental Protection Agency.

KRISTA KOSTIAL
I. ŠIMONOVIĆ
MARICA PIŠONIĆ

Institute for Medical Research,
Zagreb

Received April 16; revised July 19, 1971.

[1] Barltrop, D., *Postgrad. Med. J.*, **45**, 129 (1969).
[2] Spray, C. M., *Brit. J. Nutrit.*, **4**, 354 (1950).
[3] Kostial, K., Simonović, I., and Pišonić, M., *Nature*, **215**, 1181 (1967).
[4] Kostial, K., Duraković, A., Šimonović, I., and Juvančić, V., *Intern. J. Radiat. Biol.*, **15**, 563 (1969).
[5] Kello, D., and Kostial, K., *Summaries, 3, Yugoslav Congress of Industrial Medicine* (1971), *Arh. Hig. Rada. Suppl.* (in the press).
[6] Blaxter, K. L., *J. Comp. Pathol.*, **60**, 140 (1950).

Spanish Moss, a Sensor for Lead

THE heavy metal content of various plants has been related to sources of air pollution[1]. Trees growing near London, England, were found to contain more lead than those growing in the country[2]. Grass near a highway was found to contain more lead than grass further away[3]. These studies involved root type plants that obtain their nourishment from the soil.

More useful information can be obtained from measurements of heavy metals in epiphytes, which derive all their nutrients from the air. They may concentrate certain elements in a manner analogous to the concentration of mercury in tuna fish and DDT in penguins. Ruhling and Tyler reported that some mosses are good indicators for lead accumulation[4]. Tyler has shown that carpet-forming mosses may also be used to measure the deposition of other heavy metals (unpublished results of G. Tyler).

We wish to report some preliminary measurements made with Spanish moss (*Tillandsia usneoides*), an epiphyte and a member of the family Bromiliaceae, which promises to prove useful as a monitor of lead and other heavy metals in the atmosphere. Wherry and Buchanan analysed three samples of the ash of Spanish moss and found them to contain much soda, potash, ferric oxide, sulphur, chlorine, and silica[5]. They found a sample from the sea coast contained more chlorine than an inland sample, suggesting a higher level of atmospheric salt near the coast due to sea spray. They considered that the scales which cover the moss hold capillary water from which the plant absorbs nutrients. They found little systematic difference between washed and unwashed samples indicating that adherent dust had a negligible effect on their results. Wherry and Capen reported additional measurements of Spanish moss and Ball-moss (*Tillandsia recurvata* L.)[6].

Table 1 Lead Content in Moss Samples

Sample location	Description of sample location	Collection date	Lead content (%) (dry basis) Unwashed samples	Washed samples In sample of moss	Washed samples In wash solution
A	Adjacent to heavily travelled four-lane highway	Feb. 19, 1971	0.067		
B	On residential street along the shore of a lake in Baton Rouge	Feb. 24, 1971	0.026		
C	0.8 mile from a secondary paved highway	March 23, 1971	0.0051		
D	Adjacent to the secondary paved highway from which sample C was obtained	March 23, 1971	0.011	0.009	0.003
E	0.1 mile from an unpaved road	March 23, 1971	0.0052	Not determined	0.0005
F	Adjacent to heavily travelled four-lane highway in Baton Rouge	March 23, 1971	0.085	0.0966	0.0434
G	On residential street along the shore of a lake in Baton Rouge	March 23, 1971	0.028	0.0154	0.0071
H	0.3 mile from a secondary paved road	March 23, 1971	0.0052		

Using atomic absorption spectrometry, we have measured the lead content of samples of Spanish moss from various locations and found it extremely high (Table 1). There must be a mechanism which concentrates this lead. Samples A and F which contain the most lead were collected from the busiest highways, the next highest lead content was in samples from Baton Rouge, and the next highest on a secondary road parallel with Bayou Grosse Tete. Sample C, 0.8 mile from this road, contained approximately half as much lead as the road-side sample. Samples C, E and H, all of which were most distant from well travelled roads, had the lowest lead content.

Some of the samples were washed with an ammonium acetate solution to determine whether the lead was surficial or in the tissues. Appreciable amounts of lead were removed from samples D, F and G, but little was removed from sample E. The total amount of lead in the wash solution from sample F and the washed moss amounts to 0.14% which was more than that in the unwashed sample, possibly because of sampling technique or variability within the sample. Consistent lead values in samples remote from roads and the ineffectiveness of washing one of these samples suggest that for these samples the lead may be in the tissues of the moss.

The abundance of Spanish moss in the Gulf Coast provides the opportunity of establishing gradients from line and point sources of pollutants.

We thank Dr James Robinson, Dr Arun Shandrikar and Mr Kenneth Reiszner for advice and Dr Philip West for the use of laboratory equipment.

JOSEPH D. MARTINEZ

Institute for Saline Studies,

MADHUSUDAN NATHANY

Department of Chemical Engineering,

VENKATRAM DHARMARAJAN

Chemistry Department,
Louisiana State University,
Baton Rouge, Louisiana 70803

Received June 1; revised July 23, 1971.

[1] Lagerwerff, J. V., and Specht, A. W., *Environmental Sci. Technol.*, **4**, No. 7, 583 (1970).
[2] Warren, H. V., and Delavault, R. E., *J. Sci. Food Agric.*, **13**, 96 (1962).
[3] Cannon, H. L., and Bowles, J. M., *Science*, **137**, 765 (1962).
[4] Ruhling, A., and Tyler, G., *Botaniska Notiser*, 3 (1968).
[5] Wherry, E. T., and Buchanan, Ruth, *Ecology*, **7**, 303 (1926).
[6] Wherry, E. T., and Capen, Ruth G., *Ecology*, **9**, 501 (1928).

Recovery from Zoxazolamine Paralysis and Metabolism *in vitro* of Zoxazolamine in Ageing Mice

SENESCENCE has been viewed as the progressive loss or diminution of the ability of an organism to regulate or maintain obligatory levels of activity of its various enzymes[1–8]. The primary reason(s) for this age-dependent loss in regulatory capacity is unknown. The study of the biochemical parameters associated with senescence has been hampered by the inability to correlate a decreased functional capacity for the organism with a loss or diminution of functional capacity at the molecular level.

The hepatic microsomal enzyme system may prove useful in overcoming this obstacle. Clearly the principal function of this enzyme system is not the metabolism of drugs or other unnatural substrates which have been used to study it, but the intermediary metabolism of fatty acids and steroid hormones[9]. The system will, however, accept various substrates, use of which has made possible its separation into a variety of components[10]. All these components can be studied individually or *in toto* as a function of age.

Here we show that the ability of mice to recover from paralysis induced by zoxazolamine (2-amino-5-chlorobenzoxazole, 'Flexin', gift of Mr John Kleis, McNeil Laboratories, Inc., Fort Washington, Pennsylvania) decreases with advancing age. Furthermore, this overtly demonstrable decrease in the capacity of the organism to recover is paralleled by a decrease in the ability of the hepatic microsomal enzyme system to metabolize the drug *in vitro*.

C57BL/6J male mice of different ages were injected with zoxazolamine. The mean recovery time of young mice from drug paralysis was found to be approximately half that of 900 day old mice (Table 1), which agrees with similar studies performed *in vivo* with rats of different ages[11]. These results suggested that the age-related difference in recovery time from zoxazolamine paralysis reflect differences in the rate of metabolism of zoxazolamine, although it was possible that the observed differences reflect an age-dependent difference in the rate of clearance of the drug from affected tissues. Liver homogenates were therefore prepared from individual mice of different ages and assayed *in vitro* for zoxazolamine hydroxylase activity[12,13]. The mean rate of metabolism of zoxazolamine *in vitro* by mouse liver whole homogenate decreases as a function of age of the animal (Table 2). These data thus parallel the age-related differences observed in the recovery of mice from zoxazolamine paralysis.

Table 1 Recovery of C57BL/6J Male Mice of Different Ages from Zoxazolamine Paralysis

Age in days	No. injected	No. died	Recovery (min)	Mean recovery ±s.e.
200	10	0	12.5	37.8±4.0
			20.5	
			28.0	
			41.5	
			42.5	
			45.5	
			44.0	
			46.5	
			48.0	
			49.0	
500	10	0	44.0	56.4±2.8
			44.5	
			49.5	
			51.0	
			57.5	
			60.0	
			60.0	
			62.0	
			63.0	
			72.0	
900	8	3	49.0	74.9±12.6
			50.0	
			65.6	
			104.5	
			105.5	

All mice were injected intraperitoneally at 1000±0015 h with the drug at a dose of 0.1 mg/g body weight (as 10 mg/ml. in 0.9% NaCl). The paralysed mice were placed on their backs in individual cages, and recovery time was scored as the interval of time between drug injection and the ability to regain the "righting reflex". Young versus old—$P > 0.95$.

Table 2 Zoxazolamine Hydroxylase Activity *in vitro* of Liver Whole Homogenates from C57BL/6J Male Mice of Different Ages

Age (days)	μg zoxazolamine metabolized/g liver/0.5 h	Mean±s.e.
200	173	182±5
	175	
	177	
	186	
	200	
500	145	172±10
	157	
	168	
	189	
	201	
900	79	130±18
	93	
	150	
	157	
	169	

All animals were killed by cervical dislocation at 1000±0010 h. The livers were removed, chilled, weighed, and 10% (w/v) homogenates were prepared in 0.25 M sucrose. Zoxazolamine hydroxylase activity of whole homogenates was estimated by a spectrophotometric method previously described by others[12,13]. Young versus old—$P > 0.95$.

These results suggest that the sustaining level of activity of the mouse microsomal enzyme system decreases with advancing age, and that this decrease may be correlated with a decrease in function for the whole organism. The observed diminution of zoxazolamine hydroxylase activity may be a result of a progressive loss of genetic template accessibility[14,15]. It is also possible that this system is maintained by a certain necessary level of natural substrate, such as one or several steroid hormones. An age-related decrease in substrate pressure may result in "template atrophy" for the whole system—a gradual irreversible loss of response capacity. Finally, integrated function at different levels of biological organization requires coordinated response, which in turn necessitates efficient communication between cells, tissues, organs and systems. Senescent changes resulting from the loss of temporal organization have been discussed before[15].

M. B. Baird
H. V. Samis
H. R. Massie

Masonic Medical Research Laboratory,
Utica, New York 13501

Received April 14; revised July 5, 1971.

[1] von Hahn, H. P., *J. Gerontol.*, **21**, 291 (1966).
[2] Adelman, R. C., *Nature*, **228**, 1095 (1970).
[3] Adelman, R. C., *J. Biol. Chem.*, **245**, 1032 (1970).
[4] Adelman, R. C., *Biochem. Biophys. Res. Commun.*, **38**, 1149 (1970).
[5] Finch, C. E., Foster, J. R., and Mirsky, A. E., *J. Gen. Physiol.*, **54**, 690 (1969).
[6] Singhal, R. L., *J. Gerontol.*, **22**, 77 (1967).
[7] Kato, R., and Takanaka, A., *J. Biochem.*, **63**, 406 (1968).
[8] Baird, M. B., and Samis, jun., H. V., *Gerontologia*, **17**, 105 (1971).
[9] Conney, A. H., *Pharmacol. Rev.*, **1**, 31 (1962).
[10] Ernster, L., and Orrenius, S., *Fed. Proc.*, **24**, 1190 (1965).
[11] Kato, R., and Takanaka, A., *Jap. J. Pharmacol.*, **18**, 389 (1968).
[12] Conney, A. H., Trousof, N., and Burns, J. J., *J. Pharmacol. Exp. Therap.*, **128**, 1333 (1960).
[13] Conney, A. H., Davison, C., Gastel, R., and Burns, J. J., *J. Pharmacol. Exp. Therap.*, **130**, 1 (1960).
[14] von Hahn, H. P., *Gerontologia*, **12**, 237 (1966).
[15] Samis, jun., H. V., *J. Theoret. Biol.*, **13**, 236 (1966).

Mechanism of Cellular Drug Resistance

CELLULAR resistance to drugs or chemicals, which can be "natural"[1-4] or "acquired"[5,6], is important particularly in the treatment of infectious diseases by antibiotics and of tumour cells by cancer chemotherapeutic drugs. I have investigated the mechanism of cellular drug resistance using mammalian cell lines resistant to actinomycin D. In murine leukaemias[7], HeLa cells[8] and bacteria[9] this resistance has been related to membrane permeability barriers which prevent the interaction of actinomycin D with deoxyguanosine residues in DNA, stopping its pharmacological action. Earlier I had found that L5178Y (mouse lymphoma cell line) and L5178Y/D (actinomycin D-resistant subline) glycosidase[10] and glycoprotein : glycosyl transferase[11] activities, which are important in membrane glycoprotein catabolism and anabolism, were altered in the resistant lines. I have now shown that surface membranes are affected, particularly the glycoproteins and glycolipids and their synthesis in the cells resistant to actinomycin D. In this article I shall develop the hypothesis that acquired drug resistance occurs through selection of cells with altered membranes and altered membrane synthetic apparatus, and that such membrane changes decrease drug permeability and thus increase drug resistance.

The cell lines used were DC-3F, derived from normal female Chinese hamster lung tissue, and DC-3F/ADX, a subline of DC-3F resistant to and grown in the presence of 10 μg per ml. of actinomycin D. Details of cell culture, resistance and maintenance were as described before[12]. Initial clones were provided by Dr J. L. Biedler. All enzyme assays were performed on cells which were homogenized for thirty strokes in 5 volumes of 0.1% 'Triton X-100' in 0.1 M Tris-HCl buffer (*p*H 7.6) with a Ten Broeck homogenizer. Glycosidases, acid phosphatase, proteolytic activity, 5′-nucleotidase, UDPase, esterase, succinic dehydrogenase, monoamine oxidase, phosphodiesterase, ribonuclease, deoxyribonuclease and the highly specific glycoprotein : glycosyl transferases, collagen : glucosyl, glycoprotein : galactosyl, collagen : galactosyl, fetuin : fucosyl, PSM : fucosyl, fetuin : N-acetylglucosaminyl, and polypeptidyl : N-acetylgalactosaminyl were analysed for and are described as given in previous publications[10,11,13].

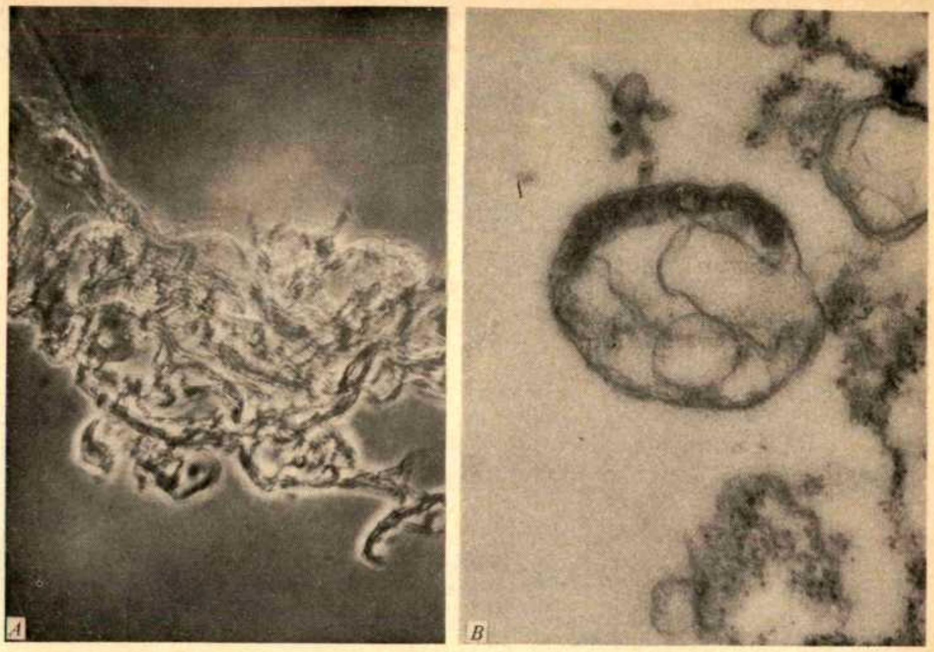


Fig. 1 *A*, Phase micrograph of plasma membrane preparation of DC-3F cells (×384). The cell membranes tend to form aggregates. Little contaminating material is present. *B*, Electron micrograph of plasma membrane preparation of DC-3F cells (×20,000). Dense particles are attached to some of the membranes as reported by Barland and Schroeder[14]. Some bilayer structure is present.

Plasma membranes were prepared by the method of Barland and Schroeder[14]. Fig. 1 indicates electron and phase micrographs of the plasma membrane preparations. The preparations were slightly contaminated with ribosomes and nuclei. Total carbohydrate was determined by the anthrone procedure[15], fucose by the Dische procedure[16] and sialic acid by the Svennerholm procedure[17].

Cell electrophoresis was performed with a thin walled cylindrical cell in a particle micro-electrophoresis apparatus, mark II (Rank Bros., Bottisham, Cambridge) according to the method of Cook and Jacobson[18]. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis of plasma membrane proteins and glycoproteins was performed without dialysis by the miniprotein method of Laico *et al.*[19]. Gels were stained with Coomassie brilliant blue for protein or a PAS technique for glycoprotein as described[20]. Gels were fractionated by a Savant autogel divider for counting.

To release material from the cell surface, cells were incubated with papain by the method of Walborg *et al.*[21] as described[11]. Analytical procedures for determining composition of the plasma membrane are as given[22].

The DC-3F cells had an electrophoretic mobility in physiological saline at 25° C of -1.65 ± 0.018 and the DC-3F/ADX of -1.75 ± 0.022 μm/s/V/cm, a statistically significant difference ($P < 0.01$), indicating more net negative charge located upon the resistant cell surface. Sizing of the cells on a Coulter counter indicated the cells were of equal volume. The drug resistant cell line is thus characterized by more charged groups

Table 1 DC-3F and DC-3F/ADX Cells and Incorporation of Radioactive Precursors into Plasma Membrane Proteins, Glycoproteins and Glycolipids

Precursor	Treatment	Whole cell, cell line DC-3F	Whole cell, cell line DC-3F/ADX	Plasma membranes, cell line DC-3F	Plasma membranes, cell line DC-3F/ADX
^{14}C-UDP-N-acetylglucosamine	TCA	114 ± 4	322 ± 19	—	—
^{14}C-UDP-N-acetylgalactosamine	TCA	206 ± 14	729 ± 39	—	—
^{14}C-UDP-glucose	TCA	120 ± 6	$1,396 \pm 72$	—	—
^{14}C-UDP-galactose	TCA	187 ± 3	801 ± 41	—	—
^{14}C-GDP-mannose	TCA	241 ± 11	$1,193 \pm 111$	—	—
^{14}C-GDP-fucose	TCA	142 ± 10	692 ± 14	—	—
^{3}H-Fucose	TCA	829 ± 14	$1,918 \pm 72$	$1,090 \pm 47$	$4,932 \pm 306$
^{3}H-Glucosamine	TCA	$3,319 \pm 171$	$13,921 \pm 716$	$2,119 \pm 82$	$6,998 \pm 172$
^{3}H-Leucine	TCA	$32,900 \pm 1,888$	$24,447 \pm 2,019$	$10,819 \pm 499$	$10,421 \pm 271$
^{3}H-Thymidine	TCA	$32,187 \pm 1,798$	$33,719 \pm 2,179$	—	—
^{3}H-Uridine	TCA	$26,187 \pm 1,827$	$27,187 \pm 1,927$	—	—
^{3}H-Fucose	$HCCl_3 : CH_3OH$	416 ± 23	619 ± 52	912 ± 18	$1,783 \pm 41$
^{3}H-Glucosamine	$HCCl_3 : CH_3OH$	786 ± 62	$1,176 \pm 89$	$1,919 \pm 107$	$3,781 \pm 406$
^{14}C-Choline	$HCCl_3 : CH_3OH$	$3,016 \pm 219$	$3,496 \pm 171$	$4,018 \pm 197$	$4,187 \pm 149$

Cells (10^7) were incubated with 0.5 μCi of ^{3}H-uridine (10 Ci/mmol), ^{3}H-thymidine (10 Ci/mmol), ^{3}H-leucine (10 Ci/mmol.), ^{3}H-fucose (10 Ci/mol), ^{3}H-glucosamine (1 Ci/mmol), ^{14}C-choline (10 Ci/mol), ^{14}C-GDP-fucose (100 Ci/mol), ^{14}C-UDP-glucose (specific activity 240 Ci/mol), ^{14}C-UDP-galactose (specific activity 240 Ci/mol), ^{14}C-GDP-mannose (specific activity 241 Ci/mol), ^{14}C-UDP-N-acetylglucosamine (specific activity 40 Ci/mol), ^{14}C-UDP-xylose (specific activity 100 Ci/mol), ^{14}C-UDP-arabinose (specific activity 100 Ci/mol), or ^{14}C-UDP-N-acetylgalactosamine (specific activity 40 Ci/mol) (purchased from New England Nuclear Corp.) in Eagle's minimal essential medium for 30 min. After incubation the mixture was made 10% in TCA, centrifuged, the pellet washed four times with 10% TCA and once with diethyl ether : ethanol (1 : 2, v/v), dissolved in 1 N NaOH, plated on a glass fibre filter and counted in a liquid scintillation counter[13]. This treatment is referred to as TCA. Alternatively after incubation 1 ml. of H_2O was added to each assay tube, the mixture was boiled for 2 min, then cooled in ice. 0.5 ml. of chloroform : methanol (2 : 1 v/v) was added to the tube and the contents were mixed with a Vortex mixer. After centrifugation at 500*g* for 2 min to break up the emulsion, the upper aqueous layer and the entire white precipitate at the interface were removed by aspiration through a Pasteur pipette and discarded. The resultant chloroform layer was then extracted with 0.5 ml. of water; the aqueous phase was discarded. To measure total lipid or glycolipid synthesis with any of the precursors, an aliquot of the chloroform extract was pipetted directly onto a glass filter, dried and the radioactivity determined in a Beckman liquid scintillation counter[13]. Data are means ±1 s.d. of c.p.m./mg protein. "Whole cell" refers to experiments performed as above. "Plasma membranes" refers to incubation as above for 1 h at 37° C with the indicated precursor followed by preparation of plasma membranes and extraction as given. (—) indicates the experiment was not performed.

Table 2 Chemical Composition of DC-3F and DC-3F/ADX Plasma Membranes and of Macromolecular Species released by Papain Digestion

Chemical composition	Cell line DC-3F	 DC-3F/ADX
	μg/mg protein	
Phospholipid	306.4	312.9
Cholesterol	190.0	186.3
RNA	8.9	9.2
Hexosamine	36.2	109.3
Sialic acid	3.6	14.4
Hexose	89.7	136.4
Papain digest*	μg/10⁹ cells	
Anthrone†	840	1,790
Fucose	200	491
Sialic acid	190	450

* Each sample represents material obtained from 10^9 cells. Papain digestion and assays were carried out as described in the text.

† Reported as μg glucose equivalents/10^9 cells.

—presumably sialic acid[18]—on its surface. To determine whether rates of synthesis of nucleic acids, proteins, glycoproteins, lipids, or glycolipids were different in the drug resistant cells, the experiments given in Table 1 were performed. The data clearly indicate that in the drug resistant cells glycoprotein synthesis was greatly increased over that in the parent cell line, while DNA, RNA and lipid synthesis (choline incorporation) were identical in the two cell lines. There was a slight decrease in protein synthesis (leucine incorporation) in the DC-3F/ADX cell line compared with the normal cell line and glycolipid synthesis was increased slightly but significantly in the DC-3F/ADX line compared with the DC/3F line. The most dramatic finding is that glycoprotein synthesis is greatly enhanced in the drug resistant cell line.

To determine whether the increased synthesis resulted in increased deposition of the glycoproteins and glycolipids in the plasma membranes of the cells, the experiments described in Table 1 were performed. The data show that in the DC-3F/ADX much greater amounts of glycoprotein and glycolipid were found in the plasma membranes even though the amounts of ^{14}C-choline in lipid and leucine in protein were similar in DC-3F and DC-3F/ADX.

Table 2 shows that the plasma membranes of the normal and drug resistant cell lines had similar amounts of cholesterol, phospholipid and RNA, but that the DC-3F/ADX had three times as much hexosamine, four times as much sialic acid, and one and a half times as much hexose as the parent cell line. That the material was near the cell periphery is shown by the data in Table 2, in which papain treated DC-3F/ADX cells released more than twice as much anthrone positive material, fucose and sialic acid as the DC-3F cells released on papain treatment.

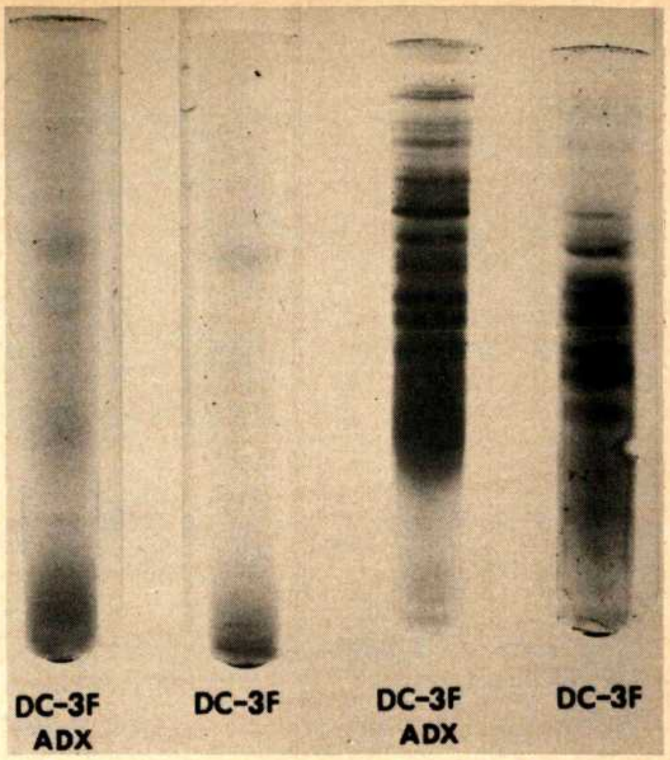


Fig. 2 Polyacrylamide gel electrophoresis of SDS extracts of DC-3F and DC-3F/ADX cell plasma membranes and identification of glycoproteins synthesized by the cells and present in the cell plasma membrane. Cells were labelled with ^{3}H-fucose and ^{14}C-glucosamine as given in Table 1. Cell plasma membranes were prepared and electrophoresis on SDS gels performed. Gels were fractionated and radioactivity was determined. Arrows indicate glycoproteins present in DC-3F/ADX not present in DC-3F. 5% polyacrylamide; *p*H 7.1, 0.1% SDS in samples, gels, and buffers.

Fig. 2 demonstrates that the DC-3F/ADX cell plasma membranes contained more protein and glycoprotein bands on

Table 3 Levels of Enzymes involved in Glycoprotein Synthesis and Degradation in DC-3F and DC-3F/ADX Cells

Enzyme	Endogenous receptors DC-3F/ADX	 DC-3F	Exogenous receptors DC-3F/ADX	 DC-3F
Collagen : glucosyl	2,800 ± 211	900 ± 27	3,100 ± 409	900 ± 49
Glycoprotein : galactosyl	5,700 ± 206	2,200 ± 107	4,100 ± 273	800 ± 36
Collagen : galactosyl	4,100 ± 310	2,000 ± 119	2,400 ± 166	1,000 ± 47
Fetuin : fucosyl	1,000 ± 42	250 ± 4	2,200 ± 217	900 ± 58
PSM : fucosyl	2,100 ± 49	1,200 ± 92	1,900 ± 88	800 ± 69
Fetuin : N-acetylglucosaminyl	3,500 ± 220	1,700 ± 43	3,700 ± 16	1,400 ± 101
Polypeptidyl : N-acetylgalactosaminyl	1,100 ± 106	480 ± 27	1,800 ± 14	1,000 ± 43
α-fucosidase	141 ± 9	300 ± 11		
β-fucosidase	1 ± 0.2	9 ± 1		
β-xylosidase	23 ± 2	25 ± 3		
α-mannosidase	19 ± 2	30 ± 2		
α-glucosidase	5 ± 1	14 ± 1		
β-glucosidase	0	0		
α-galactosidase	24 ± 2	117 ± 6		
β-galactosidase	88 ± 6	201 ± 3		
β-N-acetylgalactosaminidase	4 ± 0.3	204 ± 6		
β-N-acetylglucosaminidase	75 ± 3	1,796 ± 36		
Acid phosphatase	1,169 ± 40	1,270 ± 36		

Data for glycosidases and acid phosphatase are given as nmol/h/mg protein. Data for glycoprotein : glycosyl transferases are given as c.p.m./mg protein. Cells were extracted and assays performed as given in the text. Means ± 1 s.d. Endogenous and exogenous refer only to the glycoprotein : glycosyl transferase data.

SDS gel electrophoresis. The data in Fig. 2 show that labelling of the glycoproteins with fucose and glucosamine indicates several new glycoproteins are present in the DC-3F/ADX cell plasma membrane not present in the DC-3F cell plasma membrane.

Two means of increasing glycoproteins on the cell surface would be either to decrease glycosidase activity (enzymes which degrade glycoproteins) or to increase activity of glycoprotein : glycosyl transferases (enzymes which synthesize glycoproteins). The data in Table 3 clearly indicate that in the DC-3F/ADX cell line glycosidase activity (but not acid phosphatase) was lower than in the DC-3F cell line. Conversely, as Table 3 shows, glycoprotein : glycosyl transferase activity using either endogenous or exogenous acceptors was greatly increased in the DC-3F/ADX compared with the DC-3F cell line. Both changes would lead to enhanced content of glycoprotein in the drug resistant cells. All other enzyme levels measured (see above) showed similar activity in DC-3F/ADX and DC-3F.

The results of this communication implicate plasma membrane changes in glycoprotein and glycolipid content as determining factors in drug resistance. Whether these changes in glycoprotein content lead to permeability barriers to drug (in this instance actinomycin D) and are not simply a result of selection of drug resistant cell mutants is not known. But a mechanism—increased glycoprotein: glycosyl transferase activity and decreased glycosidase activity—has been advanced for the changes in plasma membrane glycoprotein content, although it is not yet clear whether this is general. It is, however, a working hypothesis at the biochemical level for cellular drug resistance.

This work was supported in part by grants from the US National Institutes of Health and the American Cancer Society. I am a Research Career Development awardee of the National Institute of General Medical Sciences. I thank Dr June L. Biedler for the initial clones of cells and for advice, Dr David Kessel for discussions, Mrs Gerilyn Z. Pike, Miss Melinda B. Shea and Mr Kenneth R. Case for technical assistance, Mr William Carlson and Miss Maryanne Puerner for the whole cell electrophoresis measurements, and Dr Charles Yuile for the electron microscopy.

H. BRUCE BOSMANN

Department of Pharmacology and Toxicology,
University of Rochester,
School of Medicine and Dentistry,
Rochester,
New York 14642

Received April 5; revised July 1, 1971.

[1] Hurwitz, J., Furth, J. J., Malamy, M., and Alexander, M., *Proc. US Nat. Acad. Sci.*, **48**, 1222 (1962).
[2] Haywood, A. M., and Sinsheimer, R. L., *J. Mol. Biol.*, **6**, 247 (1963).
[3] Mach, D., and Tatum, E. L., *Science*, **139**, 1051 (1963).
[4] Leive, L., *Biochem. Biophys. Res. Commun.*, **18**, 13 (1965).
[5] Schwartz, H. S., Sodergren, J. E., and Ambaye, R. Y., *Cancer Res.*, **28**, 192 (1968).
[6] Kessel, D., Botterill, V., and Wodinsky, I., *Cancer Res.*, **28**, 938 (1968).
[7] Kessel, D., and Wodinsky, I., *Biochem. Pharmacol.*, **17**, 161 (1968).
[8] Goldstein, M. N., Hamm, K., and Amrod, E., *Science*, **151**, 1555 (1966).
[9] Slotnick, I. J., and Sells, B. H., *Science*, **146**, 407 (1964).
[10] Bosmann, H. B., and Kessel, D., *Mol. Pharmacol.*, **6**, 345 (1970).
[11] Kessel, D., and Bosmann, H. B., *Cancer Res.*, **30**, 2695 (1970).
[12] Biedler, J. L., and Riehm, H., *Cancer Res.*, **30**, 1174 (1970).
[13] Bosmann, H. B., *J. Biol. Chem.*, **246**, 3817 (1971).
[14] Barland, P., and Schroeder, E. A., *J. Cell Biol.*, **46**, 622 (1970).
[15] Dische, Z., *Meth. Carbohydrate Chem.*, **1**, 478 (1962).
[16] Dische, Z., *Ann. NY Acad. Sci.*, **106**, 259 (1963).
[17] Svennerholm, L., *Acta Chem. Scand.*, **12**, 547 (1958).
[18] Cook, G. M. W., and Jacobson, W., *Biochem. J.*, **107**, 549 (1968).
[19] Laico, M. T., Ruoslahti, E. I., Papermaster, D. S., and Dwyer, W. J., *Proc. US Nat. Acad. Sci.*, **67**, 120 (1970).
[20] Bosmann, H. B., Case, K. R., and Shea, M. B., *FEBS Lett.*, **11**, 261 (1970).
[21] Walborg, E. F., Lantz, R. S., and Wray, V. P., *Cancer Res.*, **29**, 2039 (1969).
[22] Bosmann, H. B., Hagopian, A., and Eylar, E. H., *Arch. Biochem. Biophys.*, **128**, 51 (1968).

Anal Gland Secretion of the Red Fox

LITTLE is known of the chemistry of odour in higher animals[1-4] in relation to the importance which behavioural studies have ascribed to it[5-7]. Those chemical studies which have been undertaken have often lacked a concern for the behavioural and ecological implications of odour, although exceptions to this rule are becoming more numerous[2,8,9]. Here we describe preliminary chemical studies on the nature of the anal gland secretion of the red fox (*Vulpes vulpes fulva*, L.). An account of related behavioural studies will appear shortly[10]. Further chemical studies on the nature, variability and significance of the volatile components of the anal gland secretion of the red fox and of other Canidae are in progress. Microbiological studies are also planned[11].

Anal gland secretion obtained from a 1-yr-old female red fox was found to contain at least twelve volatile components including a base, trimethylamine, and a series of saturated carboxylic acids (C_2 to C_6). Among the acids, acetic acid, propionic acid, *iso*-butyric acid and *n*-butyric acid were definitely identified and evidence suggests that the remaining components included 3-methylbutanoic acid with some 2-methylbutanoic acid, pentanoic acid and 4-methylpentanoic acid.

Anal gland secretion, although sometimes released by the alarmed animal, was routinely obtained by massage of the anal glands. The slightly yellow, odorous, aqueous liquid was collected in capillaries and centrifuged to remove suspended solid. About 0.5 ml. secretion could be obtained at one time. Slight variations in secretion properties were observed from sample to sample (odour, colour, *p*H 8.2 to 8.5). Samples contained much soluble protein.

Table 1 · Gas Chromatography of Unmethylated Anal Gland Secretion *

Time (min)	Column temperature	Component	Relative molar concentration in typical sample
0.7	185	A	Minor components eluted with water
1.8	185	B	
2.7	185	C	
7.5	185	D	100
10.9	185	E	Very variable
16.9	185	F	23
28.8	200	G	9.1
32.3	200	H	13
49.4	210	I	7.3
56.4	210	J	0.95
87.9	210	K	11.4

* 'Porapak Q' column.

The centrifuged secretion was examined, without further processing, by gas chromatography using an F and M 810 chromatograph (FID) with a 6 foot × 0.25 inch silanized glass column of 'Porapak Q' (80–100 mesh) and operated at 185° C rising to 210° C with helium carrier gas. Eleven volatile components were clearly resolved although the first three, minor components with retention times similar to that of water, were not studied (Table 1).

Variations of three types were noted between secretions taken from the same animal on different occasions. First, an order of magnitude variation in the overall concentration of all volatiles was observed. Some secretions were volatile rich, others volatile poor. Secondly, although compounds corresponding to peaks D, F, G, H, I, J, K were generally present in approximately the same relative proportions, G/H and D/F variations were most pronounced. One component, E, was often a relatively minor component of the mixture, but at least once this component was observed to be present in greater amounts and on this occasion the solution was more alkaline than usual (*p*H 8.5).

Mass spectra of secretion components were obtained by combined gas chromatography–mass spectrometry. Using an LKB 9000 mass spectrometer (70 eV) with a 'Porapak Q' column and helium carrier, spectra corresponding to peaks E (*m/e*: 59, *58*, 42, 30)*, D (*m/e*: 60, *45*, 43) and F (*m/e*: *74*, 73, 57, 45) were identified as those of trimethylamine, acetic acid and propionic acid respectively following detailed comparison with spectra of authentic materials run under identical conditions and by reference to the literature[12]. The spectra of G (*m/e*: 88, 73, *43*, metastable 60.5) and H (*m/e*: 88, 73, *60*) suggested *iso*-butyric and *n*-butyric acids respectively. The spectra of I, J, K suggested higher saturated carboxylic acids. To facilitate identification of these higher acids, the secretion was acidified, extracted with ether and the extract methylated with diazomethane. The resulting solution was again examined by gas–liquid chromatography–mass spectrometry using an OV-17 column (3%) for the higher acid esters and a 'Porapak Q' column for the lower acid esters. On 'Porapak Q' at 200° C, following the solvent peak, peaks for methyl *iso*-butyrate, G (*m/e*: 102, 87, 71, 59, *43*), methyl *n*-butyrate, H (*m/e*: 102 (very weak), 87, 74, 71, 59, *43*), a pentanoic acid methyl ester, I (*m/e*: 116 (very weak), 101, 88, 85, 74, 59, *57*, 41) and a hexanoic acid methyl ester, K (*m/e*: 115, 99, 88, 87, *74*, 59, 57, 55, 43) were identified by comparison with spectra of authentic materials. Other components were either present in too small quantity to give useful spectra, or were eluted with the solvent. The presence of higher acid esters was examined using an OV-17 column at 160° C. Only one peak was observed beyond the solvent peak and this gave a mass spectrum identical with that of the final peak on 'Porapak Q'. No peaks due to higher fatty acid esters, at least as far as the C_{10} acid, were observed. In all cases, gas–liquid chromatographic retention time data were recorded with precision and compared with those of authentic samples to aid the identification of components.

A series of saturated carboxylic acids (C_2 to C_6) and the base, trimethylamine, were thus identified in the anal gland secretion. Mass spectrometry and gas–liquid chromatography retention time data clearly identified trimethylamine, acetic acid, propionic acid, *iso*-butyric acid and *n*-butyric acid as components E, D, F, G and H of the secretion. The isomers of the C_5 and C_6 acids present were not so clearly defined. The mass spectrum of peak I in the unmethylated sample, when compared with published data[12], together with a consideration of gas–liquid chromatography retention time data strongly suggested that peak I was of C_5 saturated carboxylic acid, probably a mixture of 3-methylbutanoic acid (base peak 60) with some 2-methylbutanoic acid (base peak 74). An examination of the corresponding mass spectrum in the methylated sample supported this. The mass spectrum closely resembled that of 3-methylbutanoic acid methyl ester with the exception of certain anomalous peaks. Thus the unexpectedly intense peak at *m/e* 88 could arise by McLafferty rearrangement from some 2-methylbutanoic acid methyl ester present with the 3-methylbutanoic acid methyl ester. Also the gas–liquid chromatography peak in question, although apparently "pure", was slightly shifted from the position of 3-methylbutanoic acid and this could be accounted for on the basis of the presence of an admixture of a second unresolved component of similar retention time to that of the 3-methylbutanoic acid.

The compound present in peak J was not easily identified, for no good mass spectra were obtained of this very minor component. The gas–liquid chromatography retention time and the dominance of peaks at *m/e* 60 and 73 in the unmethylated material's mass spectrum suggested the possibility of this being *n*-pentanoic acid.

Peak K was produced by a C_6 saturated carboxylic acid. Gas–liquid chromatography retention time data excluded the possibility of this being *n*-hexanoic acid. The mass spectrum of the methylated sample exhibited some differences from that of the methyl ester of 2-methylpentanoic acid and considerable differences from that of the methyl ester of 3-methylpentanoic acid. At the time of the study no authentic sample of the methyl ester of 4-methylpentanoic acid was available, but features of the mass spectra of both the methylated and unmethylated samples suggest this assignment.

The anal gland secretions were further examined by gas–liquid chromatography for the presence of less volatile odorous components using a column of 10% 'Carbowax 20M' on 'Gas Chrome Q' at 200° C. In these conditions, the retention times of 3-methylbutanoic and 3-methylpentanoic acids were 1.2 and 1.8 min respectively. On examining the unmethylated secretion, only one peak could be observed at retention time greater than 1.8 min. This was at 5.14 min (peak L). The nature of this component is now under examination. Neither indole (r_t 12.9 min) nor 3-methylindole (r_t 14.8 min) was present.

In a typical secretion sample, the relative molar concentrations of volatiles was estimated as in Table 1 by the use of gas–liquid chromatography. In this case the absolute concentration of acetic acid was 0.28 M. The trimethylamine peak was not quantified but it was clearly insufficient alone to account for the alkaline *p*H of the solution.

Using the method of Beutler *et al.*[13], the presence of thiol was sought in a secretion sample from which all solid and soluble protein had been removed. Thiol was detected at a level of 3.25×10^{-5} M. It is possible that these small quantities of thiol are not present in volatile form, but as, for example, glutathione.

We thank Dr William R. Sherman for facilities. This work was supported by a Public Health Service grant through the Center for the Biology of Natural Systems, Washington University, St Louis.

E. S. ALBONE*

Center for the Biology of Natural Systems

M. W. FOX

Psychology Department,
Washington University,
St Louis,
Missouri 63130

Received June 10, 1971.

---

* Base peaks are italicized.

* Present address: School of Chemistry, University of Bristol, Bristol, BS8 1TS, England.

[1] Comfort, A., *Nature*, **230**, 432 (1971).
[2] Michael, R. P., and Keverne, E. B., *Nature*, **218**, 746 (1968).
[3] Marler, P. R., and Hamilton, W. J., *Mechanisms of Animal Behaviour* (Wiley, London and New York, 1966).
[4] *Chemical Ecology* (edit. by Sondheimer, E., and Simeone, J. B.) (Academic Press, London and New York, 1970).
[5] Gleason, K. K., and Reynierse, J. H., *Psychol. Bull.*, **71**, 58 (1969).
[6] Bruce, H. M., *Brit. Med. Bull.*, **26**, 10 (1970).
[7] Bronson, F. H., *Reproduction and Sexual Behaviour*, chapter 21 (edit. by Diamond, M., *et al.*) (Indiana University Press, 1969).
[8] Brownlee, R. G., Silverstein, R. M., Müller-Schwarze, D., and Singer, A. G., *Nature*, **221**, 284 (1969).
[9] Müller-Schwarze, D., *Nature*, **223**, 525 (1969).
[10] Fox, M. W., Albone, E. S., Levy, J., and Mitchell, C., in *Social Behaviour and Ecology of Canines* (edit. by Fox, M. W.) (Van Nostrand, Princeton, in the press).
[11] Hoyt, C. P., Osborne, G. O., and Mulcock, A. P., *Nature*, **230**, 472 (1971).
[12] Cornu, A., and Massott, R., *Compilation of Mass Spectral Data* (Heyden, New York, 1966) and Supplements.
[13] Beutler, E., Duron, O., and Kelly, B. M., *J. Lab. Clin. Med.*, **61**, 882 (1963).

BOOK REVIEWS

Industrial Crystallization

Industrial Crystallization from Solutions. By Jaroslav Nyvlt. Translated by Paul Feltham. Pp. 189. (Butterworth: London, July 1971.) £5.50.

CRYSTALLIZATION, the practice of which dates back to antiquity, is one of the major processing techniques of the chemical industry. Yet it is only in recent years that serious attention has been paid to this old established and long neglected unit operation of chemical engineering.

Without doubt, crystallization is an exceedingly complex operation. It is a simultaneous heat and mass transfer process with a strong dependence on fluid and particle mechanics. It takes place in a multi-phase, multi-component system. It is concerned with particulate solids whose size and size distribution, both incapable of unique definition, vary with time. Nucleation and growth kinetics, the governing processes in this operation, can often be profoundly influenced by mere traces of impurity in the system.

It is, perhaps, no wonder that crystallization has been called an art rather than a science. Nevertheless, industrial crystallizers have to be designed and operated, in spite of the fact that the foundations are somewhat insecure. Any publication, therefore, which attempts to increase available knowledge of this complicated subject is more than welcome.

The author is head of the industrial crystallization section at the Czechoslovak Research Institute for Inorganic Chemistry at Usti Nad Labem. The book, which is an extensively revised version of the original 1967 Slovak language edition, is in two parts. The first deals with theoretical analyses of phase equilibria, material and thermal balances, nucleation and crystallization kinetics and growth behaviour in multiparticle systems. Experimental techniques for measuring kinetic data and equilibria are also described. The second part covers the industrial practice of crystallization. Several basic types of crystallizer are described, and pathways leading to the design of these units are explored. A number of useful worked examples are given.

Most of the mathematical models utilized in this book were devised by the author, and it is very interesting to compare and contrast them with those that have been derived in the past decade or so by research workers in the United States and the United Kingdom. In any case, a large proportion of the author's publications have appeared in Czechoslovakian journals only, so it is most instructive to have this summary of them in the English language. Furthermore, another valuable feature of this book is the fact that it introduces the reader to a considerable amount of virtually inaccessible information published in a wide variety of East European journals.

J. W. MULLIN

Statistical Mechanics

Statistical Mechanics at the Turn of the Decade. Edited by E. G. D. Cohen. Pp. viii+235. (Marcel Dekker: New York, January 1971.) $12.50; £5.95.

THIS book is a record of the symposium held at North Western University, Evanston, Illinois, in October 1969 to celebrate the seventieth birthday of Professor G. E. Uhlenbeck. It consists of a verbatim record of the eight lectures given at the symposium, and forms a fitting tribute. The style of the lectures is lucid and contrasts favourably with that of the usual formal layout of research publications: they are decidedly readable. I particularly like the way some of the authors review the major work of past papers in their topics with a frank and searching criticism not usually found, and anticipate the direction of further developments in their fields. Each lecture is well referenced, and for the most part they all contain brief (sometimes too brief for the uninitiated) introductions. If the book has to be faulted, it could be said that it is too broadly based, in that I feel that most people working in statistical mechanics and allied fields will find something of interest, but they may not be conversant with the work outlined in many of the sections.

In the section on statistical mechanics and ergodic theory, A. S. Wightman contributes a cohesive and well documented discussion of developments in classical statistical mechanics, including good introductory sections, and remarks on the seeming paradox of microscopic reversibility giving macroscopic irreversibility. He goes on to consider certain Hamiltonian flows and K and S systems in some detail.

The generalization of the Boltzmann equation to higher densities is covered by E. G. D. Cohen in a very well written section outlining the investigations into the density distribution functions for moderately dense gases, incorporating the concepts of multiple particle collisions. The author concludes with astute remarks on the major difficulties of the problems.

D. Ruelle contributes a short section on the C* algebra approach to statistical mechanics. Time evolution of quantum systems and, eventually, decomposition equilibrium states are dealt with. Possibly this discussion would be better fitted to a book on quantum mechanics.

The lectures on the Curie point, given by C. Domb, occupies the longest and most readable section of the book, with a comprehensive literature survey. The introductory parts could well be used for undergraduate reading. The principal consideration here is with the position of the attempted reconciliation of the approaches of Onsager and Van der Waals to this transition.

F. J. Dyson provides a good sequel to the talk of Professor Domb with a section on phase transitions in ferromagnets. He considers some interesting and inter-related problems concerning phase transitions for the Ising model of a ferromagnet, and how these relate to the Heisenberg model.

The varying fortunes of the Weiss ferromagnetic theory and the Van der Waals theory of gas-liquid systems, and their status in the light of modern developments are the topics of A. J. F. Siegert's contribution.

P. C. Martin reviews superfluids and superconductors, first describing superfluidity and its thermodynamics and hydrodynamics. He concludes with a discussion of non-equilibrium problems of vortex motion, supercurrent decay and fluctuations near the transition temperature.

Finally, P. C. Hohenberg deals with dynamic phenomena near a critical point. First there is an exposition on the static properties near a critical point as given by Professor Domb; then an interesting, but non-rigorous, approach to dynamic critical phenomena which gives good agreement with experimental results.

J. E. JONES

Fluorine Journal

Journal of Fluorine Chemistry. Edited by H. J. Emeléus and J. C. Tatlow. Pp. 128. (Elsevier: Lausanne, July 1971.) n.p.

THE quality of the papers in the first issue of the new quarterly, *Journal of Fluorine Chemistry* is much the same as that found in most learned society journals.

Each paper was carefully scrutinized by one senior referee and by one of the two distinguished editors-in-chief. The editors have informed me that, if they themselves have any doubts about papers submitted for future issues of the journal, the manuscripts will also be examined by a second referee. Refereeing procedures, therefore, should be as tough as those employed by the more reputable journals.

Provided the determination of the editors to stop this journal becoming a depository for papers rejected elsewhere is maintained, and, if the editorial board continues to exercise a critical vigilance, there is every reason to hope that the standard of the first issue will be upheld.

I note with concern, however, that this is yet another specialist journal which now has to be perused. The proliferation of specialist periodicals during the last few years is a trend to be abhorred, partly because of their tendency to lower standards and partly because their very nature encourages narrow specialization which has a detrimental effect on the progression of science. Furthermore, in the present economic situation, libraries are being forced to become more and more selective in what they purchase and inevitably, if this trend continues, some journals must fail. A relatively expensive, specialist quarterly, such as *Journal of Fluorine Chemistry*, must surely be one of the most vulnerable.

J. H. HOLLOWAY

School Physics

PSSC Physics. By Uri Haber-Schaim, Judson B. Cross, John H. Dodge and James A. Walter. Third edition. Pp. 674. (Heath: Lexington, Massachusetts, and Farnborough, Hampshire, March 1971.) £4.

THIS is the third edition of a really most attractive elementary introduction to physics, a text which by now is firmly established in the United States. It is essentially aimed at a school leaving standard more or less corresponding to our A-level. Its 674 pages cover quite an extensive range, including optics, gravitation, energy, electrostatics, electromagnetics, elements of atomic structure, quanta and matter waves. The illustrations throughout the book are magnificent. The text is mainly descriptive, depending heavily on well drawn, and often amusing, illustrations, backed with a solid sprinkling of admirable photographs, some in colour and all very much to the point. The very backbone of the whole text is the many fine illustrations, for there is hardly a page but that it has one or more of these, all well drawn, cleverly designed and very informative.

There are perhaps one or two weaknesses in the text. The optics section is largely purely descriptive, at times a little superficial. Although, as elsewhere, the 160 pages devoted to optics have numerous delightful illustrations, yet there is, I think, a decided lack of derivation of elementary formulae, indeed not enough for O-level, much less A-level physics. However, when the reader moves straight on to the next sections (motion in a straight line and vectors) this defect is corrected. Especially clear and attractive are the sections on Newton's laws of motion and on gravitation, with just the right loading of mathematics for the A-level candidate. Since this quite essential amount of mathematics is correctly included here, all the more does one feel the weakness due to the paucity of simple mathematical treatments in the earlier fairly large section on optics. Much clever use is made of stroboscopic and interrupted photographs of moving objects to illustrate dynamical principles.

There follows a short section on the kinetic theory of gases which is excellent. There is a curiosity in the succeeding electrical section. It is perhaps a criticism that the description of the cathode ray tube and the use of the oscilloscope come even before the derivation of Coulomb's law in electrostatics. This seems to be giving in to modern popular expectations at the expense of a logical teaching sequence. A similar curiosity appears as early as page 5 of the text. Surely this is much too early to illustrate a large bevatron and even to write about "a common place Geiger counter". Such excitements should be left for later. This reminds me of a school I visited in the United States where boys were "familiar" with the use of a Geiger counter before they had been taught Ohm's law. Surely there is a very real educational danger in by-passing basic fundamentals early for the sake of modern (admittedly exciting) applications.

The sections of the text dealing with the magnetic field and electromagnetics are practical, well illustrated and backed up by just the right amount of mathematics. The chapters on the Rutherford atom, photons, atomic spectra and the wave theory of matter are all compact, adequate and treated with sympathetic understanding for the difficulties the schoolboy will encounter.

Each chapter has a set of problems and a book list for further reading. My only real criticism is that there is no section on acoustics, which lends itself admirably to the illustrative type of treatment used throughout this book. Production is first rate and the price reasonable, although high by school standards in this country. Yet this is a text of which there should certainly be more than one copy in every school library in this country. I welcome this new edition. S. TOLANSKY

Ionic Interactions

Ionic Interactions: From Dilute Solutions to Fused Salts. Vol. 1: Equilibrium and Mass Transport. Edited by S. Petrucci. (Physical Chemistry: a Series of Monographs, Vol. 22.) Pp. xiii+407. (Academic: New York and London, February 1971.) $19.50; £9.10.

THE word "Interactions" in the title must be read in nearly all of its connotations in order to connect the miniature monographs in this volume. It means the long range mutual electrostatic interactions of many ions in dilute solutions; it means the short range interactions which, depending on the forces and partners involved, lead to ionic association, solvation or complexation; in the second volume, interaction of ions with radiation will be included. Two types of interaction are omitted—chemical interactions of ions with other ions or molecules, and electrochemical interactions at the electrode–electrolyte (fused or solution) interface.

The first chapter, by H. Falkenhagen and W. Ebeling, presents a theoretical treatment of the equilibrium properties of dilute solutions of strong electrolytes. The classical limiting laws of Debye and Hückel for osmotic and activity coefficients are derived, first for point charges, and then for charged rigid spheres in a continuum. After a brief discussion of the limitations imposed by the linearized Poisson–Boltzmann equation, general molecular distribution functions are derived by the method of Bogoliubov; these lead to the thermodynamic properties as functions of concentration. Finally, ionic association as a consequence of electrostatic attraction is considered; the association constant is evaluated by requiring identity of corresponding coefficients in equations derived respectively for the physical model and the chemical model. The second chapter, by H. Falkenhagen, W. Ebeling and W. D. Kraft, summarizes the theory of electrolytic conductance as developed by the electrolyte school at Rostock University. First, the equation for the limiting tangent is derived, following the classical Debye–Hückel–Onsager approach. The authors then proceed to review their attack on the problem of irreversible processes in electrolytic solutions (conductance, in particular) which begins with a derivation of the fundamental equation of continuity from the exact Liouville equation of statistical mechanics. Solution of the former for the pair correlation function leads to the relaxation force. The electrophoretic effect is derived from the generalized diffusion equation of Falkenhagen and Ebeling. Combination of these two long range interionic effects then gives the conductance function for unassociated electrolytes. Finally, the consequences of ionic association due to electrostatic attraction are treated by a method which parallels that used in the first chapter for reversible processes.

In the third chapter S. Petrucci reviews the various statistical and thermodynamic approaches to the still incompletely resolved question of ion association. These include Bjerrum (1926), Fuoss (1934), Fuoss (1958), Denison-Ramsey (1955), Gilkerson (1956), and Bruckenstein-Pettit (1966). Complexes of ions and neutral ligands are next discussed, from the point of view of crystal field theory. The chapter concludes with a description of the methods of calculating association constants from experimental data on galvanic cells, conductance, and absorption spectra.

In the fourth chapter J. Braunstein describes the transition from very dilute aqueous solutions (the domain of the first three chapters) to fused salts with concentrated solutions, hydrate melts, and melts with less than enough water for complete hydration as intermediate stages. Theory of the thermodynamic properties of these systems requires a change of model as one goes from dilute solution to fused salt; a cell model begins to replace the continuum in the moderately concentrated range, and finally fused salts are best represented by the randomized crystal lattice. Transport properties of fused salts are the subject of the fifth chapter, by C. T. Moynihan. Experimental results on conductance, diffusion, viscosity (shear and bulk), and transport numbers are first reviewed in sufficient breadth to set the stage for a critical presentation of the many theories which have been advanced to describe irreversible processes in fused salts. These include the Stokes–Einstein equation, the Nernst–Einstein equation, transition state theory, significant structure theory, the hole theory, statistical mechanical theories, the free-volume theory, and the Adams–Gibbs theory. The chapter concludes with sections on relaxation phenomena in fused salts and transport processes in fused salt mixtures.

Raymond M. Fuoss

Antimicrobial Action

Biochemistry of Antimicrobial Action. By J. T. Franklin and G. A. Snow. Pp. ix+163. (Chapman and Hall: London, July 1971.) £2.25.

The field of antimicrobial mechanisms of action has developed rapidly in the past twenty years. This book describes chiefly the principal known features of the mechanism of action of antimicrobial drugs. It is well up to date but, unfortunately, does not deal, in most cases, with details of the experimental approaches leading to the main discoveries. In spite of this, the book should be very useful for beginners because it provides important basic information about the biochemical action of antimicrobial drugs.

The book comprises seven chapters dealing sequentially with (1) a general outline of the development of the field and different approaches to investigate the biochemical action of antimicrobial agents; (2) drugs affecting synthesis of the bacterial cell wall peptidoglycan; (3) antiseptics and antibiotics interacting with the membrane; (4) inhibitors of nucleic acid synthesis; 5) inhibitors of protein synthesis; (6) antifolic agents and inhibitors of respiration, inhibitors of metabolite uptake and miscellaneous inhibitors; and (7) resistance to antimicrobial agents.

In dealing with the different drugs there is, throughout the book, an emphasis on the compounds important for clinical use. In this respect, however, there are a number of important omissions, including colistin and gramicidin A (in the group of antibiotics acting on the membrane), streptovaricin and streptolydigin (inhibitors of bacterial RNA polymerase), daunomycin and adriamycin (intercalators between the base pairs of DNA), doxycicline, spiramycin and oleandomycin (inhibitors of bacterial protein synthesis) and emetine (inhibitor of eukaryotic protein synthesis).

Editing and presentation of the book are good. The list of references is reduced but moderately well selected. The book is very pleasant to read and data are presented carefully and clearly. It can be easily understood even by readers without a solid background in the subject. I particularly enjoyed the seventh chapter which deals with the problem of resistance to antimicrobial drugs. I strongly recommend this book as an introduction to the field for students of biology and medicine.

D. Vazquez

Kidney Hormones

Kidney Hormones. Edited by J. W. Fisher. Pp. xviii+665. (Academic: London and New York, March 1971.) £8.

Dr J. W. Fisher has edited a first-rate book on kidney hormones. I am puzzled, however, as to why these hormones, so disparate in function, are included in one volume. Perhaps because the enzyme renin is closely associated with erythropoietin in the juxtaglomerular cells, this is thought to be reason enough. And then the kidney is involved in the anaemia which accompanies chronic nephritis, and erythrocytosis is associated with some renal tumours. Nevertheless, like so many such conglomerates there are chapters whose relevance even to kidney hormones is questionable. Blood flow, oxygen utilization and distribution of blood flow are interesting and well exposed but it is not made explicit why they belong in a book on renal hormones.

The authors of the twenty-five sections are among the most competent in their fields, ensuring the quality of their judgment and writing. Two hundred and thirty-eight pages of the book are given to erythropoietin, 179 to angiotensin and 118 to prostaglandins, vasoactive renal lipids and kinin-forming enzymes. An interesting cross-over is provided by Gallagher's chapter on polycythaemia and erythropoietin production in hypertensive patients. Emphasis, however, is largely on the chemical and pharmacological aspects of the hormones, indicating that the book is written for basic scientists by excellent scientists: clinicians beware!

At a cost of £8 and the large amount of time required to read the book one wonders how many investigators will buy it or peruse it. Those of us who live by research in these fields are glad to have the review, even though most of the material is long familiar. The literature on erythropoietin is far less well known to most of us than is that on angiotensin and, therefore, the more welcome.

The editor could have profitably used his blue pencil to the discomfort of his authors in some of the chapters. For example, one wonders about the nearly two full pages in "Studies on the Renal Erythropoietic Factor (REF)" listing in detail the numbers of rats, rabbits, sheep, dogs, pigs, persons, carp, frogs and ducks ("five ducks, one white Peking duck") that were studied but neglecting to tell the reader the results!

It is not the prerogative of a reviewer to expound his own views on needs of investigators: nevertheless, I shall do so. Books being as expensive as they are and the volume of publication so great, the time is overdue for a thoughtful discussion of what constitutes an effective literature review. The range of opinion is still very wide, largely, I think, because it has not been adequately debated. It varies from authors who believe the editor has no more rights than to see to it his manuscript is published, to editors who believe each article must receive the most scrupulous revisions of fact, rhetoric and evaluation. Often to the surprise of both, the reader feels he has a stake in the discussion.

There is a place for short, readable overviews and for comprehensive ones as in *Physiological Review*. There is also room for that rare volume which digests and evaluates the vast literature of such a subject as angiotensin and presents it as a critical, structured review. But I am dubious of reviews which fall between. They are too complicated for the student and clinician, too elementary for the expert and too expensive for those who merely want to give prestige to their bookshelves.

I do not mean that this all applies to Dr Fisher's book but some parts of it might. For what it is, however, I liked the book.

IRVINE H. PAGE

Aldosterone

Aldosterone. By Edith Gláz and Paul Vecsei. (International Series of Monographs in Pure and Applied Biology, Vol. 6.) Pp. 626. (Pergamon: Oxford and New York, June 1971.) £10.00; $27.

THIS review by Gláz and Vecsei has been divided into two sections, bringing together the scientific and clinical aspects of aldosterone research. Vecsei begins his section with a chapter describing the significant publications which led to the suggestion of a salt retaining hormone and eventually to the isolation of aldosterone. For the historically minded this makes interesting reading. The second chapter is concerned with a detailed description of the various indirect and direct methods in the assay of aldosterone. A critical analysis is given in the third chapter of aldosterone metabolism, from its biosynthesis to its circulation in different body compartments and its removal from the body. A chapter is then devoted to the mode of action of aldosterone in various body tissues while the final chapter of this section is used to describe the mechanisms regulating the release of aldosterone.

The section written by Gláz begins by describing the disease of aldosteronism. A wealth of information is provided on the syndromes observed which is followed by an assessment of the diagnostic tests used in studying aldosteronism. Finally, several pages are devoted to the therapeutic measures used in alleviating aldosteronism. The seventh chapter contains sections describing the various diseases where the condition of secondary aldosteronism arises, for example, Bartter's syndrome; nephrotic syndrome; and also sections dealing with the rationale behind these diseases, for example, experimental renal hypertension; experimental neurogenic hypertension. Other forms of aldosteronism and hypoaldosteronism are then discussed in two short chapters. The final chapter concerns the drugs used to treat aldosteronism and their effectiveness, either singly or together, are described.

Although the book is generally well written, there are frequent errors of language. Often the definite article is missing, and phrases such as: "As for a long time . . ." page 134), "It is to be assumed that, thus, also . . ." (page 268) and even an incomplete sentence (page 242) tend to distract the reader. Several of the figures (for example Figs. 22, 28, 30 and 31) need fuller footnotes for them to be understood.

Far more serious is the criticism that in places the text is already out of date and in need of revision. For example, the concept of "adrenoglomerulotrophin" is no longer generally accepted (fifth chapter). Similarly, Gláz fails to state that in 1968 Kinson and Singer (*Endocrinology*, **83**, 1108; 1968) were able to demonstrate angiotensin stimulation of aldosterone secretion in the rat, but only in the sodium deficient state. Again, Vecsei's emphasis on paper chromatographic systems in aldosterone assay is too strong. Today many laboratories are using thin-layer chromatography and gas chromatography in their assay systems, therefore more information on these techniques would have been useful. Indeed, at present methods of protein binding and radio-immunoassay are becoming increasingly important in this field and the application of these procedures could have been discussed.

In spite of these criticisms I can only agree with the aims of the authors as expressed in the preface, that the review is "comprehensive and detailed". They have certainly maintained a "balanced standpoint" on many of the unresolved problems in aldosterone research. This volume is extremely well based and should prove very useful to both newcomers and those deeply involved in this area of interest.

EDWARD J. JOHNS

One Man's Nerves

The Autonomic Nervous System: Morphological, Comparative, Clinical and Surgical Aspects. By Joseph Pick. Pp. xv+483. (Lippincott: Philadelphia; distributed in Great Britain by Blackwell Scientific: Oxford, 1970.)

THIS posthumous work covers the personal research and reading of one man. The book is divided into four sections. The first, on fundamentals of the autonomic nervous system, is the largest part and covers the principles of the system, its development, central connexions and the histology of the autonomic neurones. The whole presentation is largely morphological but the author points out that the increasing complexity of the autonomic nervous system parallels the increasing environmental stresses imposed by the evolution from sea to land. One of the most valuable and interesting chapters is that on the history of the discovery of the autonomic nervous system, based acknowledgedly on the work by Sheehan.

In the second section the author covers the autonomic nervous system in animals ranging through the animal kingdom from *Amphioxus* to the monkey. Throughout these detailed chapters the aim is to show the relationship between the autonomic nervous system of these forms and the more complicated nervous system of man.

The remainder of the book, the third and fourth parts, is devoted to man, the third part being a morphology of the autonomic nervous system in man. This is profusely illustrated with a dissection of major nerve systems using the Zeiss operative microscope, all the fine dissections being checked histologically. Most of the regional autonomic dissections have been done on one cadaver but the microscopic pictures are beautifully reproduced. There is a particularly interesting account of the electron microscopy of the peripheral autonomic system and especially interesting is the electron microscopy of man's autonomic nervous system from foetal life through adolescence, maturity and senescence.

Mary Lorenc, the illustrator, has produced splendid line drawings, some of great simplicity yet serving to clarify the theory most admirably. The black-and-white illustrations are superb and the illustrations of the micro-dissection of plexuses in relation to levels is strikingly brought out in black-and-white with red for the vessels. In places, however, a striving for realism in some of the half-tone plates has obscured the aim of anatomical illustration to clarify and depict a relationship. There is an unusual but extremely effective series of illustrations, combining diagrams against photographic insets of the nerve trunks dissected. All in all, the work owes almost as much to the artist as to the author and of the 300 illustrations very few could be faulted.

The clinical section is exceptionally comprehensive and valuable and the detailed anatomy of many procedures is described and illustrated. Though there is a large section on denervation of the head and extremities by sympathetic ganglionectomy there is no mention of intracranial procedures such as nervus intermedius section or resection of the petrosal nerve, for example. The place of the author in the surgical world is shown by the distinguished surgeons who gave advice and the section on clinical surgery—White, Smithwick and LeRiche to mention a few.

The whole work is a superb reference book which is a "must" for the libraries and which many who work in this field will use as an essential work of reference for many years. It is to be hoped that in spite of the author's death further and up-dated editions will appear.

EDWARD HITCHCOCK

Pure Chemistry Library
SCIENCE COLLEGE
92 Upper Circular Road
CALCUTTA

CORRESPONDENCE

Rapid Publication

SIR,—Your editorial, "Publish and Be Damned a Second Time," is of especial interest because of the experience we had in the US space programme. The answer you arrived at on the question of newspaper reports *vis-à-vis* scientific journals was "publish quickly". This may be the only course for the journals.

In December 1962 the results of the Mariner spacecraft which had journeyed to Venus were ready for dissemination but the scientists involved made a strong case against a press conference until articles could be published in scientific journals. This meant delays of weeks or months and a rather haphazard way to release results from a number of related experiments.

An internal struggle in NASA with the public affairs people arguing for timely release of data (gathered at the taxpayers' expense) versus scientists who insisted on reporting first—and surely not *via* the news media—to the scientific community ensued.

A compromise was worked out using *Science* magazine as a base: press conferences such as the Mariner would be held on the publication day of the magazine. Letters would be submitted, stating the urgency of the matter, to *Science* on a Monday, prearranged immediate attention would be given the letter, publication of preliminary results accepted (or rejected) for that very week and a press conference could be arranged Thursday or Friday. The experimenter would state to the news media that the preliminary results to be presented had been submitted for publication. Acceptance for publication in these cases meant immediate publication, not acceptance today and actual publication months later. Additional later publication of refined results appeared often in other journals, including *Nature*. The experimenter afforded himself protection under this procedure with two *caveats*: publication and the word "preliminary".

As a result, the practice has become increasingly more liberal and, although there have been a few faint cries of "instant science", now the real time reporting of (preliminary) scientific results from space probes has become a practice. Viewers will see in the current Mariner 9 Mars probe real time interpretation of Mars television pictures and press conferences throughout the encounter phase without regard to publication. Publication will come later when careful analysis with all the data is available.

Yours faithfully,

JULIAN SCHEER

Elmwood,
R.D. 2, Box 24,
Catlett,
Virginia 22019

Announcements

University News

Dr D. L. Lee, Houghton Poultry Research Station, has been appointed professor of agricultural zoology and **Dr A. Care,** Rowett Research Institute, has been appointed professor and head of the Department of Animal Physiology and Nutrition, in the **University of Leeds.**

Dr G. Edsall, Harvard School of Public Health, has been appointed to the chair of microbiology, tenable at the London School of Hygiene and Tropical Medicine, and **Professor J. F. M. Middleton,** New York University, has been appointed to the chair of African anthropology, tenable at the School of Oriental and African Studies, **University of London.**

Dr David Evans, University of Sheffield, has been appointed to the new chair of computing in the Department of Mathematics, **Loughborough University of Technology.**

Professor R. H. Tuck has been elected dean of the Faculty of Agriculture and Food at the **University of Reading.**

Miscellaneous

Representatives of university departments and other qualified organizations and scholars are invited to apply for grants in aid of archaeological research to be carried out in the county of Somerset during 1972. The grants will be allocated from the **Maltwood Fund** by the Royal Society of Arts, and will total £1,000. Further information can be obtained from J. S. Skidmore, Royal Society of Arts, John Adam Street, Adelphi, London WC2N 6EZ.

Applications are invited by the **Lalor Foundation** for the 1972 postdoctoral awards, offered for research in certain aspects of mammalian reproductive physiology. This year preference will be given to work which is aimed at detection and exploration of dysgenic factors in ovum or foetal development, evaluation of the genetic factors involved and means towards their disposition. Research on non-traumatic cervical dilation and on various aspects of abortion will also be considered. Applicants may be of any nationality but should be under 41 years of age. The research may be undertaken at the applicant's own laboratory or elsewhere. Further information and forms of application can be obtained from the Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware 19805, USA. The closing date for applications is January 15, 1972, and awardees will be notified on or before March 15, 1972.

Errata

IN the article "Science, Statistics and Society" by A. W. F. Edwards (*Nature*, **233**, 17; 1971), the last sentence of the second paragraph under the subheading "Causation, Correlation, Classification", page 19, should read: "Even though this is all the information I possess about you, it enables me to make a statement about your height, thus: the probability that you are between 5 feet 8 inches and 5 feet 8.5 inches tall is 0.08, if I take as my datum the fact that Englishmen are normally distributed in height with a mean of 5 feet 8 inches and a standard deviation of 2.5 inches".

IN the article "Inhibiting Effect of Some Antimalarial Substances on Glucose-6-Phosphate Dehydrogenase" by D. W. K. Cotton and A. H. M. Sutorius (*Nature*, **233**, 197; 1971), the second figure in the first column of Table 1 (NADP 0.002 M in buffer (ml.)) should read 0.01. Moreover, the name of the second author is incorrectly spelt in the contents list.

IN the News and Views article entitled "Fooling the Experts" (*Nature*, **232**, 442; 1971), the implication that the Basel Museum has purchased or is in possession of forged Etruscan paintings on terracotta is incorrect. The plaques in question only passed through the museum for examination when their authenticity was already in doubt. The Munich plaques are in a private collection. The Berne plaque is in the possession of the Archaeology Seminar of the University of Berne. There are genuine Etruscan plaques in the British Museum, the Louvre and the Villa Giulia Museum in Rome.

British Diary

Monday, October 25

Sensation and Neural Code (5.30 p.m.) Professor H. Hensel, University of London, in the Edward Lewis Lecture Theatre, Middlesex Hospital Medical School, Mortimer Street, London W1.

Tuesday, October 26

Air Pollution: How Dark the Shroud? (1.20 p.m.) Professor T. J. Chandler, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Concepts of Structural Safety (5.30 p.m.) Dr K. O. Kemp, University of London, in the Engineering Theatre, University College London, Malet Place, London WC1.

The Chemical Engineer's Contribution to Air Pollution (7.30 p.m.) Mr C. J. Stairmand, Institution of Chemical Engineers, at the Unicorn Hotel, Prince Street, Bristol.

The Library, The Scientist and the Humanist (6 p.m.) Dr Robert Shackleton, University of London, in the Beveridge Hall, Senate House, London WC1.

The Wright Brothers: Inventors of the Aeroplane (1.30 p.m.) Mr C. H. Gibbs-Smith, University of London, at Queen Mary College, Mile End Road, London E1.

Treatment of Glass Surfaces for Composite Manufacture (6 p.m.) Dr B. E. Gillespie, Society of Chemical Industry, Plastics and Polymer Group, at 14 Belgrave Square, London SW1.

Wednesday, October 27

Discussion Meeting (5.30 p.m.) Society for Analytical Chemistry, Microchemical Methods Group, at Imperial College, London SW7.

Innovation in Industry—a Factor for Growth (6 p.m.) Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Relevance of Animal Feeding Trials to Human Dietary Requirements (6.15 p.m.) Dr K. L. Blaxter, Society of Chemical Industry, Food Group—Nutrition Panel, at 14 Belgrave Square, London SW1.

The Shape of Things to Come (5.30 p.m.) Professor E. R. Laithwaite, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Teacher and the Taught: The Changing Roles in the Progress of Science (1 p.m.) Dr Frank Greenaway, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Thursday, October 28

Is There a Future in Computers? (7.30 p.m.) Mr R. J. Redding, Institute of Measurement and Control, at Stanley Palace, Watergate Street, Chester.

Photography One Hundred Years Ago (5.30 p.m.) Mr Arthur T. Gill, Royal Institution, Library Circle, at 21 Albemarle Street, London W1.

Physics in the Service of the Engineer (5.30 p.m.) Mr L. A. Thomas, Institution of Electrical Engineers, at Savoy Place, London WC2.

Solid State Detectors (2.30 p.m. discussion meeting) Society for Analytical Chemistry, Radiochemical Methods Group, at the Laboratory of the Government Chemist, Stamford Street, London SE1.

The Problem of Addressing New Display Materials (6.30 p.m.) Mr A. Colchester, Institution of Electronic and Radio Engineers, at the University of Cambridge Engineering Laboratories, Trumpington Street, Cambridge.

Friday, October 29

Curved Boundary Problems and Diffusion Approximations (5.30 p.m.) Professor H. E. Daniels, University of London, in the Chemistry Auditorium, University College London, Gower Street, London WC1.

Rutherford: a Century of Nuclear Energy (9 p.m.) Dr T. E. Allibone, FRS, Royal Institution, at 21 Albemarle Street, London W1.

The Forth-Tay Estuaries—an Environmental Assessment (10 a.m. symposium) Royal Society of Edinburgh, at 24 George Street, Edinburgh.

Type II Photocleave Reactions in Aromatic Thioesters (1 p.m.) Dr J. Wirz, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Saturday, October 30

Dinosaurs—The Terrible Reptiles (3.30 p.m.) Mr W. T. Blows, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Monday, November 1

Modern Food and Food Additives (6.30 p.m.) Dr D. W. Kent-Jones, Society of Chemical Industry, Food Group, at the Scientific Societies Theatre, 23 Savile Row, London W1.

Some Aspects of Drying Oils Technology (7 p.m.) Mr G. Hutchinson, Oil and Colour Chemists' Association, at the Queens Hotel, George Street, Hull.

Tomorrow's World in Telecommunications (7 p.m.) Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A. & Canada
Nature (Friday)	**£14**	**$48**
Nature + Nature Physical Science	**£24**	**$83**
Nature + Nature New Biology	**£24**	**$83**
All three editions	**£29.50**	**$108**
Annual Index	**£1**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number : 01-836 6633. Telegrams : Phusis London WC2R 3LF
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number : 202-737 2355
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number : Basingstoke 29242
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone : 01-242 6264/01-405 4743
Telegrams : Textualist London WC2A 2ED
Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, October 22 1971

NATURE Volume 233
OCTOBER 29 1971

Should Reproductive Biology be Encouraged?

FERTILIZING human eggs in the test tube and using prostaglandins as an abortion pill are spectacular ideas which have captured attention recently. But in the past year or so there have been advances in almost all aspects of human reproductive biology, although this work has been more in the nature of pinpointing the areas which can most profitably be exploited than in providing any definite answers. In this context, it seems likely that virtually all scientists will regard as timely the plans of the World Health Organization to inject considerable funds into some aspects of research on human reproduction. (An account of the programme will be found in *Nature New Biology* next Wednesday.)

Reproductive biology is, inevitably, a controversial subject and the promise to some, a threat to others, that research will make a revolution in social as well as in scientific thinking is reflected in last week's attacks by two Nobel Laureates, Dr James Watson and Dr Max Perutz, on the work of Dr R. G. Edwards and his colleagues from Cambridge. There is every prospect that, if successful, Edwards's work will lead to the development of procedures to allow human eggs to be fertilized *in vitro* and the embryos then to be reimplanted at an early stage of their growth. But although this technique may be able to overcome some forms of infertility, Watson and Perutz argue that it involves very great and quite unjustified risks of producing a defective embryo.

But whether or not what seems likely to develop into a campaign to stop such work is successful, other changes in reproductive biology are almost certainly in train. It will not be surprising, for example, if recent developments in distinguishing sperm carrying a Y-chromosome from those bearing an X-chromosome should lead to a ready technique for separating sperm according to whether they determine a male or female child. Coupled with the use of artificial insemination this finding implies that it may become possible to choose the sex of progeny.

Not everyone will agree on the value of advances of this nature, but research on embryogenesis itself has in general produced results which can universally be applauded. Immunological studies of foeto-maternal relationships, for example, have already proved their use in helping rhesus-positive babies to survive in rhesus-negative mothers. Indeed, histo-incompatibility studies on foetal development may lead to a better understanding of the processes which may go wrong (of which there seem to be many) in earlier embryogenesis. Studies on the presence of antigens on sperm may also be fruitful.

Spermatogenesis and the ovulatory cycle are ill-defined by comparison and it is this area of research which will benefit most from the expanded programme by which WHO hopes to increase understanding of the processes of human reproduction. The aim of the programme is to develop safe and effective methods for regulating human fertility. Research is planned on a broad basis. One reason for this is that WHO must take account of social mores which may mean that particular methods of contraception may not be acceptable in some countries. Another is that it is at present difficult to decide just what will prove to be the best ways of improving physiological contraception.

The decision is a sensible one to concentrate research on the basic processes which lead to sperm formation, maturation and transport and on the release, fertilization, implantation and early development of the egg. Even the stages of the ovulatory cycle are not at present known in much detail and just a slight increase in knowledge could cast a valuable light, for example, on what are the dangers of the rhythm method of contraception. A large part of the research will concentrate on the biochemistry of steroid action on tissues of the female reproductive system which certainly seems to offer the best prospects for improving contraception in the short term; fears about present contraceptive pills may be eased and better ones may be developed. The programme is also strong in that there will be centres which will conduct clinical trials of any possible fertility regulating agents.

It is all to the good that WHO seems to have decided that it is not sufficient just to conduct research and then to let the results look after themselves. An integral part of the programme is to help disseminate to other scientists the results which emerge from research on reproductive biology. There seems to be less certainty over just how this is to be achieved than there is about the research to be promoted but scientists will hope that a decision is taken and put into effect soon; plans for WHO to establish a Documentation Centre will no doubt be watched as an example of how communication can be improved in specific research areas.

How can WHO best support this ambitious programme of research? About $500,000 per year is at present spent by WHO on research in various aspects of human reproduction and this programme will continue. The new programme, however, with a projected income for its first year of nearly $7 million is likely to have more impact. Funds for the programme are being gathered from many sources and scientists will be anxious to see how well the plans work to organize research on rather closely defined lines.

Because of its international nature, particular problems must face WHO in organizing this programme. One of WHO's weaknesses is at the same time a strength; to be successful, the programme must take into account the susceptibilities of the member states of WHO in general and the funding agencies in particular. This means that justice must be seen to be done in apportioning funds to research institutions in different countries. The first consideration of the programme must, of course, be to ensure that the research supported is of proper quality. But it is no use providing answers if no one is willing to listen to them. The strength of WHO's approach is that any successes of a programme organized on such a wide basis will be likely to have an equally wide influence. Those who would judge the programme, therefore, should be duly sympathetic if at times it seems that it will take longer than it might to come up with the answers.

Poor Report for the Post Office

POSTS and telecommunications are no more suited to being part of the same enterprise than are ocean liners and aircraft. Yet history has lumped them together and organizations like the British Post Office must make the best of it. The best is none too good, as the report of the Post Office's first full year as a public corporation shows (*Post Office Report and Accounts, 1970–71,* HMSO, £1.50). So great were the losses on the postal services that the astonishing profits of £93.5 million on the telecommunications side left the corporation with a total profit of only £20.5 million, the lowest turned in since 1964–65.

To be sure, the seven-week strike of early 1971 hurt—all considered, it cost the corporation about £13 million—but the bulk of the postal loss of nearly £73 million was owed simply to the facts that it costs a great deal of money to hire people to carry messages by hand and that the public demands that its messages be carried cheaply. Mr Bill Ryland, the Post Office's new chairman, has said that increased postal charges are inevitable and the choice must be made between substantial price increases or lower quality service. He refused to be pessimistic about the future of the postal services while taking comfort from the health of the telecommunications service. But it is hard to share his pleasure in the telecommunications performance. What is so depressing about the corporation's first full report is the utter lack of promise of a sophisticated telephone system for Britain in the near future. While the vast profits from telecommunications are a great solace to the Post Office, they have not done much for the telephone subscriber. In the past year, the waiting list for new telephones has grown from 108,000 to 121,000, while the quality of the subscriber trunk dialling (STD) service deteriorated. The Post Office figures, always more conservative than those of the ordinary frustrated telephone user, concede that 8.7 per cent of calls failed because of faults in the system and calls made through the operator were also slightly more troublesome. In 1970–71 during the day it took the operator nearly 6.5 seconds to answer—the poorest record since 1966–67.

The Post Office admittedly is embarked on an extensive programme to expand and modernize the telephone service, and it is now spending about £500 million a year to make up for the mistakes—the Treasury's as well as its own—of the 1950s and early 1960s. Yet the report does not have cheerful things to say about the progress in modernizing telephone exchanges which are the bottleneck to any drastic improvements of the present system. Because so much existing equipment is of the old fashioned step-by-step Strowger variety, the Post Office had to spend £5.5 million in 1970–71 to buy more of the same, to keep the system running. Moreover, its independence as a corporation has not helped it find a solution to its chronic problem of the late delivery of equipment by the British telecommunications industry. In fact, in the past year, fewer orders were filled than the year before, and the average delay on exchange contracts was thirteen months, with the newer, more desirable crossbar exchange equipment even further behind schedule than the Strowger.

Perhaps the most worrying statements of the Post Office's report are "the relative cheapness of the telephone is creating a very heavy demand which is increasingly difficult to meet with present resources." "Waiting lists are bound to increase and service deteriorate until prices can be adjusted." What irks the Post Office is that while telephone calls are profitable, the average return on the rental of a telephone set is not. To put this right it would like to raise the rental—and drive some of those prospective customers away. The Post Office has not yet learned to concentrate on the golden eggs and forget the cost of buying the geese.

It is worrying that the Post Office misinterprets the demand for telephones. The waiting list refuses to diminish—not because telephone rentals are cheap but because the public is recognizing that telephone calls are cheaper and often superior (because they are instantaneous and two-way) as a form of communication than the letter. This recognition could be one of the Post Office's most valuable assets, if it chose to make it so. Because it is saddled with running both a postal service and a telephone system, why does it not argue publicly the advantages of using one over the other? British telephones are under-used and telephone calls on which the Post Office made so much profit last year were made from instruments which are used, on the national average, only twice a day. For that matter, the Post Office should be putting more effort into making cheap, throwaway telephones, and at least, insisting that telephone wires and outlets be installed in all new buildings.

The most essential task for the Post Office is to recognize that it is in the telephone service, not the moribund post, where the real emergency lies.

100 Years Ago

THE Inaugural Meeting of the Newcastle College of Physical Science on Tuesday last week was a great success. The Council decided unanimously, on the application of upwards of seventy ladies, to make no distinction of sex in the admission of pupils, placing all on a footing of exact equality. The total number of students admitted up to the time of the inaugural ceremonial, was fifty-one. In contrast to this we may note that last week the governing body of the University of Edinburgh rejected by a small majority Dr. Alexander Wood's motion, "That, in the opinion of this Council, the University authorities have, by published resolutions, induced women to commence the study of medicine at the University; that these women, having prosecuted their studies to a certain length, are prevented from completing them for want of adequate provision being made for their instruction; that this Council, without again producing any opinion on the advisability of women studying medicine, do represent to the University Court, that, after what the Senatus and Court have already done, they are at least bound in honour and justice to render it possible for those women who have already commenced their studies to complete them."

From Nature, 5, 13, *November* 2, 1871

OLD WORLD

RESEARCH AND DEVELOPMENT

Constructive Advice

THE Institution of Professional Civil Servants feels that the British government should provide increased support for its industrial establishments so that Britain can compete on equal terms with other European countries. This advice is given by the IPCS to the Department of Trade and Industry as a contribution to the DTI's review of industrial research establishments (*The Role of the Government's Industrial Research Establishments in the 1970s*, IPCS).

The IPCS stresses that the advantages of such reviews are nullified if they are held too often and the institution feels that this has been the case recently—several establishments are now being reviewed for the third time in ten years. This almost continuous series of reviews coupled with changes that have placed some establishments under the aegis of four different departments in the same period has, in the view of the IPCS, led to uncertainty about jobs and prospects and a loss of morale.

The establishments now under review are the Laboratory of the Government Chemist, the National Engineering Laboratory, the National Physical Laboratory, the Safety in Mines Research Establishment and the Warren Springs Laboratory together with the United Kingdom Atomic Energy Authority establishments at Harwell and Risley. The IPCS sees a scope for increasing the work of these establishments, in particular by an increase in their contract work for individual companies. For this to be achieved, however, it will be necessary to increase the powers of the directors of the establishments so that they can exercise commercial judgment and develop the work as they see fit.

In his introduction to the report, Mr William McCall, general secretary of the IPCS, is possibly previewing Lord Rothschild's review of civil science when he calls for public research and development establishments to contribute more to the industrial and economic well-being of Britain. He also re-states the institution's view that these establishments should not diversify but should continue their work in basic long-term research.

The report comes out strongly against the idea that these establishments should pay their own way and it criticizes modern innovation theory, which holds that all industrial research should be self-sufficient. To substantiate this position Bell Telephone Laboratories Inc. is cited as a large company that has failed to keep its research and development self-sufficient. A plea is also made for more research and development resources to be made available to industry to supplement its own efforts and the IPCS points out that the industrial research establishments could well fit this bill.

The central theme of the report is that the present support of research and development by the government is biased towards defence, aerospace, electronics and nuclear power whereas industries that contribute most to the balance of payments—the mechanical and engineering industries for example—have been sadly neglected. The institution says that this imbalance should be corrected by providing more support for these industries at government establishments, provided however, that this is accompanied by a corresponding increase in research and development effort within the industry itself.

Perhaps the most important plea made by the IPCS is for increased support for industrial research and development so that Britain will not be handicapped in competition with other countries, in particular members of the European Economic Community. While emphasizing that support should not be increased merely because other countries are supporting both civil and military technology to a greater degree than Britain, the institution feels that British industry would be severely handicapped if there were no increase in support for industrial research and development.

EDUCATION

A-level Broadening

MORE schoolchildren are opting for A-level courses involving both science and other subjects, according to a report of the Department of Education and Science (*Statistics of Education*, 1, HMSO; £1.70). At the beginning of 1970 31.0 per cent of the 254,000 A-level pupils in schools in England and Wales were following courses comprising science subjects alone and a further 50.9 per cent were studying non-scientific courses; 18.1 per cent of the courses, however, contained both science and non-science subjects. In January 1969, the proportions were 31.0 per cent, 52.1 per cent and 16.9 per cent respectively.

During 1969 the total number of pupils of all ages rose by 2.4 per cent to 8.6 million, of which about 8 million were in maintained schools. The total teaching strength increased by 3.3 per cent and the pupil–teacher ratio dropped from 27.7 to 27.4 in maintained primary schools and from 17.9 to 17.8 in maintained secondary schools while in direct grant schools the ratio is 15.5.

The proportion of 15-year-old children continuing their school careers still shows large regional variations, but the figure for the north region, 46.9 per cent on January 1, 1970, compared with 44.9 per cent a year before, reflects a larger increase than that for the south-east region (63.8 per cent compared with 62.5 per cent). In 1966, 53.2 per cent of 15-year-old pupils in the south-east region stayed on at school beyond the statutory leaving age.

Reliving the Past

OVERHEARD at a reception at 21 Albemarle Street, London, last week: "I've not visited this place before; it's rather marvellous, isn't it?" Not such an extraordinary remark in the normal course of talk at a social gathering, but the speaker and the occasion gave it special meaning. With true British feeling for the past, the 172-year-old Royal Institution of Great Britain stepped back in history on October 20 and for three hours became once more the assembly rooms of London society. It had thought to ask to a party (no doubt with an eye, somewhat disguised, on some generous benefactions) as many descendants as could be traced of its founders, early proprietors and officers to meet some of the present members, to hear something of its history and functions today and to see its collection of archives and historic scientific apparatus.

Because so many distinguished and wealthy people directly associated themselves with the Royal Institution in its early days and supported it with subscriptions, it is not surprising that the list of descendants at the party read like an extract from *Debrett's Peerage* (although, of course, it is just these people who can most easily be traced). Few of the guests were practising scientists, although among them was the engineer Sir Richard Young, a descendant of Thomas Young who was one of the institution's first professors. The Percy family have long had an honourable connexion with the Royal Institution—the fourth, sixth, seventh and eighth Dukes of Northumberland were all presidents as was Lord Eustace Percy—and it was therefore fitting that the guest of honour was the tenth Duke of Northumberland, at present chairman of the Medical Research Council.

RESEARCH AND DEVELOPMENT

Summer Lightning

RESEARCH has begun into the possibility of providing Britain with an electricity transmission system of up to one and a half million volts. Since August, scientists at the Central Electricity Research Laboratories (CERL), using a gantry forty metres high and a Ferranti impulse generator capable of an open circuit output of 5.2 million volts, have been working on the basic problems of such a system.

The CEGB's new 400 kV supergrid is not yet fully commissioned but CERL has started work on ultra high voltage (uhv) because the time required to make a system operational means that research must be many years ahead of possible application. So far, each time a higher voltage network has been added, the voltage has roughly doubled —from 132 to 275 kV in 1950 and from 275 to 400 kV in 1960—but it has been suggested that the next step ought to be a trebling rather than a doubling of present levels—to 1,300 kV rather than 800 kV—because of the capital cost involved. It is also unlikely that demand will exceed the capacity of a 1,300 kV network in the foreseeable future.

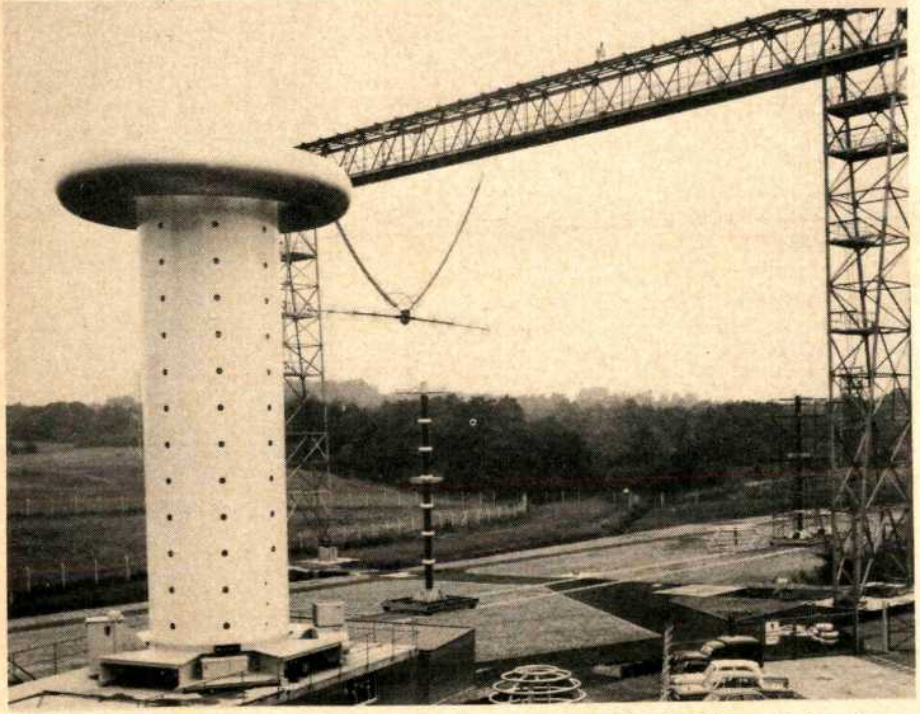
Work on the basic data for an 800 kV system has already been completed and CERL is at present trying to find insulators capable of resisting lightning strikes and the surges resulting from switching in a uhv system. With higher voltages there are ever increasing difficulties with insulation and it has been suggested that uhv transmission will prove impossible; at CERL, however, they are confident it can be done and hopeful that it will prove possible to carry uhv on a single circuit eight conductor bundle on towers no higher than those used at present.

The porcelain insulators at present under test have withstood simulated lightning strikes of up to 5 million volts without breakdown but switching surges, which can be expected to reach two and a half times the nominal rating of a transmission system, are tending to break down at about 2.25 million volts.

The facilities, sited at Leatherhead on the edge of the Surrey green belt, are the world's first completely all-weather uhv test facilities; they will be used in close cooperation with the CEGB's Brighton site where work will shortly begin on the other main problem of transmission, the reduction in insulation efficiency due to pollution. Salt in the atmosphere is the worst enemy, and the Brighton site, right on the coast, is ideal for studying this.

The capital cost of the Leatherhead and Brighton sites is about £500,000. Cost was one of the two main factors that resulted in outdoor sites—to put only the Leatherhead facilities indoors would have cost another £2 million. The other factor was the firm belief of Dr John Forrest, director of CERL, that a uhv grid will finally have to work outdoors so it might as well start that way. The power frequency tests, which need to be done indoors, can be accommodated later in the existing laboratories.

It is in fact doubtful if a grid of more than a million volts will ever be built. If the predicted 5.5 per cent per annum load growth rate for the later 1970s continues, then the present 400 kV network will be good for the next 20 years. Whether a 1,300 kV network (the likeliest voltage to be adopted over one million volts) will then be built will depend on the nature of power transmission by the 1990s; centralization resulting in fewer, bigger generating plants might well demand it, but a change in public attitudes to the siting of nuclear power stations near large towns, for example, would make it unnecessary. Whatever happens, uhv will not replace but only supplement the 400 kV network, and it is likely to be used only for the large-scale, long-distance transmissions from, say, the Midlands to London.



The CERL facilities at Leatherhead showing the Ferranti 5.2 million volt impulse generator and the test gantry that permits possible tower designs to be examined, and substation plant to be tested alongside. Eight conductors in a metre bundle are suspended from the gantry on the porcelain insulator strings.

SOVIET SCIENCE

Industrial Research

THE recent "Scientists for Industry" seminar held in Rostov-on-Don on October 13–15, 1971, has again brought to the fore the apparent lack of contact between research and development and industrial application in the Soviet Union. The Rostov seminar was devoted to problems of expanding research in technical colleges, in accordance with the dictates of industry, but background material published in the Russian press suggests the existence of a more urgent problem than was dealt with at the Rostov meeting.

Writing in Pravda (No. 278, 1971), Academician Aleksandr Ishlinskii, chairman of the All-Union Council of the Scientific-Technical Society of the USSR, stresses the need for the development of research and development laboratories within factories that could provide one of the chief links in technological progress. Unfortunately, in many establishments, they are poorly equipped outbuildings, unsuitable for serious research and many factory directors consider them to be second-class departments. Although specialists working in these laboratories are entitled to a similar salary to that of scientists in pure research institutes, very few directors are willing to implement such payments. Accordingly, there is a serious leakage of scientists from industry, particularly in the highly qualified classes.

A closer cooperation between science and industry was one of the principal resolutions of the Twenty-Fourth Party Congress held earlier this year, and the factory laboratory has often been commended as an excellent means of implementing this cooperation. But, without more attention and funds, they may act as a deterrent to cooperation.

SCIENTIFIC INFORMATION

One Step Nearer

First plans to help keep scientists and engineers in touch on a world-wide basis with the latest published work in their field have been approved at a UNESCO conference.

At a meeting in Paris earlier this month delegates from eighty-three countries and thirty-nine international organizations approved the recommendations made in October of last year to establish a World Science Information System, known as UNISIST (see *Nature,* **230**, 544 ; 1971).

The intention behind UNISIST is that it will help overcome the "information explosion" which has resulted in about two million scientific and technical articles being published each year in 70,000 specialized journals ; it will also make the work already published more easily available. UNISIST is the joint brainchild of UNESCO and the International Council of Scientific Unions, and its protagonists claim that its advantages are, first, that it is a world-wide organization that involves both governments and scientific organizations and, second, that unlike earlier plans for world-wide systems it is comparatively flexible, coordinating existing networks rather than establishing a new central organization. The chief problems that UNISIST faces are how to interconnect existing systems of information retrieval, whether conventional or computerized, and how to solve the problems of classification.

The recommendations that the conference discussed—and passed with only minor modifications—were the work of the UNESCO-ICSU committee that was created in January 1967, and final approval of the scheme now rests with the UNESCO general conference which meets in the autumn of 1972.

After the conference the chairman, Dr Harrison Brown of the United States National Academy of Sciences, said that there was "strong east–west cooperation" in the preparation for UNISIST. The French government is to establish a centre for an International Serials Data System, and Poland is providing an international training centre to help meet the shortage of information specialists. At present UNISIST is confined to science and technology, but it is hoped to extend it to cover the social sciences and humanities if the system proves successful.

A recommendation that allowance be made for UNISIST in the 1973–74 budget of UNESCO was passed to the director general, and although no figure was named, £750,000 to £1 million as recommended in October 1970, is thought to be a likely figure.

Whether UNISIST will ever function efficiently remains to be seen and doubts have been expressed as to whether UNESCO is the best body to run such an organization. The problem of accommodating the views and interests of all the countries involved is tending to produce more talk than action, and it has been suggested that intensification of the interchange of information between existing organizations might in the end prove more effective than the more ambitious UNESCO plan.

ARCHAEOLOGY

Old Boats, New Ideas

from a Correspondent

Boats excavated in Britain and dating from before the Norman conquest were discussed at an international symposium held earlier this month at the National Maritime Museum, Greenwich. The Ferriby boats (1600 BC to 800 BC) are the oldest yet found in northern Europe and were described by their excavator, Mr Edward Wright, who admitted that, apart from the last of the three excavations in 1963, the rescues had been archaeological disasters. The exception, Ferriby 3, is still being treated at Hull museum.

Although the ages of the Ferriby boats are quite different, they were found within a hundred yards of each other in the Humber foreshore and have unique common structural features. They are flat-bottomed with a hinged plank structure stiffened by poles pushed through cleats. Mr Wright suggested that these boats were relics from a prehistoric boatyard that had been located at the same site for centuries.

The rescue of the Ferriby boats has proved to be a cautionary tale. This time last year another ancient boat rescue was attempted, but under very different circumstances from Mr Wright's pioneering efforts. The preconquest Graveney boat was raised in seventy-three parts from a Kent marsh by a joint task force from the National Maritime Museum and the British Museum, and has since been the subject of a meticulous scientific examination.

The wealth of information now emerging from this painstaking work was sketched by the archaeological director of the project, Valerie Fenwick of the British Museum. Much is being learnt about trade contacts with Europe in the ninth century AD, the importance of the sea route as a highway between London and Canterbury, the relevance of the local saltworks to the battering which the boat received and so on. Several intriguing constructional details have also been deciphered ; it seems, for example, that the boat was built right side up in the open. Such information could not have been obtained without the most exhaustive study of the boat's timbers, involving the drawing of every mark on both sides of each fragment—a nine-month task.

One question raised at the symposium was how the Greenwich unit should deploy its still slim resources (see *Nature,* **233**, 226 ; 1971). Should it, as proposed by Mr Wright, deliberately excavate selected sites such as the sixth century BC Brigg raft still buried in a Lincolnshire brickyard or should it prepare itself for the next rescue? Ancient boats often emerge during land developments in Britain and, typically, have to be abandoned for lack of recording power and conservation facilities. Within weeks of the Graveney operation, for example, two boats of almost equal interest were found during cofferdam works in the Thames near the Mermaid Theatre. Although part of one of them was recovered, much valuable information was lost.

MUSEUMS

Keeping in Touch

History that is barely history is displayed in the Science Museum's new telecommunications gallery opened this week by Christopher Chataway, Minister for Posts and Telecommunications. The new gallery covers the development of electrical communication from the early nineteenth century to the days of colour television and a glimpse into the future is also shown.

The gallery, with an area of 10,000 square feet, provides about twice the area previously available for this collection, and is laid out on either side of a central avenue with the side bays going into particular aspects of the subject in greater detail. The north side of the gallery deals largely with line communications, the south side with radio, television and radar.

Among the more interesting exhibits are Cooke and Wheatstone's needle telegraph instruments, examples of the first transatlantic cables, and the equipment used in the Daventry test to prove the feasibility of radar in 1935, which is accompanied by a tape recording of an account of the experiment by A. F. Wilkins, who assisted Watson-Watts with the test. What is believed to be the only surviving example of the world's first centimetric radar equipment is included in the exhibition, as is the first commercially marketed video recorder.

Among the recent developments on show are a video cassette and a brief description of the possibilities of wave guides and optical fibres for carrying telecommunications signals.

NEW WORLD

New AEC Broom Begins to Sweep

by our Washington Correspondent

THE nuclear power industry is by now used to accusations that it is abusing the natural environment and endangering the health of countless Americans. Mere accusations that its quality control and safety standards are not all they might be would, in normal circumstances, scarcely merit a single raised eyebrow, but last week these accusations caused considerable consternation in the nuclear industry, for they were made by James R. Schlesinger, the new chairman of the Atomic Energy Commission. And, to rub salt in the industry's wounds, Schlesinger added that the nuclear companies need not look to the AEC to fight their battles, for the commission's most important job will be to protect the interests of the public.

These remarks were made not to an appreciative audience of environmentalists, but to representatives of the nuclear industry itself who listened with growing alarm to what Dr Schlesinger had to say in his first public speech. The remarks have been widely interpreted as marking a fundamental shift in the AEC's policies, but what they mean in effect is that the Atomic Energy Commission is fully prepared to stand by the new regulatory procedures set up in response to the appeals court decision on the Calvert Cliffs reactor (see *Nature*, **233**, 85; 1971). In a sense, Dr Schlesinger will be seeking during the next few years to build up public confidence in the AEC's power to regulate the nuclear industry, and in so doing, he is likely to help the cause of nuclear power much more effectively than by siding with the nuclear industry.

The burden of Dr Schlesinger's message was that the nuclear industry has now grown past the stage at which it needs to be nurtured by the AEC, and that it ought now to be capable of standing on its own feet. "From its inception, the Atomic Energy Commission has fostered and protected the nuclear industry," he said. "Looking back, one can, I think, say that this was the right policy for that historical epoch . . . but that industry should now be on a self-sustaining basis. Those of you who regard the response to Calvert Cliffs as indicating a climatic change in the relationship between the industry and the AEC could well be right, though perhaps for the wrong reason. The move toward greater self-reliance for the industry had a certain historic inevitability."

The AEC has been consistently criticized in the past for allowing nuclear power companies to get away with substandard engineering and in consequence the purchasing utilities have bought plants which are unduly susceptible to breakdown and poor performance. But in future, if Dr Schlesinger lives up to the promises made in his speech, the AEC will tighten its control considerably over important but unglamorous engineering details. "The AEC's role is . . . primarily to serve the public interest," Dr Schlesinger told his audience, and "those engineering details are the heart of the problem". The AEC will need to be assured that piping is of the highest quality, that pumps work and that valves are properly designed. "Those in industry who have chided us —quite properly—about the AEC regulatory process know full well that you have reason to blush regarding some of these aspects of quality assurance," Dr Schlesinger told his audience.

But the AEC will not expect to fight all the battles for the purchasing utilities for ever, for Dr Schlesinger told them they should acquire the technical expertise and trained personnel to make sure that they receive good value for their money from the builders of power plants. If the utilities acquire this expertise, it will help "to avoid power disruptions, to insure that plants can and will be properly maintained and, among other things, to avoid relying on the Atomic Energy Commission to perform this critical task".

Much of what Dr Schlesinger had to say was a bitter pill for his audience to swallow, and the nuclear power industry could perhaps be excused for choking on some of his suggestions, if only because they are reminiscent of many of the charges levelled at the industry by some of its more vocal opponents. Indeed, Dr Schlesinger had more than a good word to say for the environmentalists who have pitted their armour against the industry in the past. "A number have bad manners," he said, "but I believe that broadside diatribes against environmentalists to be not only in bad taste but wrong. I believe that we shall receive from responsible environmentalists considerable assistance in resolving our present difficulties." Dr Schlesinger refused even to be drawn into the argument over whether or not society ought to curb its appetite for nuclear power, saying that "it would be inappropriate for the Atomic Energy Commission to take a position on this issue".

In a sense, the AEC has already demonstrated to the nuclear industry its new role, for in spite of great industrial pressure to appeal against the Calvert Cliffs decision, the AEC chose instead to amend its regulations. Dr Schlesinger pointed out that the new regulations present no insuperable difficulties, and "while they are tough, they are workable". He chided the nuclear industry for advising the AEC to evade the court mandate, saying "it is not the AEC's responsibility to ignore on your behalf an indication of Congressional intent". There is a clear option open to the industry if it believes the new regulations to be too harsh, and that is through the courts. There will evidently be nothing to be gained by complaining to the AEC itself.

Finally, Dr Schlesinger set about dismantling a popular misconception. "It is not the responsibility of the AEC to supply power," he said, and he went on to infer that those who have criticized the AEC for fostering the demand for electricity would do better levelling their complaints at the utilities themselves. "The AEC does not sell power reactors. We are a bystander, sympathetic I trust. The selling of power reactors is a concern of the vendors: the decision to buy that of the utilities."

Set against remarks made by Dr Glenn Seaborg, Dr Schlesinger's predecessor as chairman of the AEC, Dr Schlesinger's speech marks a change of attitude, if not of policy. In a speech to the International Platform Association, in July, for example, Dr Seaborg had little hesitation in jumping into the debate about society's need for power, and he spent much of his speech extolling the virtues of nuclear power as opposed to other forms of energy.

Why did Dr Schlesinger choose to hit out so strongly and controversially in his first public pronouncements as head of the AEC? Perhaps one answer lies in his statement that "it is to industry's long run advantage that the public has high confidence that the AEC will appropriately perform its role".

SPACE

Martian Accord

by our Washington Correspondent

THE expected simultaneous arrival in orbit around Mars of NASA's Mariner-9 and the USSR's Mars 2 and 3 probes next month will set the stage for a far-reaching agreement on space matters between the United States and the Soviet Union. The fruit of a series of meetings in Moscow in August, the agreement provides for exchange of information between NASA and the Academy of Sciences of the USSR almost as soon as the information is relayed back to Earth from the Mars spacecraft.

This exchange of information will, however, be merely the first tangible evidence of a number of agreements hammered out between NASA and the Academy of Sciences of the USSR in recent months. The agreements, the terms of which have recently been made public by NASA, provide for cooperation on space activities ranging from planetary exploration to monitoring the Earth's environment by satellite.

The exchange of Mars data will allow particularly interesting phenomena to be studied by the spacecraft of both countries, and it will also allow some areas of Mars to be studied simultaneously by two probes. The idea is that experimental data will be relayed between NASA and the Academy of Sciences of the USSR by telex, by giving the nature, time and location in agreed Mars coordinates of phenomena such as changes in the colour of the Martian surface, or movements in the atmosphere. It is hoped that this exchange of information will be followed by a bilateral discussion of the preliminary results of the missions in March next year, and by a deeper review of the results a year later.

If this agreement is successfully carried out, it could pave the way for implementation of the other agreements reached in Moscow last August, and it could also help the negotiations aimed at development of compatible docking systems on US and USSR spacecraft (see *Nature*, **233**, 160; 1971). The fount from which this new accord springs is a meeting on space cooperation, held jointly by NASA and the Academy of Sciences of the USSR in January this year, as a result of which a number of joint working groups were set to work on areas thought to be particularly ripe for cooperative efforts. Recommendations from three of these working groups have recently been approved by NASA, and they provide for a variety of means for sharing information, and even for joint planning of some projects.

As far as exploration of near Earth space is concerned, one working group recommended that joint seminars be held whenever the need arises, to analyse data and coordinate programmes. On a slightly more practical note, the working group also suggested that a study be made of the possibility of transmitting magnetometer data from a USSR ground-based station, to the proposed US ATS-F geostationary satellite. The idea is that these data would then be relayed back to Earth with data from an on-board magnetometer and used for correlating magnetic variations in space with those on the ground.

The working group also recommends the holding of bilateral discussions on the results, objectives and strategy for planetary exploration and, in addition to sharing information on the Mariner and Mars 2 and 3 probes, that by the end of the year, the USSR will provide NASA with 700 time-of-flight data points for Venus, in exchange for about 900 post-1965 data points on Mars. On lunar exploration, representatives of the USSR will attend the Lunar Science Conference in Houston in January, and the working group has suggested that a lunar conference be held in the USSR in February or March 1973 so that future lunar exploration can be discussed. The agreement also provides for the exchange of photographs and maps of the lunar surface, and finally, the working group recommends that the two organizations continue to discuss the "possibilities of complementary lunar exploration activities".

To work towards a closer accord on environmental monitoring, NASA and the Academy of Sciences of the USSR have agreed to conduct remote sensing of designated sites in the US and the USSR either by satellite or by aircraft, and to exchange the results in the form of technical reports. The agreement also provides for exchange of infrared temperature measurements taken over the oceans, so that these measurements can be compared with actual measurements taken on board ships, and both organizations have agreed to work towards cooperation on programmes designed to monitor sea surface roughness, ocean biological productivity and sea ice conditions.

Finally, on meteorology, NASA and the USSR Academy of Sciences have agreed to exchange technical notes on temperature sounding by satellite, and to organize a joint experiment on temperature sounding over Western Europe for 1973–74. One NASA official said last week that the working group recommendations have all been accepted in principle by NASA and the Academy of Sciences of the USSR, but the practical details still need to be sorted out. What happens in orbit around Mars could therefore be important to future cooperation on all kinds of space activities.

COMSAT

For Services Rendered

by our Washington Correspondent

THE Communications Satellite Corporation (Comsat) has been undercharged to the tune of $6.1 million by the US government for the launching of communications satellites between 1965 and 1969, according to an investigation carried out by the General Accounting Office. The GAO recommends that $31,000 should be recovered from Comsat, and that the basis on which the company should be charged for all the Intelsat IV launches (including the first launch last January) should be changed so that all the costs are included. NASA and the Air Force (which launches satellites for Comsat) are in general agreement, but the Department of State and Comsat itself do not see eye to eye with the GAO's recommendations.

Chief reasons for the undercharging were deficiencies in the accounting methods used by the Air Force and an agreement between NASA and the Department of Defense to the effect that Comsat would not be charged for research and development associated with the launches. The upshot was that Comsat entered into an agreement with NASA which left the US Government paying for both the depreciation and the research and development associated with the launching of Intelsat satellites. The GAO report suggests that the basis on which Comsat was charged for the launching of Intelsat I, II and III cannot now be changed, but the company was not charged for at least $31,000 worth of services covered by the agreement. The GAO believes that this amount should be recovered, and that the agreement to launch Intelsat IV satellites should be renegotiated to include full launch costs.

The issue is complicated for several reasons. First, the Air Force, which launches the satellites, charges NASA, which in turn charge Comsat for the launch and support services. The basis for these bills is set out in agreements between NASA and Comsat, but the Intelsat IV agreement includes a clause to the effect that changes in the Air Force charges may be renegotiated. On the other hand, Comsat is the managing company for the International Telecommunications Satellite Consortium—a group of 80 nations interested in telecommunications satellites — which has had some severe internal problems during its first few years of existence.

The Department of State is therefore chary of causing an international dispute by an unseemly wrangle over $31,000.

The GAO argues that special services provided by the US government should be charged at their full cost, and that Comsat is now a viable commercial concern which ought to pay its full share (it recently reported a net income for the first quarter of 1971 of $4,978,000). A Comsat official said last week that the GAO report is being considered by the company, and pointed out that $31,000 should be compared with the $30 million it costs to launch each satellite. Comsat would, however, resist any attempt to change the basis for charges incurred by the launching of Intelsat IV satellites.

TUBERCULOSIS

Ribicoff lashes CDC

by our Washington Correspondent

SENATOR Abraham Ribicoff has, in the past few weeks, shown little respect for the agencies which he regulated when he was Secretary of Health, Education and Welfare in 1961–2. Two weeks ago, he lashed the NIH's Division of Biologics Standards for allegedly relying on dubious techniques for testing influenza vaccines, and last week the Food and Drug Administration and the Center for Disease Control were the subjects of his wrath for the part they played in the mass TB immunology programme on Capitol Hill last year, during which two people died of hepatitis. Senator Ribicoff's chief complaint is that the Center for Disease Control, which supervised the programme, gave 300 mg single doses of isoniazid—the drug commonly used for treatment and prevention of tuberculosis, instead of the usual treatment of three separate 100 mg tablets.

Based on an investigation conducted by the General Accounting Office, Senator Ribicoff's allegations were made in a stinging speech delivered in the Senate. He said that in 1964 the Center for Disease Control asked for permission to start investigational use of a 300 mg tablet of isoniazid. Under FDA rules, the centre should have supplied information derived from animal tests, and an outline of the planned investigation. "CDC supplied virtually none of this information to the FDA," Ribicoff claimed, "Instead, the CDC doctors started an accelerated experimental programme on a wide range of human subjects." Part of this experimental programme included the mass treatment of Capitol Hill employees last year after some cafeteria staff had contracted tuberculosis.

A spokesman for the FDA last week, however, although admitting that the agency "did not push the CDC for its experimental results", suggested that the CDC's action in the Capitol Hill programme simply followed accepted medical practice. He also pointed out that the two people who died from hepatitis during the programme showed symptoms of the disease eight months after they began taking the drug. Nevertheless, the latest bulletin of the FDA points out that it has recently changed the labelling on isoniazid to the effect that the drug should not be used if hepatic disease is suspected, and that the patient should be closely monitored. Alongside the announcement, however, the FDA bulletin contains an editorial by Gordon M. Meade, formerly medical director of the American Thoracic Society, who points out that "isoniazid remains a powerful and very effective antituberculosis agent which merits continued therapeutic and prophylactic use even though liver dysfunction can be associated with its use".

Short Notes

by our Washington Correspondent

Fort Worth

A CONFERENCE committee set up to decide the future of the Public Health Services Hospitals in general, and the drug rehabilitation centres at Lexington in Kentucky and Fort Worth in Texas in particular, reached deadlock on October 7. The Department of Health, Education and Welfare, frustrated in its plans to transfer the Fort Worth facility to the Bureau of Prisons, seized its chance and ordered the facility closed and all patients sent back for treatment in their home communities within twenty-four hours. The result: Congress was presented with a *fait accompli,* and, according to Mr James C. Wright, Texas representative in the House of Representatives, at least half of the ninety-two addicts discharged were taking drugs again before the weekend was finished.

The trouble was that in its desire to transfer the facility to the Bureau of Prisons, the Department of Health, Education and Welfare overlooked the fact that October 8 was a Friday. Consequently, many of the addicts could not be reached by representatives of their local rehabilitation centres for nearly three days, and not surprisingly many returned to the habit—none had been under treatment for more than four months. Mr Wright, who set his committee investigators to look into the fate of the addicts, found one pushing drugs in New Orleans; another smashed up a car and was jailed for speeding and was later found to be high on drugs. Mr Wright described HEW's actions as "a flagrant breach of good faith with Congress". He said, "I was stunned that a Federal agency would stoop to using drug victims as pawns in a power play to thwart the will of Congress."

Science in School

FIGURES released this week by the National Science Foundation show that the number of schoolchildren in public schools opting for science subjects increased by a factor of 2.5 between 1948–49 and 1969–70—faster than total enrolment which for grades 9 to 12 increased 2.3 times over the same period. "The steady proportional growth of enrolments in such basic core courses as introductory algebra, introductory geometry, biology, and chemistry indicates a basically healthy state of science interest at the high school level," the NSF claims. The figures released, however, unfortunately include no discussion of recent trends which may reflect the growing disillusionment with science and technology in the present state of concern about the environment, and the NSF's optimism is therefore difficult to question. One fact which mars the optimism is that enrolments in physics have lagged dramatically during the past two decades—in 1949, 28 per cent of the 12th grade were enrolled in physics courses, but in 1970 the percentage had dropped to 18.

Table 1 Enrolment in Selected Science and Mathematical Courses in Grades 9 through 12 in US Public Secondary Schools as a Percentage of Total Enrolment in the Grade in which the Course is Usually Offered

Course	Grade in which usually offered		Per cent	
		1948–49	1960–61	1969–70
Natural sciences				
Biology	10	71	85	96
Chemistry	11	33	39	39
Physics	12	28	23	18
Selected social sciences:				
Economies	12	25	17	32
Sociology	12	18	17	19
Psychology	12	5	8	13
Mathematics:				
Introductory algebra	9	64	66	75
Introductory geometry	10	40	45	46
Adv. mathematics	11–12	27	32	31

Source: National Science Foundation adapted from Office of Education data.

NEWS AND VIEWS

Cholinergic Receptor Sites in Muscle

THE transfer of excitation from nerves to muscle fibres is the most thoroughly studied example of chemical transmission in the nervous system. A great deal is now known of the mechanisms responsible for the synthesis, storage, release and subsequent metabolism of the transmitter substance, acetylcholine, which is involved in the transmission of impulses at neuromuscular junctions. Until recently, much less was known of the post-junctional receptor mechanisms which are activated by acetylcholine to cause excitation of the muscle fibres. The exploitation of the extraordinary properties of the substance α-bungarotoxin, however, seems likely to lead to rapid progress in understanding the nature of such receptors.

Bungarotoxin is a protein, of molecular weight 8,000, which can be isolated from the venom of the Formosan snake *Bungarus multicinctus*. The protein seems to be the affinity label *par excellence* for acetylcholine receptors in vertebrate muscle, and in analogous structures such as the electric organs of electric fish and eels. The snake seems to possess a "receptor probe" molecule with near ideal properties, because bungarotoxin seems to bind with extremely high affinity and almost complete specificity to muscle and electric organ cholinergic receptor sites.

By using bungarotoxin labelled with radioactive iodine-131, it has already proved possible to perform preliminary studies towards the isolation and purification of cholinergic receptor molecules from electric organ tissue (Miledi, Molinoff and Potter, *Nature*, **229**, 554; 1971). In this issue of *Nature* (page 599), Miledi and Potter describe the application of bungarotoxin to studies of cholinergic receptors in amphibian and mammalian muscles. The extensive pharmacological studies of Chang and his colleagues in the past decade had shown that bungarotoxin is a powerful cholinergic antagonist in vertebrate muscles. Miledi and Potter confirmed that the exposure of frog or rat nerve-muscle preparations to low concentrations of bungarotoxin (1 μg/ml.) abolished neuromuscular transmission within a few minutes. The action of the toxin was irreversible, because no recovery of transmission or responses to applied acetylcholine occurred even after washing such preparations for up to eight days.

When nerve-muscle preparations were exposed to ^{131}I-labelled bungarotoxin, some label became firmly bound to the muscles; this binding was saturable and reached plateau levels within about two hours. If the binding of bungarotoxin is specific for cholinergic receptors, it should occur only on those areas of the muscle surface which underlie nerve terminals, because it is well known that non-innervated regions of muscle are virtually insensitive to acetylcholine. In diaphragm muscle, the innervated regions of muscle can be fairly simply dissected from non-innervated regions, because the neuromuscular junctions of adjacent muscle fibres are quite precisely aligned in such preparations. When such dissection was performed it was found that more than 90 per cent of the bound labelled bungarotoxin was present in the innervated region of muscle, which represented only one sixth of the muscle weight. This high degree of localization of toxin binding to innervated regions of muscle speaks strongly in favour of the specificity of such binding to receptor sites.

Assuming that labelled bungarotoxin does bind with such specificity, then the amount of toxin bound under saturating conditions can be used to estimate the number of cholinergic receptor sites in various muscles. Using this approach Miledi and Potter obtain values of 10^9 sites per neuromuscular junction in frog sartorius muscles, and between 1 and 4×10^7 sites per junction in mouse and rat diaphragm muscles. The dimensions of the area of muscle membrane underlying nerve terminals are known from previous studies so that the density of receptor sites in frog sartorius junctions can be estimated as approximately 100,000 per square micron of membrane. The recent neurophysiological studies of Katz and Miledi (*Nature*, **226**, 962; 1970) suggest that the depolarization of the muscle membrane which occurs in response to nerve stimulation is composed of many virtually simultaneous "single shot" activations of cholinergic receptors. Katz and Miledi have estimated that each individual receptor activation or "single shot" event gives rise to a depolarization of 0.22 μV amplitude; to cause the overall depolarization observed in response to a single nerve impulse would require approximately 600,000 such events in the frog sartorius muscle. Even if as many as ten receptor molecules were involved in each "single shot" event, the calculated receptor population at frog sartorius junctions of 10^9 suggests that only a minute proportion of these receptors are activated by each nerve impulse.

The examination of bungarotoxin-^{131}I binding in muscles which had been surgically denervated has also yielded important information on the changes in cholinergic receptors which occur in such preparations. Previous studies by Miledi (*J. Physiol.*, **151**, 1; 1960) and by Axelsson and Thesleff (*J. Physiol.*, **147**, 178; 1959) have shown that a curious spread of acetylcholine sensitivity occurs in muscles which have lost their normal innervation. In normal muscles only the region of membrane directly underlying the neuromuscular junctions responds sensitively to applied acetylcholine; denervation is followed by a gradual spread of this area of acetylcholine sensitivity radially over the muscle membrane, so that after several weeks the entire surface of the muscle membrane may become highly sensitive to externally applied transmitter.

Using labelled toxin, Miledi and Potter have now confirmed that a large increase in toxin binding sites occurs after surgical denervation of muscles; increases of up to twenty times the normal binding were observed three weeks after denervation. Furthermore, by dissection of innervated and non-innervated regions of diaphragm muscles it was found that toxin binding sites became uniformly distributed along the muscle fibres in the denervated preparations. Regions of muscle which are not normally innervated showed increases of up to 200-fold in their ability to bind bungarotoxin after denervation. When denervation was effected by crushing the motor nerves, damage to the muscle innervation is quite rapidly reversible, as the nerves will regenerate to re-innervate the muscle fibres within a few weeks. When such re-innervation occurs the changes in acetylcholine sensitivity of denervated muscles are reversed, and the preparations revert to their previous state of precisely localized acetylcholine sensitivity. Miledi and Potter have found that the spread of toxin binding sites is also reversed when denervated muscle preparations become re-innervated after nerve crush—showing again that the behaviour of bungarotoxin exactly parallels the inferences previously drawn about cholinergic receptor sites from neurophysiological evidence.

A curious and still mysterious aspect of the results reported by Miledi and Potter concerns the differences which seem to exist between the binding of bungarotoxin and curare to muscle receptors. Curare has for long been known as the classical antagonist drug for muscle acetylcholine receptors. Small concentrations of *d*-tubocurarine completely abolish neuromuscular transmission and cause total block in responses to applied acetylcholine. When curare was used in the presence of labelled bungarotoxin, however, it prevented the binding of only 50 per cent of the toxin, even when high concentrations of *d*-tubocurarine were used. The suggestion that some 50 per cent of toxin binding might be unspecific seems to be ruled out by the precise localization of toxin binding sites at neuromuscular junctions. It would seem therefore that curare can interact with only half of the receptor sites present in muscle; the nature of the difference between the curare-sensitive and insensitive sites remains obscure.

The results obtained by Miledi and Potter throw some light on the phenomenon of receptor desensitization. Previous studies have established that prolonged exposure of muscle preparation to high concentrations of acetylcholine leads to a long lasting decrease in the ability of the muscles to respond to acetylcholine. Such desensitization could be attributable to a variety of causes. The receptor mechanism, for example, might retain the ability to bind acetylcholine, but failure might occur in the subsequent events leading to increased membrane permeability to ions. Miledi and Potter have now shown, however, that after prolonged exposure to acetylcholine the binding of labelled bungarotoxin is reduced by as much as 80 per cent. This suggests that the desensitization phenomenon involves some persistent change in the conformation of the receptor molecules.

Finally, Miledi and Potter report that the use of labelled bungarotoxin may be applied to muscle receptor isolation, as previously reported for the electric organ. Labelled toxin can be used to follow the isolation and purification of the toxin-binding substance. Preliminary experiments show that the labelled material can be solubilized from muscle preparations by the use of 'Triton X-100', and that the solubilized material can be further disaggregated by sodium dodecyl-lauryl sulphate.

EVOLUTION

Natural Selection and Industrial Pollution

from our Population Genetics Correspondent

ONE of the problems for many species of animals and plants is how to adapt to man's rapidly changing industrial environment. Until about 1800, man can have had only a slight effect on the environments of most species. Organisms evolved in response to the evolutionary changes of others and the generally slow changes of the physical environment. There was usually time for even the slowest breeders to adapt themselves to new conditions. But in the past few hundred years many species have become extinct and many more are threatened. This is a direct effect of man's activities.

An animal can adapt itself to a new environment provided the change is not so drastic that a large proportion of the population is eliminated by selection and not replaced by reproduction. Genetic variations in fitness are also necessary, of course, if fitness is to increase in the new environment. For an animal with a long generation time, the environmental change, if it is very rapid, may have gone so far in a single generation that the population cannot adapt to it. But an organism that produces many generations in the same period will easily be able to adapt to the relatively slight changes that take place in each of its brief generations. This is the problem with the use of pesticides. The pests are often organisms such as insects which produce a new generation every few weeks and they can usually adapt to the increasing quantities of insecticides the farmers throw at them. But the larger predators, such as birds of prey with their long generation times, are in danger of being completely eliminated. When predators are eliminated, their prey, if they are highly resistant to the pesticide, may increase rapidly and produce population explosions because there may be nothing but disease and lack of food to check them. The biological environment thus becomes very unstable and greater and greater use of an increasing variety of pesticides is necessary to control the pests.

Pesticides are merely the most drastic of the selective agents produced by man. Many other selective agents are produced unconsciously as a byproduct of the industrial society. The evolution of industrial melanism in various moths is a well known example of selection favouring a dark variety of the species which will escape the attentions of predators in industrial environments. The selective forces in favour of the melanic are quite strong—of the order of a 50 per cent advantage to the melanic form. The value of smoke abatement is demonstrated by the almost immediate decline in the frequency of the melanics where smoke control zones are introduced (Clarke and Sheppard, *Proc. Roy. Soc.*, B, **165**, 424; 1966).

Many other species of insect show examples of melanism, for example, the two-spot ladybird (*Adalia bipunctata*). The melanics, which are black with red spots, are found in high frequency in industrial areas. But the melanism cannot have evolved by selection by predators, for ladybirds are poisonous and are rejected as food. Presumably the melanics are simply more tolerant of the toxic elements in industrial pollution. Creed (in *Technological Injury*, edited by J. Rose; Gordon and Breach, 1969) certainly found a very close correlation between the frequency of melanic ladybirds and smoke concentration. In the latest issue of *Evolution* (**25**, 290; 1971) he describes the results of a survey of the frequencies of melanic ladybirds in various parts of Birmingham and compares them with their frequencies of a few years before.

In some areas of the city, the frequency dropped by as much as 50 per cent in four years. On average, from 1962 to 1967, the frequency of the black ladybirds dropped from about 45 per cent to 25 per cent. In the same period, as a result of the introduction of smoke control zones the level of smoke pollution fell by 40 per cent. There is no direct proof that selection for black ladybirds is caused by smoke pollution, but the circumstantial evidence is very strong.

Assuming that smoke abatement is responsible for the decline in the melanic ladybirds, it is possible to work out from Creed's figures the selection against the melanics in the less polluted environment of Birmingham. If about eight generations have passed since the introduction of smoke control zones, the selective coefficient against the melanics comes out at about 0.12. This means that the melanics suffer a mortality of 12 per cent over and above that of the normal, non-melanic ladybird in the new environment.

Although these selective forces are much smaller than those produced by pesticides, it is important to remember that they are the selective forces involved in the change back to a more natural environment. The original selection for melanism may have been much more drastic. Ladybirds can obviously adapt themselves to these industrial changes without much difficulty. Birds and mammals may not find it so easy. If the partial abatement of one aspect of industrial pollution can have such selective effects in thus raising the fitness of the normal red form of the ladybird, there can be no doubt of the benefit of the abatement of pollution in general in improving the natural environment. The leading article in the issue of *Nature* for October 15 (**233**, 437 ; 1971) made the point that pesticides have brought great benefits to man, as well as a certain amount of harm to the environments of many species: on balance "DDT may be good for people". Smoke and other avoidable effluents seem to offer no worthwhile benefits in compensation.

ANIMAL BREEDING

Keeping Fossils Alive

from a Correspondent

For ten years an *ad hoc* group of biologists has laboured to gain support for a scheme that would systematically conserve breeds of farm animals that are in danger of extinction. Several breeds of cattle, sheep and poultry were kept in a "gene bank" at Whipsnade (*Nature*, **202**, 131 ; 1964) but these had to be dispersed when the Zoological Society of London needed the space.

In spite of lack of funds, some of the livestock were taken over by the Royal Agricultural Society of England at its National Agricultural Centre, Kenilworth. The organizing group became a working party under the auspices of this society, and it organized a conference on rare breed survival on October 15 to draw attention to the problem.

Mr W. Longrigg (Chief Livestock Adviser, MAFF) outlined the decline of breeds, which has chiefly been caused by changes in demand. Belatedly, geneticists are beginning to realize that the scientific approach to animal production should not be governed by current profitability, but by the need to maintain variability for future selection.

But economic value is only one aspect of the case for preservation which was presented by Dr P. A. Jewell (University College, London). He pointed out that every breed, like a wild species, represents unique genetic material of importance in archaeological, historical and scientific research as well as being a living museum of educational value. Once lost, a breed cannot be re-created. Dr Jewell described work in which bones from surviving primitive breeds such as the Soay sheep had been used in archaeological comparisons, and outlined research on the blood types of cattle in which primitive survivors had widened the range of study. He pointed out that Darwin used observations on domestic animals in his theory of evolution.

Dr M. L. Ryder (Animal Breeding Research Organization, Edinburgh) showed how survivals of sheep from prehistory, the Middle Ages and the nineteenth century had enabled a study of their development, and particularly of fleece evolution, to be carried out.

Professor J. C. Bowman (University of Reading) gave the results of a survey carried out in 1970 to ascertain the exact status of breeds. It was found that some well known sheep breeds had declined alarmingly to numbers lower than other breeds already designated "rare", for example, the Wensleydale (260) and Wiltshire (400) compared with the "rare" white-faced woodlands (700). He considered that it should be possible to keep a breed viable with as few as fifty individuals, provided the ratio of males to females was kept much higher than in normal husbandry. But two breeds of pigs (Tamworth and middle white) had fewer than the critical number of ten males.

The papers created considerable discussion and comment and it was decided to establish a society for the preservation of rare breeds, the functions of which would include the registering of animals, exchange of stock and technical advice. Further information can be gained from the Royal Agricultural Society of England.

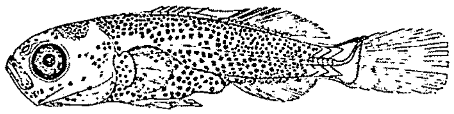
GOBIES

Two Species or One ?

from our Marine Vertebrate Correspondent

The diminutive goby *Lebetus* has attained some degree of distinction among European fish as the smallest vertebrate in the fauna, a claim which has even ensured it a place in the *Guinness Book of Records* (although parts of the entry are sadly erroneous). Now, however, its interest is reinforced by a remarkable uncertainty as to the number of species within European waters.

Neclâ Demir and F. S. Russell have reported recently (*J. Mar. Biol. Ass. UK*, **51**, 669 ; 1971) that two forms of *Lebetus* postlarvae were distinguished in the collections of young fish at the Plymouth Laboratory. These two types of postlarvae show certain differences in the concentration of pigment cells, and also show quite distinct differences in fin development at comparable sizes. Thus in one form the dorsal and anal rays are well defined when the young fish is 5.0–5.5 mm long but are only just noticeable in the other form at this length. Pelvic fin development also shows similar differences, and even more significant are the numbers of vertebrae, 25–26 in one form, 27–29 in the other.



A drawing of one of the largest specimens of the Fage form of *Lebetus* postlarvae, 8.1 mm long.

The interest in this discovery, paradoxically, is that it confirms what was discovered by two other workers almost half a century before. In 1919 the Danish marine biologist C. Petersen identified the postlarvae of a goby as *Lebetus scorpioides*, and a year before L. Fage had found postlarvae of the other known species, *L. orca*. Both species had been described from Scandinavian waters in 1874 by Collett. Later work by A. V. Tåning on *Lebetus* from Iceland and Faroes confirmed that the postlarvae of the two named species could be distinguished in several ways, notably in their length at development of certain features.

It was thus widely accepted that there were two species of *Lebetus* in European waters, an assumption which was demolished by P. Miller in 1961 (*Nature*, **192**, 675 ; 1961) who postulated that the two named species were sexually dimorphic forms of the same species. His hypothesis was exhaustively reinforced in a later paper (*Bull. Brit. Mus.* (*Nat. Hist.*), **10**, 205 ; 1963), in which he showed that, having examined more than eighty specimens of full grown *Lebetus*, including the types of both species, and dissected much of the material, he could find only male specimens of *L. orca*, and immature male and female *L. scorpioides*. This seemed to prove a classic case of sexual dimorphism in *Lebetus* (and other gobies are known to be sexually dimorphic), and Miller's conclusion has been generally accepted.

The work of Demir and Russell, however, has shown that *Lebetus* cannot be dismissed as a monotypic genus, for in addition to the morphological differences they have demonstrated biological differences. Collections made in 1970 of young fish in the western English Channel revealed forty-two "Petersen type postlarvae" and more than four hundred of the "Fage type", and it was established that the Fage type were more abundant between February and August, whereas the former were found from June to September only. The 1970 survey also showed that Petersen postlarvae were most common inshore; the other form was caught only at offshore stations.

It now seems therefore that there are two *Lebetus* species in the European Atlantic. If Miller's view of *L. orca* as a synonym of *L. scorpioides* (the only two named forms in the genus) is correct, then clearly one of the forms described from postlarvae by Demir and Russell is so far unrecognized and unnamed as an adult. On the other hand, it may be that both *L. orca* and *L. scorpioides* are good species, and if so it is to the credit of the earlier workers, Collett, Petersen and Fage, that their observations have been supported by this recent work on the postlarvae of *Lebetus*.

PLANT PATHOLOGY

Host-Parasite Contact

from a Correspondent

THE need for studies, on very short time scales, of the reactions arising from host-parasite contact was repeatedly stressed during a symposium on fungal pathogenicity and the plant's response, held at Long Ashton Research Station, University of Bristol, from September 22 to 24.

In his inaugural lecture, Professor R. K. S. Wood (Imperial College, London) emphasized the importance and specificity of the hypersensitive response (using the term in its botanical context). Originally applied to the death of cells in an incompatible relation between an obligate pathogen and a resistant host, it is now clear that the general concept may be more widely applicable. Of particular interest in this context was the demonstration by Drs A. Kaars Sijpesteijn and A. van Dijkman (TNO Biochemical Department, Utrecht) of the effect of two near-isogenic races of *Cladosporium fulvum* in causing leakage from cells of two tomato cultivars, which showed complementary host-parasite reactions. Other contributions were devoted to the nature of the trigger system which initiates the biochemical and structural reactions that occur when a plant is challenged by a potential pathogen. The complexity of compounds implicated in disease resistance makes it difficult to resolve the sequence of cause and effect.

The intensive studies during the past 30 years on the role of ethylene were reviewed by Dr E. C. Hislop (Long Ashton Research Station) who drew attention to the possible implications of ethylene in altering the rate of disease development, and Dr Barbara Lund (Food Research Institute, Norwich) demonstrated that bacterial enzymes probably activate the production of ethylene by a route already present in normal tissue.

By original definition, phytoalexins are absent in the unchallenged host plant but the symposium suggested no such clear-cut distinction between preformed and induced resistance factors.

Because obligate pathogens cannot be grown in axenic culture, it is particularly difficult to examine the pathogen component of the host-pathogen system. The use of tissue culture has been of some value but, as shown by Dr D. S. Ingram and Dr Inez Tommerup (University of Cambridge) with *Peronospora farinosa* on sugar beet callus tissue, it is not necessarily typical of the system *in vivo*. Electron microscopy has been increasingly used to study the structure of the host pathogen interfaces, where interchanges of nutrients, regulatory compounds, proteins, and perhaps even genetic material must occur. The participants were impressed by the elegance of such ultrastructural studies seen in the illustrations of Professor P. H. Williams (University of Wisconsin), Professor C. E. Bracker (Purdue University) and Dr L. J. Littlefield (North Dakota State University), Professor Williams showing how, in cabbage clubroot, the bullet-like "stachel" from encysted zoospores penetrate in a matter of seconds, but minutes are apparently required for the host to respond.

In his closing remarks, Professor P. W. Brian (University of Cambridge) considered that the present era of plant pathology has the same advancing frontiers as molecular biology and plant biochemistry. The study of host-pathogen interactions has not so far aided the chemical control of plant disease, but the symposium illustrated the delicacy of the balance of such interactions, better knowledge of which may lead to new ways of combating the pathogen.

Mutating the Rous Sarcoma Provirus

FURTHER evidence, if any were needed, that the replication of RNA tumour viruses involves a DNA replicative intermediate, the provirus, is reported by Bader and Brown in next Wednesday's *Nature New Biology*. These authors have exploited the reverse transcription of Rous sarcoma virus genomes to obtain mutants of this virus which are temperature sensitive for transformation. They argued that if chick cells are exposed to 5-bromodeoxyuridine, the mutagenic analogue of deoxythymidine, when they are infected with Rous sarcoma virus some BUdR should be incorporated into the DNA provirus as it is synthesized, presumably by the reverse transcriptase in the infecting virus particles. Furthermore, when the DNA proviruses containing BUdR residues in place of some of their thymidine residues are in turn transcribed to yield progeny RNA genomes, guanine for adenine base substitutions should be induced and some mutant progeny Rous genomes would be expected.

Bader and Brown found that if chick cells are simultaneously infected with Rous sarcoma virus and exposed to BUdR, but not if the exposure to BUdR is 12 hours before or 12 hours after infection, temperature sensitive progeny virus can indeed be isolated from the transformed cells some 24 hours after infection. Such progeny virus stocks transform chick cells more efficiently at 36° C than 40.5° C, whereas the converse is true of wild type Rous sarcoma virus. This observation immediately suggested that many of the progeny virus particles liberated from the cells exposed to BUdR contain temperature sensitive lesions in the gene or genes responsible for transformation.

To prove this point, Bader and Brown isolated the progeny virus produced by 119 clones of cells transformed in the presence of BUdR and found that of these 20 yielded mutant virus temperature sensitive for transformation. Because BUdR is not metabolized into RNA by chick cells this result must mean at the very least that that part of the Rous sarcoma genome which determines the ability to cause the transformation of chick fibroblasts must be replicated by way of a DNA intermediate. Moreover, because cells transformed by these mutants at their permissive temperature and then shifted to the nonpermissive temperature continue to produce progeny virus, the temperature sensitive lesion cannot be interfering with the replication and packaging of the mutant genomes.

LIPIDS

Protomembranes

from our Molecular Biology Correspondent

IT seems that the study of hydrocarbons will for some time continue to provide a comfortable livelihood for many a physical chemist in the guise of a molecular biologist. The possibilities in liposomes are still far from exhausted, and McConnell especially has shown how much structural and dynamic information can be extracted from his spin-label experiments on such systems.

Cadenhead and Katti (*Biochim. Biophys. Acta,* **241**, 709; 1971) have tried to step on the cake by showing that a molecule carrying a large and ugly spin-label group will not necessarily have the same physical properties as the virgin form. There is no complete counter to this criticism, though it can be argued that inasmuch as spin-labelled lipids can be biosynthetically incorporated in membranes, they seem to be at least compatible, if not identical in behaviour, with normal lipids. Moreover the results of other methods, including NMR and X-ray diffraction, seem to be converging on much the same conclusions. Cadenhead and Katti, however, have looked into the effects of oxyloxazolidine-substituted cholesterol-like steroids on the surface properties of films, in which they are mixed with myristic acid. The polar spin-label group, they find, changes the effect on the surface isotherms considerably, compared with the parent compound; they suggest that this will generally be the case and recommend caution in visiting the results of spin-labelled onto unlabelled systems.

At the same time Birdsall *et al.* (*ibid.*, 693) offer support for the conclusions of Hubbell and McConnell concerning configurational freedom of lipid chains in bilayers. Instead of introducing the relatively large and polar spin-label, they have prepared a series of stearic acids containing a fluorine atom at various positions in the paraffin chain. These derivatives were then mixed with lecithin and dispersed in water as vesicles. NMR signals from the ^{19}F nucleus revealed that the line-widths changed drastically with the distance of the fluorine from the head group, indicating a large increase in freedom of motion going from the outside to the centre of the bilayer. This is by now a heavily laboured conclusion. It may be hoped, however, that a more complete analysis, including an estimate of exchange line-broadening effects, will make possible a quantitative treatment, so as to allow quantitative comparison with the results of other methods.

The most interesting current development in the application of NMR to the study of such systems comes from the Moscow group (Bystrov *et al., Chem. Phys. Lipids,* **6**, 345; 1971), who have examined the effect of paramagnetic perturbants on the proton magnetic resonance spectra of lecithin vesicles. When vesicles are prepared in water (or actually D_2O) containing manganous ions, there is a spectacular broadening effect, primarily of the signal from the quaternary amine methyl hydrogens, because these are in contact with the aqueous medium on the inside and outside of the vesicle. The ions do not penetrate the bilayer, however, so that when the vesicles are made in water and the paramagnetic ions are only afterwards added, a quite different effect is observed: superimposed on the shallow broadened background is a sharp peak of diminished magnitude. The polar heads on the inner surface are not in contact with the ions, and thus give rise to the sharp unperturbed resonance, whereas those on the outer surface generate only the broadened pedestal. When such preparations are sonicated, the residual sharp line from the internal head groups vanishes into the broadened background. An equally spectacular effect is observed with europium ions, which elicit a large contact-shift in the head-group resonance, so that when they are added to preformed vesicles, two sharp signals, one from the internal, the other from the external head groups, are observed.

Bystrov *et al.* also prepared micelles of lecithin in benzene, and found that these could be made to take up water. Again the introduction of the paramagnetic perturbent perturbed only the head groups exposed to water on the inner surface. These experiments offer good promise for the separate observation of events at different surfaces both of models and of real cell membranes.

A review by Kuhn and Möbius (*Angew. Chem.*, **10**, 620; 1971) of their remarkable work on monolayer systems, and the use of excitation transfer to measure critical distances, will give workers in the biological membrane field much food for rumination. The scope of this work takes in many areas of chemistry, but some examples of biological interest have been included in the survey, in particular the measurement of membrane thickness by introducing the membrane between emitter and sensitive layers and measuring the efficiency of excitation transfer and the construction to order of various arrangements of lipid and protein monolayers.

Rescuing Mouse Sarcoma Virus

IN next Wednesday's *Nature New Biology,* Klement, Nicolson and Huebner report that they have rescued mouse sarcoma virus particles from rat cells which although transformed by this virus do not support its replication until they are exposed to appropriate concentrations of 5-bromodeoxyuridine (BUdR).

This discovery, which follows hard on the heels of those of Rowe's group (*Science,* **174**, 155; 1971) and Aaronson, Todaro and Scolnick (*ibid.*, 157), who have used BUdR and IUdR to rescue respectively mouse leukaemia virus from non-producing AKR strain mouse embryo cells and a C-type virus, of which the oncogenic potential has yet to be tested, from normal BALB/C 3T3 fibroblasts, is a powerful shot in the arm for the devotees of the oncogene hypothesis of malignant transformation. And of course the induction with BUdR of IUdR technique provides a new approach to the search for other mammalian, including human, RNA tumour viruses.

The experiments of the three groups, which are essentially identical, indicate that the drugs BUdR and IUdR, for reasons that are far from clear at present, stimulate the active replication of dormant RNA tumour virus genomes in rat and mouse cells. The virus which Rowe's group have activated in embryonic cells of the AKR strain, a high leukaemia strain, seems to be identical to that isolatable from adult AKR mice with the disease. The virus which Todaro's group have activated in 3T3 cells has all the vital statistics of a C-type RNA tumour virus and it will be fascinating to learn whether or not it is indeed oncogenic. And Huebner's group believe that the rescue of mouse sarcoma virus from transformed but non-producing rat cells is most probably a consequence of the activation of a dormant rat "leukaemia" virus which can provide the helper function required for the replication of mouse sarcoma virus and normally provided by mouse leukaemia virus.

The avian oncogenic virologists, and Weiss in particular, have, of course, already shown that a C-type virus, probably an avian leukosis virus, can be activated in the cells of domestic chicks and wild jungle fowl by various chemicals including carcinogens, and so the betting must be that all vertebrate cells have the genetic potential to specify C-type viruses at least some of which are oncogenic. And that is what Huebner and Todaro's oncogene hypothesis predicts; perhaps all cells do indeed carry genes which can render them malignant.

ESTUARIES

Inaugural Meeting

from a Correspondent

THE inaugural meeting of the Estuarine and Brackish Water Biological Association was held at the Zoological Society of London on October 13. After the formal founding of the association by Dr D. S. Ranwell (NERC Coastal Ecology Research Station), it was announced that the steering committee would act as the first council and that the constitution would be approved at the first annual general meeting in April 1972. Some participants suggested that the word "biological" should be deleted from the name of the association, because the association plans to encourage an interdisciplinary approach to studies of estuaries. Mr R. Bassindale (University of Bristol) serves as the first president and Dr R. S. K. Barnes (Central Electricity Research Laboratories, Fawley, Southampton) is the secretary, from whom full details of membership may be obtained.

A symposium on the estuarine environment was held during the remainder of the meeting. The symposium was opened by Professor W. D. P. Stewart (University of Dundee), who reviewed the sites of estuaries in the United Kingdom and estuarine research projects which are in progress. Professor Stewart stressed the importance of estuaries in relation to man's activities and the need to develop management policies and a strategy for research.

Professor L. C. Beadle (University of Newcastle upon Tyne) discussed physiological adjustments shown by organisms in relation to extreme fluctuations of ionic conditions which may occur in estuaries. He stressed the need for information on the time which organisms take to adjust to salinity stress, how this affects growth rates and the extent to which changes in ionic fluxes may be used as indicators of salinity stress before more adverse effects of stress on organisms are apparent.

Mr A. J. Phillips (Central Electricity Research Laboratories, Fawley) stressed that although there is a considerable body of scattered information on the chemistry of estuaries, there is a lack of guiding principles. He also emphasized the differences in the chemistry of sea water and river water, not only in relation to ionic activities but also in relation to different ionic species present in the two types of solution. He suggested that the amounts of trace elements present in sea water and estuarine water may be underestimated by the use of some methods, because a fraction of these elements is bound to particulate matter carried in suspension. The particulate matter tends to flocculate in the presence of high salinities.

The last speaker in the morning, Dr K. R. Dyer (University of Southampton), spoke on sedimentation in estuaries. Salinity levels are extremely important, besides topography, current flow and volume of water, in determining rates of sedimentation. Various degrees of mixing may occur between river and sea waters, resulting in the formation of completely stratified to completely mixed bodies of water. The principal minerals in sediments are illite, montmorillonite and kaolinite which have different fall velocities that in part are dependent on temperature and salinity. A mean sedimentation rate for estuaries in Britain is approximately 0.2 cm per annum. Under some conditions, fluid muds are present close to the bottom of estuaries which during ebb and flow tides may give rise to peaks in turbidity.

In the afternoon, Dr R. L. Jefferies (University of East Anglia) reviewed the research on salt marshes. He emphasized the high net productivity of marshes and suggested that plants growing under high salinities are often perennial, show vegetative reproduction and have a limited but rapid growth period. He also discussed the possibility of assaying adaptive enzymes such as nitrate reductase in plants in an attempt to measure the level of nitrate-nitrogen available for plant growth in enriched estuaries.

Dr P. R. Walne (Ministry of Agriculture Shellfish Laboratories) spoke on the importance of estuaries to commercial fisheries. He concentrated on the shellfish industry which has an annual turnover of £0.5 million in Britain and examined methods for improving yield such as the moving of shellfish or encouraging sprat to settle.

Dr H. Milne (University of Aberdeen) examined the trophic relations at the upper end of the food chain in an unpolluted estuary, the Ythan in Aberdeenshire. Although the results are preliminary, he has been able to determine the annual energy budget of mussel populations in commercial beds and to show that predation by birds together with loss of energy as a result of metabolism during the winter cause the principal reductions of energy in the standing crop.

Mr M. J. Barrett (Water Pollution Research Laboratory, Stevenage) discussed changes in the oxygen levels in the waters of the Thames during a period of 80 years and how in the past 15 years conditions have improved so that anaerobic reaches are no longer present.

In the closing contribution, Professor D. R. Arthur (King's College, London) emphasized the need for interdisciplinary studies on estuaries and stressed the approach used by systems analysts in gaining a better understanding of estuarine systems.

Dilatons and Gravity

IN the continuing attempt to establish connexions between the microscopic and macroscopic worlds of physics, it is becoming fashionable to explore gravitation from the standpoint of elementary particle physics; and in next Monday's *Nature Physical Science*, Y. Fujii of the University of Tokyo describes a contribution to this approach. The starting point of his work is the prediction of the Goldstone theorem that the invariance of the world under scale transformations (dilatations) can be realized physically by the existence of a massless boson, known here as the "dilaton". Any breaking of scale invariance results in a non-zero mass κ for the dilaton, with a consequent finite range κ^{-1} for any physical effects. If the dilaton is now coupled to gravity, it would manifest itself by a modification of the usual Newtonian force law in the range κ^{-1}. The choice of κ is entirely arbitrary, but a characteristic mass may be constructed from gravitational and elementary particle parameters, and this leads to values of κ^{-1} of a few km or less.

Fujii's concern is to devise experiments to detect any such short range non-Newtonian component, if it exists. Several suggestions are made for laboratory and larger scale searches for this component. The chief difficulty with all these experiments is to achieve sufficient sensitivity. The most direct method—the Cavendish experiment in its refined form—could almost detect a component with $\kappa^{-1} \sim 10$ m. An amusing terrestrial experiment is also suggested in which the change in gravity due to the lowering of the water level in a large semicircular reservoir is measured. Other terrestrial approaches would be to compare the size of the Earth as measured by astrogeodetic techniques with the radius calculated from the gravitational force at the Earth's surface. Or the variation of gravity with altitude could be explored. Although astrophysical considerations might be expected to be the most appropriate for testing gravitational theories, only one extra-terrestrial experiment is mentioned, that of observing the behaviour of artificial lunar satellites which orbit close to the surface of the Moon and would be perturbed by a short range force.

Unfortunately, in all these large scale experiments it is impossible to make correct allowances for the many other complicated effects, such as those due to inhomogeneities beneath the terrestrial and lunar surfaces. Nevertheless, Fujii places present limits on κ^{-1} of either between 10 m and 1 km, or less than 1 cm.

PEAT

Pennsylvanian Deposits

from a Correspondent

WETLAND ecosystems are particularly prone to human interference. Either they are drained for agriculture or they are exploited for peat, but rarely are they left untouched. The reason for this is probably that it is difficult to justify mire conservation except in terms of the intrinsic scientific interest of the habitat, which appeals only to a relatively small minority of the population. The interest of these habitats lies both in their distinctive wildlife and also in that they build up a record of their own development in the peat which they form. Peat is an accumulation of the remains of dead vegetation which once grew upon the surface of the mire. Waterlogged, anaerobic conditions discourage microbial activity so that decay processes become impaired. Such remains are often recognizable and document the vegetational succession on the site. Furthermore, the peat contains pollen which may have travelled considerable distances in the air before being deposited on the mire surface and from this can be reconstructed the vegetational history of much larger areas surrounding the mire. Such information can be valuable in the study of climatic changes in a region.

Peat has long been valued as an energy reserve, a fossil fuel of recent origin. But in recent times its value as a horticultural commodity has risen very considerably, a value which derives from certain of its physical properties. In the first place it is a source of organic matter which, when added to a well aerated soil, acts as an energy source for soil microbes and thus improves soil structure. The water holding capacity of peat is very high, its value depending upon the vegetation from which it was formed, for example, *Sphagnum* moss peat can hold 800 per cent of its dry weight in water, and for sedge peat the figure is about 400 per cent. Peat also has properties of nutrient retention which make it valuable when added with fertilizers to soil. This horticultural demand for peat has placed a new stress on the world's harassed wetlands.

A recent bulletin of the United States Geological Survey (C. C. Cameron, Bulletin 1317–A; 1970) is devoted to the peat deposits of the Appalachian Mountain region of north-east Pennsylvania. The author calculates that the resources of this 900 square mile area are more than 13 million short tons of air dried peat. This estimate is based on a study of ninety-five peat deposits most of which are less than 100 acres in area and less than 16 feet deep. In spite of the small size of these resources (between 10,000 and 100,000 tons each), the proximity of the Atlantic seaboard and metropolitan markets for peat products makes their exploitation an economic possibility. In 1966 the United States imported peat to the value of $11½ million. With a demand of this size it is understandable that the Department of the Interior should seek a home supply of peat. If economic needs demand the exploitation of an area such as north-east Pennsylvania, this should be accompanied by the conservation of selected sites representative of the wide range of mire types found in that area. In this way the irretrievable loss of valuable ecological information can be avoided.

PLANT GROWTH

Izmir Institute

from a Correspondent

"NEVER has so much been said by so many on a subject about which we know so little." This remark, which was made at the 1955 Wye College conference on plant growth regulation, is perhaps as true today as it was then. The NATO Advanced Summer Institute on the same subject, which was held at Izmir in Turkey from September 20 to October 1, highlighted the complexity of the interactions between the classical auxins and the gibberellins, cytokinins, abscisic acid and ethylene. It also stressed the need for "translating" results obtained on segments of plants to whole plants.

The organizers arranged for a general review to precede the more detailed accounts of research on specific topics. This met one of the objectives of the NATO institute; that is, to provide students with a general survey of the field while at the same time providing a forum for research workers to present original work. Dr I. D. J. Phillips (University of Exeter) opened the proceedings with a review of the principles of promotion and inhibition of growth in plants. This was followed by an original paper by Professor E. C. Cocking (University of Nottingham) on specific discussion of growth in isolated protoplasts. Other contributions from the United Kingdom included reviews on the gibberellins (Dr J. Macmillan, University of Bristol), some recent studies on gibberellin-like substances in higher plants (Dr J. L. Stoddart, Welsh Plant Breeding Station) and growth regula-

How the Molecules of Collagen are Assembled

NEXT Wednesday's *Nature New Biology* will see the publication of the third article in the past few months on a longstanding problem in molecular biology; how the molecules of collagen are assembled in three dimensions to produce functional connective tissue. Each approach starts with different data and it is interesting that they all point to the same solution—a five-fold microfibril.

The latest study comes straight to the point that any answer must finally explain. How is the molecular packing related to the molecular structure? Segrest and Cunningham start with the tropocollagen molecule, a three-strand rope formed by supercoiling two identical helices termed α_1 and α_2. They speculate that the primordial collagen may have contained three identical chains of general structure (gly-pro-X)- where X is apolar. Fibril formation would likely take place by more or less random parallel aggregation of such molecules. They then suppose that a mutation in the nucleic acid led to a charged side-chain at the same level in all three α chains, followed by a second mutation which resulted in the appearance of a second charge side-chain, complementary to the first and separated by a suitable distance along the molecule. If the relative positions of the two new complementary groups are correct, fibril growth may proceed by lateral accretion of staggered molecules.

In addition to appropriate position along the length of the molecule, several factors are involved in the correct relationship of mutations for fibril growth. When parallel cylinders are packed they touch along edges of contact. For helices, points which lie along such an edge must be separated axially by integrals of the pitch of the helices. Thus mutations can only occur along these edges for formation of a regular fibril. Segrest and Cunningham list the types of packing which could result from various mutation positions. One of these is a hexagonal array; another is an extended pleated sheet. The third is the five-fold microfibril suggested independently on other grounds. The two identical α_1 chains in the tropocollagen molecule are related by an aximuthal rotation of 108°, so that the existence of contact edge on one chain automatically generates an identical edge 108° round from the first. If complementarity of these edges can be obtained by an axial stagger, then a second molecule would add to the first at an angle of 108° which is the internal angle of a pentagon.

Model building has its limitations. Without further information it is frequently only able to indicate possible structures. It is gratifying to see how the microfibril suggested by X-ray diffraction and electron microscopy arises readily from the structure of the component molecules.

tion in a whole plant (Professor A. J. Vlitos, Tate and Lyle, Ltd, Keston), and some aspects of growth control in cereal seedlings (Dr L. L. Mer, Imperial College, London).

The transport of auxins, gibberellins and cytokinins was discussed by several participants. Professor W. P. Jacobs (Princeton University) presented evidence for the polar transport (in *Coleus*) of all of the three classes of growth regulators. Professor T. K. Scott (University of North Carolina) demonstrated that the movement of gibberellins in sugarcane tissues is strongly influenced by auxins and perhaps also by other endogenous growth regulators. Dr H. Kaldewey (University of Saarlandes) presented good evidence for polar movement of radioactive ^{14}IAA in the stems and in the roots of intact cotton seedlings. An original concept for explaining apical dominance was presented by Professor Y. Vardar (Ege University, Turkey). Professor H. Bürstrom (University of Lund) demonstrated quite conclusively that the anatomy (that is, cell type) of a given tissue determines to a considerable extent the velocity of movement of growth regulators through the tissue. He also demonstrated the histological effects on cells after treatment with various classes of growth regulators.

Interactions between the various classes of endogenous growth regulators were reviewed by Professor A. W. Galston (Yale University) and by Professor A. C. Leopold (Purdue University). Both speakers stressed that auxins, gibberellins, cytokinins, abscisic acid and ethylene operate in a dynamic system where the presence of one type of growth regulator influences the physiological activity of another. Auxin, for example, may predispose a cell to respond to a gibberellin, and in some instances treating a tissue with a gibberellin induces it to produce more auxin; in turn this may lead to a release of ethylene. Ethylene may produce a host of physiological responses including the induction of flowering in tissue cultures of higher plants as was demonstrated by Dr C. Nitsch (CNRS, Gif-sur-Yvette).

Dr O. M. Heide (Makerere University) reviewed some of the physiological effects of cytokinins with special reference to the induction of rooting and initiation of buds. He demonstrated that these effects were mediated by temperature. Of particular interest was the demonstration that rooting of cuttings can be stimulated by cytokinins under certain conditions.

The role of ethylene in plant growth was discussed in some detail by Dr S. P. Burg (University of Miami). As one delegate remarked, "Ethylene can do anything an auxin can do and even better". Dr Burg demonstrated that ethylene is indeed a very active plant growth regulator but an extremely difficult and elusive compound to study, chiefly because it is a gas.

A thorough review of the auxins and other auxin-like substances by Professor K. V. Thimann (University of California, Santa Cruz), followed by a more specific contribution by Dr J. Shen-Miller (Argonne National Laboratory), set the stage for lively discussions on whether the classical auxins, as studied in isolated plant parts and in dark rooms, are as relevant to the growth of intact plants as was once thought. Dr Mer indicated, in discussion, that some of the published results may be interpreted in more than one way and that extrapolations to whole plant physiology from such results are invalid.

Inhibitors of growth, some new synthetic growth regulators and some interesting and aberrant physiological effects of auxins on growth were also discussed. Dr K. Dörffling (State Institute for General Botany and Botanic Garden, Hamburg) reviewed the status of abscisic acid as a naturally-occurring plant growth regulator.

A thorough description of the synthetic growth regulators, known as morphactins, was presented by Dr G. Schneider (E. Merck AG, Darmstadt). These substances retard the growth and development of dicotyledonous species and in the future may have important practical applications.

Professor L. Lona (University of Parma) presented evidence that indole-3-butyric acid (IBA) acts as a gibberellin-like substance in certain species. He stressed the need for evaluating the physiological effects of any given growth regulator on a wide range of species before attempting to classify the regulator.

Heterogeneous Nucleation from a Polymer Melt

THE ingenious researches of David Turnbull in the nineteen-fifties stimulated a great deal of work on the mechanisms and temperature dependence of nucleation in supercooled metallic and molecular melts. The classical Becker–Volmer theory of homogeneous nucleation is now firmly established for these categories of materials, but at the same time it has become clear that nuclei form homogeneously only under very exceptional circumstances: normal melts contain specks of impurity that act as nucleation catalysts. *A fortiori*, crystals forming from polymer melts would be expected to be heterogeneously nucleated always, in view of the extreme improbability that a sufficient length of polymer chain should align spontaneously in the correct configuration to constrain further growth in the proper pattern. But so far no heterogeneous nucleation catalyst for a polymer has been properly characterized and, generally speaking, this aspect of polymer science is confused.

In next Monday's *Nature Physical Science*, S. Y. Hobbs casts some light on this problem. Hobbs crystallized spherulites of isotactic polypropylene from the melt, using short lengths of carbon fibre as nucleation catalysts. These were standard 'Morganite' fibres made from a polyacrylonitrile precursor, and two fibre variants were used —high strength (type 1) and high modulus (type 2). Type 1 fibre is known, from electron microscopy and small-angle X-ray scattering, to consist of very small turbostratic graphite crystallites of random orientation, whereas type 2 contains larger, highly oriented crystallites, with the basal graphite planes parallel to the fibre axis. Hobbs found that type 2 fibres were highly efficient nucleation catalysts, whereas type 1 fibres were ineffective.

The polymer chains in the crystalline structure of polypropylene are arranged in straight spirals. Hobbs showed that such a spiral can be accurately keyed to a graphite basal plane through some of the hydrogen atoms which (according to recent molecular orbital calculations) are preferentially chemisorbed at the midpoints of C–C bonds in the graphite structure. For effective nucleation the spiral has to be "set", perhaps along one complete repeat period, and this requires the graphite basal plane which serves as substrate to exceed a certain minimum dimension, which Hobbs estimates at 50 Å. In type 2 carbon fibres, the constituent crystallites are more than 100 Å across measured in the basal plane, whereas in type 1 they are only ~25 Å across. The inefficacy of type 2 fibres is thus readily understood.

Type 2 carbon fibres have a crystallographic "fibre texture", which implies that the normals to the basal plane are randomly distributed in a plane normal to the fibre axis. It would be interesting to continue Hobbs's work with single crystals of graphite or alternatively with highly oriented pyrolytic graphite. On the basis of Hobbs's interpretation of his observations, polypropylene would be expected to crystallize on such a substrate in two-dimensional spherulites, all polymer spirals being constrained to lie parallel to the exposed graphite basal plane. This form of nucleation would generate a unique form of preferred orientation with, presumably, strongly anisotropic properties.

Outlook for Controlled Fusion Power

J. L. TUCK
University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico

Fission and fusion are compared as a source of energy for the future. Energy reserves for the two are similar, as are their pollution and ecological characteristics. A remote integrated site policy is recommended for fission.

UNTIL now mankind's chief energy source has been fossil fuel. Within the next century, if the population continues its present growth, rising energy demands and falling fuel reserves will force us to find new sources of energy and, although several possibilities exist[1-4], it looks at present as if the chief energy source of the future is likely to be either nuclear fission or nuclear fusion.

Comparison of Fission and Fusion

The advantages of nuclear fission as an energy source are, first, that the fuel reserves are large. The fuel used in fission reactors at present is obtained from rich ores, at a cost of ~\$5.00 per pound of UO_2, for which the reserve is small—a hundred years at most. But for weak ores (containing ~10 p.p.m. of UO_2), which would be uneconomic to extract at the present time, the reserve is large. Thus, using the customary energy unit $Q(=10^{18}$ BTU $=10^{21}$ J), the reserve at the 10 p.p.m. level will be about 10^6 Q if the uranium is used efficiently in breeder fission reactors. The present annual energy consumption rate of the world is 0.2Q, and it has been estimated[3] that for a world with an asymptotic population of 7×10^9 this might rise to 2 to 3Q a year. This asymptotic world has then about one million years supply of energy in its reserve of accessible fuel for fission.

The second advantage of fission is that non-breeder fission reactors are available and already producing power competitively. Breeder fission reactors exist only as prototypes at present but are in a state of intense development. They are expected to come into wide commercial use in the next decade and to be competitive power sources. The third advantage is that fission reactors pollute less than coal burning power plants.

The disadvantages of fission as an energy source are, first, that the plants are hazardous (because of possible melt down, power excursions and so on). But a major bomb-type explosion is probably impossible and precautions, involving heavy containment vessels and fail-safe cooling equipment, are taken to prevent escape of radioactive material into the atmosphere as a result of imaginable accidents. But no matter how safe they are made, they can probably never be made entirely safe from technically informed sabotage. It seems unlikely, therefore, that they will ever be installed within cities.

The second disadvantage is that the plutonium by-product is a material fundamental to nuclear weapons. In an asymptotic world running on fission, there would be enough to make tens of thousands of nuclear bombs a year. It follows then, that the rise to power of, say, a Hitler would assure a Götterdämmerung finale. Third, the plutonium is valuable, saleable, and therefore likely to be stolen. Mankind's past behaviour suggests that, sooner or later, somebody will "cobble" a crude fission bomb—of a kiloton or so—and fire it in a city centre. Fourth, fission products are biologically hazardous and probably should not be transported over long distances for fear of dispersion by accident.

The first fusion plants will have to operate on the, thermonuclearly speaking, less difficult, deuterium–tritium (DT) cycle. The factor determining fuel reserves will not be deuterium but lithium and the reserves of this are about 2×10^6Q or enough to last about 10^6 yr; ultimately fusion plants operating on the DD cycle and fuelled by deuterium may be achieved. Deuterium is present to the extent of 1 part in 50,000 by weight of the oceans of the Earth and constitutes an energy reserve of 10^9Q—a fuel reserve for about 10^9 yr at the asymptotic Q rate. Second, fusion reactors are incapable of running out of control and it follows that they could, if necessary, be located inside urban complexes. They also generate no fission products and, although the tritium is valuable and subject to theft, it is not adaptable to the construction of illicit bombs.

The disadvantages of fusion are, first, that fusion power is not yet available; second, that it seems as if fusion reactors will be more complicated than the basically very simple fission reactor, though this may not necessarily always be the case and it is too early to be sure. This suggests, however, that it will take time for fusion power to become competitive with fission. Third, although a fusion power reactor produces no long term radioactive fission products, it will produce about ten times as many neutrons kWh^{-1} as a fission reactor. This means that a high level of neutron activation will take place in some components of the reactor and precautions in plant design and hygiene will have to be taken to prevent the creation and escape of biologically hazardous nuclei. Fourth, tritium will be present and will be processed in large quantities in a fusion reactor. Although tritium is a biological hazard, it is less dangerous than most radioactive species because of its rapid dissipation in the atmosphere and because of the low penetrating power of its radiation.

Fifth, a fusion reactor operating on the DT cycle will contain large quantities of fused lithium in the form of a layer of thickness 1 m around the reaction space. Lithium is a chemical fire hazard in the same way as the sodium in fission reactors.

Even a technically expert saboteur in a fusion power plant could do no more than stop it and, in the case of a DT cycle plant, set off a lithium fire and release tritium in quantities that would not be catastrophic but would depend on the construction of the plant.

Fission, with all its disadvantages, could be improved by a wise siting policy. This would be to install plants on remote sites with their own fuel element processing and fission product disposal (and preferably, for its thermodynamic advantage, with cold water cooling). Fuels would enter such a plant but no radioactive or fissionable material would leave it. This reduces the hazard of fissionable material dispersal by theft and is also advantageous because of the low density of the surrounding population. Such plants would naturally form the power input points of a nationwide power grid. Many advanced countries today have such grids and, although a linking of privately owned power companies is taking place in the United States, the formation of a national power grid is somewhat overdue.

The objections made to such an obviously logical course by power plant operators have been, first, the high losses in long distance bulk power transmission, and, second, the high right-of-way costs of bringing transmission lines into cities. Both these arguments are valid if only on account of many years of neglect of development in the field of power generation and transmission. Overhead high voltage d.c. transmission as developed in Sweden is being tried in the United States (Bonneville to Los Angeles). The transmission of high voltages underground, ultimately by superconducting lines, is obviously the way to bring power into cities, both from the point of view of the amenities and the cost of right-of-way.

According to a recent *Fission Power Reactor Review*[12], there are more than 300 large fission reactors in existence or being built. Thus, fission power not only exists but is in a flourishing state. The disadvantages fission has will have to be lived with and it is not too late to do better. Fusion does have advantages over fission, and, in my opinion, will ultimately supersede it. The critical question is—when might this takeover occur ?

Time Scales

December 1942 is the birth date of fission, when the first pile went critical. Two years later, under the wartime thrust of the Manhattan project there came into existence a 1,000 MW (thermal) fission reactor (Hanford). It is less easy to put a firm date on the achievement of economic fission power—1965 might be correct for the United States, but it came earlier in Britain where power costs are higher and competitiveness easier to achieve. Accordingly, the development time of fission power from birth to usage will be taken as twenty years.

The term feasibility has been widely used in the search for a comparable birth date for fusion. The energy balance for a fusion reactor plasma is determined first by the ion temperature T_i which must be above a certain minimum value—$\sim$10 keV for most reactor geometries using the DT cycle but as high as 100 keV for the special case of the mirror machine with direct conversion. The second determining factor is the Lawson energy balance criterion $n\tau$, where n is the plasma density and τ the plasma confinement time. These two parameters can evidently be traded off against one another and so there can be a considerable range of parameters capable of satisfying the $n\tau$ reactor criterion, that is low density and quasi-steady state (with $n \sim 10^{15}$ ions cm^{-3}, $\tau \sim 1$ s) up to high density, pulsed state (with $n \sim 10^{23}$ ion cm^{-3} and $\tau \sim 10^{-8}$ s). The energy break-even point for a DT reaction is at $n\tau \sim 10^{14}$ ion cm^{-3} s, and the achievement of this has been called feasibility. For a reactor, one would need to achieve $n\tau \sim 10^{15}$ say, which would give an output of about ten times the input.

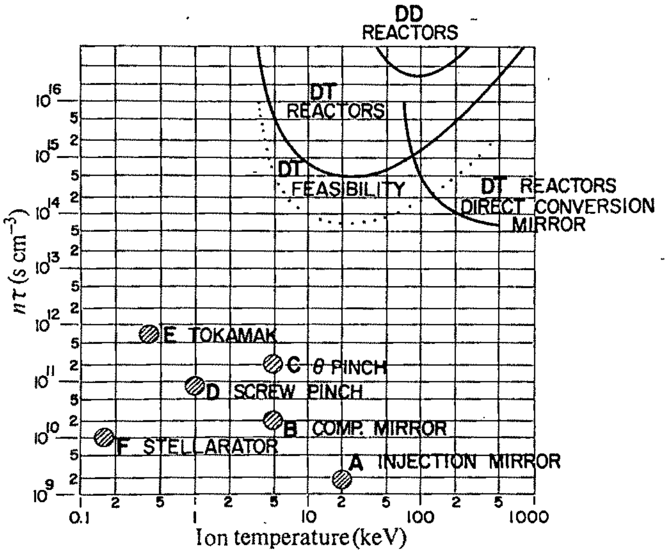


Fig. 1 Status diagram of fusion approaches to power reactors. *A*, Injection mirror machine Phoenix (Culham, UK); *B*, compression mirror machine 2*X* (Livermore, USA); *C*, θ-pinch Scylla 4 (Los Alamos, USA), also ISAR I (Garching, W. Germany); *D*, screw pinch ISAR IV (Garching, W. Germany); *E*, Tokamak T-3 (Moscow, USSR) since duplicated by S.T. (Princeton, USA); *F*, Stellarator-C (Princeton, USA).

In Fig. 1, these quantities T_i and $n\tau$ are plotted on an achievement chart for the plasmas at present obtained in reactor-oriented experiments. (The laser-heated DT pellet approach (for example) is, however, too recent for significant plasma achievements to have been made.) Although at this early stage it is probably not significant, the θ-pinch is at present leading the race to reach feasibility by a factor of 2 in T_i and 100 in $n\tau$. But an increase in $n\tau$ by a factor of 10^3 is still necessary before reactor status can be achieved. For the special case of mirror machines with direct conversion, the reactor target area is different, with a much higher T_i, >100 keV, but a lower $n\tau$, $\sim 10^{14}$.

For the benefit of those unaware that a controlled thermonuclear reaction had been achieved at all in the laboratory, it should be noted that the Scylla 4 θ-pinch plasma can generate (DT) a gross output of 100 kW of thermonuclear heat for a few μs. It is much too early to predict which of the contenders will reach the target first—the next steps forward at Tokamak (T–10) and at the θ-pinch (Scyllac) will, hopefully, advance $n\tau$ by a factor of 50.

The rate of achievement will depend in some less than linear way on the degree of financial support: a reasonable extrapolation at the current rate of progress suggests that feasibility might be reached by several routes in, say, 1980. Such an achievement would in no sense be a reactor; it would merely be a plasma which put out as much potentially recoverable energy as had been put into it—the engineering complexities, walls, heat exchangers would still lie ahead.

Starting from 1980 as the birth date for fusion, we now have to estimate the development time. It is hard to imagine anything simpler than a fission chain reacting pile, but a fusion reactor is bound to be much more complicated. It took twenty years to develop fission, but then there was no previous experience with radioactivity on a large scale.

I shall take the two factors of increased fusion complexity and increased engineering experience with radioactivity as mutually compensating, and the development period for fusion to be the same as for fission—twenty years. Competitive fusion can, therefore, be expected by the year 2000.

Fusion could have undoubted advantages over fission socially but from the point of view of ecology and pollution there is actually very little to choose between them, and both are greatly superior to fossil plants (especially those fired by coal).

Perhaps because of a confusion between the social and the ecological roles, fission has come under widespread attack from ecologists and anti-pollutionists recently, whereas fusion is being held up as the ideal. This use of fusion as a stick with which to beat fission seems also to have created a political polarization which is ill-founded technically and, if allowed to continue, may work to the disadvantage of fission, fusion and mankind generally.

For a fusion reactor to produce power, it must achieve the requisite ion temperature ($\sim$10 keV for DT, $\sim$50 keV for DD) for a sufficient $n\tau$ for the energy output from the thermonuclear reaction ($\propto \varepsilon n^2 \tau\, Q \overline{\sigma v}$ cm^{-3}) to exceed the input energy investment in heating the plasma ($\propto nT$ cm^{-3}); Q is the energy yield of the nuclear reaction, $\overline{\sigma v}$ is the nuclear reaction cross section averaged over the Maxwell distribution of velocities in the plasma and ε is the Carnot efficiency of the heat-electricity conversion. The aim of the magnetic confining system (bottle), if there is one, is to be as economical as possible both in its construction and energy running costs. This implies that the magnetic field B shall confine the maximum possible plasma pressure, or that the ratio of plasma pressure nkT to magnetic field pressure $B^2/8\pi$ shall be as near to unity as possible. A plasma confined by an external magnetic field has the fraction $(1-\beta)^{1/2}$ of that field inside. Some characteristic le I · Characteristic Parameter Values for a Possible DT Reactor Plasma

n temperature	T_i	10 keV ($\equiv 114 \times 10^6$ K)
lectron temperature	T_e	10 keV
on density	n	10^{15} ion cm^{-3}
lectron density	n	10^{15} electron cm^{-3}
Confinement time	τ	1 s
Plasma pressure	$2\,n\varepsilon T$	3.2×10^7 dyne cm^{-2} ($\simeq 32$ atm.)
Magnetic field	$\beta = 1$	21 kgauss
	$\beta = 0.01$	210 kgauss
Velocity of a 10 keV deuterium ion		10^8 cm s^{-1}

parameter values for a possible DT reactor plasma are given in Table 1.

The last parameter is an indicator of the rapidity with which the plasma moves when free to do so or with which it escapes when an instability defeats the magnetic confinement.

Tokamak

Tokamak is a large toroidal system with thick copper walls, and a toroidal winding encircling a magnetic core. A large current is induced in the plasma encircling the core, which heats the plasma resistively, and a large longitudinal magnetic field from the toroidal winding stabilizes the z pinch-like configuration; additional stabilization is provided by the conducting wall. The approach was devised early in the days of controlled thermonuclear research at the Kurchatov Institute, Moscow, by Artsimovich and his co-workers. From the 1950s onwards, a series of models of increasing size were produced but they aroused little interest among the main stream of plasma physicists. The breakthrough occurred in 1969 when an ion temperature of 0.4 keV was achieved in the T–3 machine; the pulse duration was 20 ms (ref. 5). The density is in the region 2 to 5×10^{13}, giving a value of $n\tau$ of 10^{12}, the largest yet achieved anywhere. The result was surprising, according to conventional theory, because such temperatures are not accessible by Joule heating with classical plasma conductivity. The resistivity of the Tokamak turned out to be 5 to 10 times the classical value and this has been explained in terms of a plasma turbulence. There is a critical limit to the current that can be passed through the plasma (in terms of the longitudinal magnetic field), which is such that the spiral executed by the resultant magnetic field does not close on itself in one circuit of the torus (Kruskal-Shafranov limit). This implies a very low value of β for the plasma. A visit by Artsimovich to the United States convinced physicists there of the validity of the results—the first time thermonuclear temperatures have been achieved in a slow, low-β system. This produced a somewhat surprising landslide toward Tokamaks in the United States with the result that five machines of differing designs have been authorized (at Oak Ridge National Laboratory, Princeton Plasma Physics Laboratory, Austin, Texas, MIT and Gulf-General Atomic at La Jolla). In the meantime, the construction of a much larger Tokamak, T–10, has commenced in the USSR.

The Tokamak is a very real experimental advance which reflects great credit on Artsimovich and co-workers. It is far from clear how the Tokamak can be extrapolated to a reactor, as the Russian physicists readily concede. For one thing the present method of Joule heating will not continue from 0.4 keV to the required 10 keV even with anomalous resistivity although supplementary heating processes, such as ion cyclotron heating, have been proposed. It is hard to imagine that with the present low value of β ($\sim 10^{-3}$) a reactor could be economic, so some method of confining stably more plasma and exceeding the Kruskal–Shafranov limit seems to be needed. The plasma density can also be increased by reducing the torus aspect ratio—making it fat. At the most recent Fusion International Conference at Madison, Wisconsin, in June 1971, a most ingenious proposal was made for self-driving of the longitudinal plasma current by the outward diffusion of new fuel injected into the axis. This may greatly reduce magnetic core requirements, and even offers the possibility of a steady state reactor. Other basic problems of extrapolating Tokamak into a reactor arise from the presence of the multiplying blanket and from synchrotron radiation by the hot electrons. The 1 m thick blanket must be interposed between the heavy torus windings—which are too thick to transmit neutrons—and the plasma. The presence of the 1 m standoff weakens the stabilizing effect of the wall so other stabilizing means are being sought; a scale size for a reactor in the 10,000 MW_e range is thus established. Fig. 2 is a Russian schematic drawing of a Tokamak power reactor showing the scale and general appearance; the heat exchangers, turbine, injection and processing auxiliaries are not shown.

The synchrotron radiation flux from the electrons of the plasma in a magnetic field has been estimated theoretically but the flux has not yet been observed experimentally. The radiation is an individual process and therefore emitted at a rate proportional to the density—by contrast with the thermonuclear fusion reaction itself which proceeds at a rate proportional to the square of the density. Consequently high densities favour the desired fusion over the undesired radiation. In a reactor situation, synchrotron radiation increases as β^{-3} and becomes the dominant process at values of β less than about 0.01. The subject of synchrotron radiation is theoretically difficult and tends to have been treated superficially in reactor studies. The radiation will have a wavelength in the region 0.01 cm and this is easily reflected by metal mirrors with an efficiency of 99%. By completely surrounding the plasma with such a reflector, the synchrotron losses can be greatly reduced though the radiation intensity inside the shield rises above its previous value. The problem is that, quite apart from the effect that the enhanced radiation will have on the stability of the plasma it is certain that engineering requirements pumping ports and so on, will make it very difficult to achieve 99% perfection in the reflecting enclosure. A theoretical calculation for the Tokamak indicates that synchrotron radiation loss would be a significant factor in the energy balance for a Tokamak without reflectors[6].

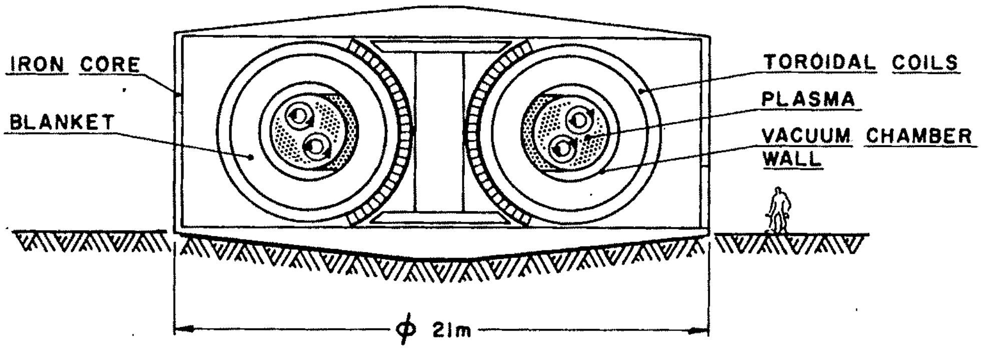


Fig. 2 Tokamak reactor. Fuel, D+T; thermal power, 5 GW; electrical power, 2 GW; major plasma diameter, 14 m; maximum magnetic field, 100 kgauss; plasma density, 3×10^{14} cm^{-3}; containment time, 0.7 s; $\beta = 0.3$. Two of many ion orbits are shown in the plasma region. (After Golovin, "Energy 70", Las Vegas, 1970.)

Direct Conversion

Most (~14 MeV) of the total Q of 18 MeV associated with the DT reactions appears in the form of neutron energy. This

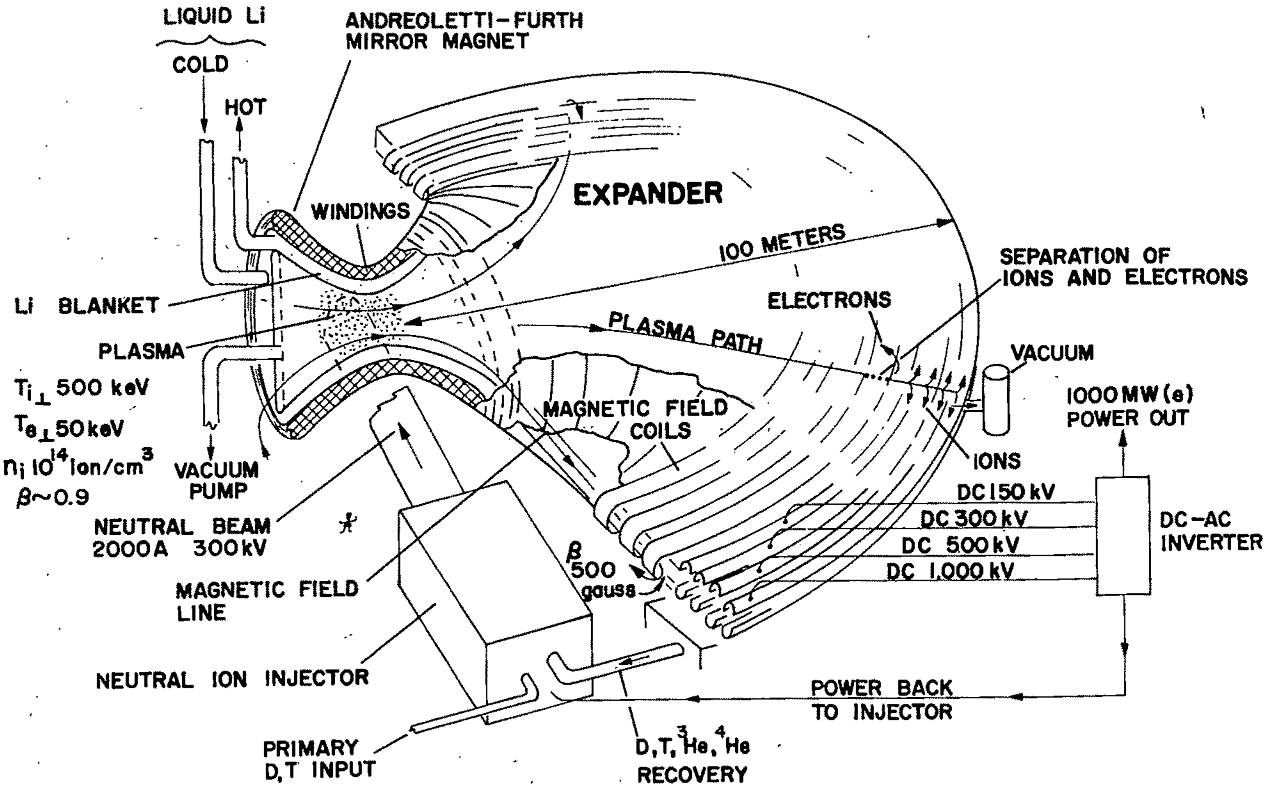


Fig. 3 Direct conversion mirror fusion reactor.

energy can be utilized only by depositing it in a target as heat and passing the heat through a thermal cycle (for example, a Carnot cycle). The efficiency of a Carnot cycle between upper temperature T_1 and lower temperature T_2 is $(T_1 - T_2)/T_1$ and, for practical upper temperature ~750 K and lower temperatures 373 K, this value is about 0.3 to 0.4. This efficiency appears in the Lawson break-even equation for $n\tau$ as $(1-\varepsilon)/\varepsilon$ or, with $\varepsilon = 0.3$, a factor ~2.3. In the DD reaction, only 38% of the Q of ~4 MeV appears in the neutron, so that most of it is evident in the form of α particles, protons and ^{3}He nuclei. In the case of the D^{3}He reaction, all the energy of the reaction is emitted as charged particles. Now although the neutron energy can only be converted into power by the low efficiency thermal cycle described previously, charged particle energy could, in principle, be converted much more efficiently either by allowing the plasma of charged particles to do work on a magnetic field directly or by separating the charged components of the plasma and discharging them into conductors, that is, direct conversion. In either case, the upper temperature of the Carnot cycle becomes, in effect, the plasma temperature $\sim 10^8$ K. Taking T_1 as 10^8 K, this gives a value of ε of 0.999, so $(1-\varepsilon)/\varepsilon \sim 10^{-3}$; this brings the Lawson criterion $n\tau$ down from its previous value by a factor of 2,300. According to the calculation for this ideal, DD fusion with direct conversion would be easier, in terms of $n\tau$, than DT fusion without it.

Post[7] has made the ingenious suggestion that the exhaust plasma from a mirror machine should be expanded by a large factor. This has the effect of converting the initially random motion of the plasma into a quasi-ordered beam, that is, an isentropic expansion. The beam of plasma is separated into its ion and electron components by magnetic deflexion, and the charges in the two components collected in stages to provide high voltage d.c. power. The expander is of considerable size (Fig. 3) but the elimination of the tiresome and inefficient heat exchanger-boiler-turbine combination characteristic of other fusion reactors, and the achievement of a power plant with no moving parts, would be more than worth while.

Not only does the mirror machine have to operate at a much higher ion temperature ($\gtrsim 100$ keV) than other systems but the electron temperature has to stay low ($\lesssim 30$ keV) and decoupled from the ions; this is a thermodynamic disequilibrium which invites instability. If instability should occur, then the electron temperature will rise, bringing trouble from increased synchrotron radiation. Furthermore, in mirror-machine reactors the fraction of plasma burn-up during one passage of the plasma through the machine is small so a large amount of energy (compared with the useful output energy) has to be recirculated—that is, plasma is reinjected into the machine. This means that the achievement of a positive power balance depends critically on a very high efficiency, >90%, in the stages—output beam-to-electricity converter and electricity-to-input-ion beam accelerator—through which the large recirculating power passes. These values are based on a classical mirror confinement time. There is evidence that the loss out of the mirrors is 10 to 15 times faster than this because of the effect of theoretically predicted instability. If this instability cannot be overcome, the stage efficiency requirement will become even more stringent—to the extent, possibly, of being unrealizable.

It is a most interesting idea whose application may extend to the thermonuclearly more efficient high β, open-ended, linear Scylla-type reactor. This is already capable of yielding a positive power balance using a DT fuel at 10 keV temperature and the usual low 0.3 efficiency for ε. If direct conversion, with an efficiency $\varepsilon = 0.9$, were applied, $n\tau$ could be lowered by a factor of ~20 to 30—which might yield a positive power balance even with DD. The blanket could then be omitted and the whole system made much simpler.

Scylla-Scyllac θ Pinch

Plasma in a long tube is subjected to a rapidly rising longitudinal magnetic field. This has the effect of first shocking the plasma, and then adiabatically compressing it. The elegant aspect of this approach is that the heating is applied directly to the ions, where it is needed; the heating is not resistive (which would heat the electrons first) and does not fail when the temperature is very high and the plasma is exceedingly con-

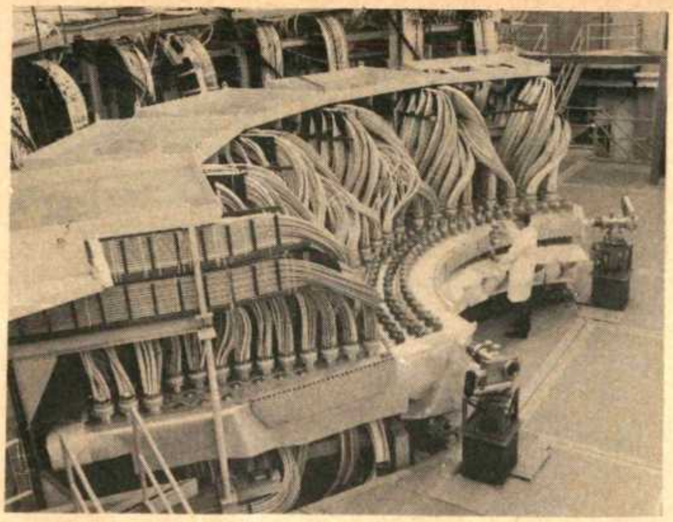


Fig. 4 Completed 120° arc of the Los Alamos Scyllac toroidal θ pinch.

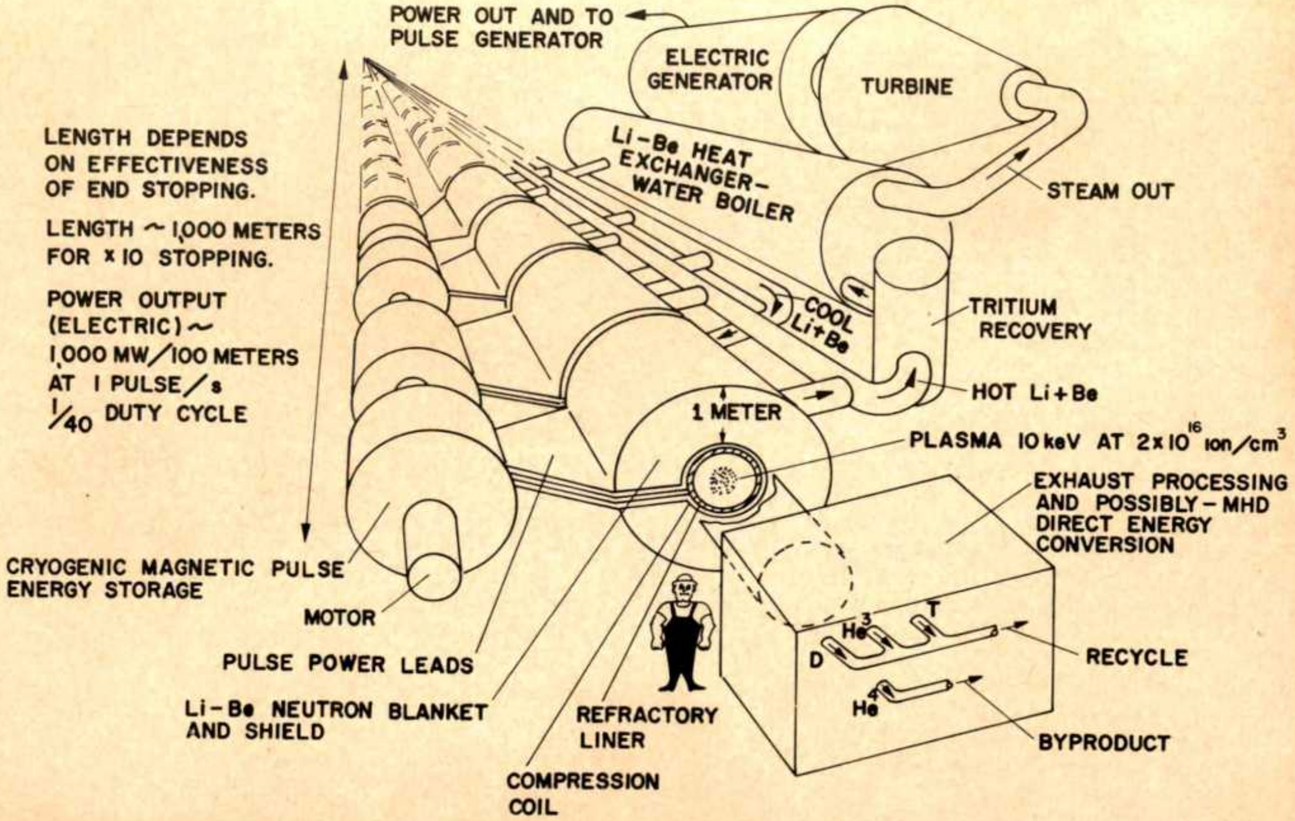
ductive. Unlike many other approaches, the hotter the plasma gets the better behaved it has been found experimentally to become. This device in its early form (Scylla I) produced the first laboratory thermonuclear reaction in 1957, although it was not until 1961—after many experiments—that the thermonuclear character was established beyond doubt. Achievements with Scylla IV are T_{ion}, 5 keV; n, 5×10^{16} ion cm^{-3}; τ, 5 μs; $n\tau$, 2.5×10^{11} ion cm^{-3} s; (β (max) ~ 0.9; β (av) ~ 0.3).

Although the Scylla θ pinch was originally conceived[8] only as an experiment to make thermonuclear plasma, it was later shown[9] that, in principle, it could become a reactor. The idea was to make the compression coil thin enough to be essentially transparent to the 14 MeV neutrons. This allows the T regenerating blanket of thickness 1 m, which is also the flowing Li-Be heat removing fluid, to be outside the coil.

The temperature achieved is already within a factor of two of the target temperature for a reactor, and there is no obvious difficulty in raising it further. At present, the confinement time ~5 μs is determined by free escape of the plasma from the ends of the compression coil. There are two ways to extend this time. One is to close the compression coil on itself to form a torus. This distortion introduces field gradients, disequilibrium, and calls for entirely new countermeasures to give stability and to hold the plasma in the centre of the tube. It is not yet known whether stability will be obtainable statically or whether dynamic or servo methods may have to be used. In its toroidal form, this device is known as Scyllac (Scylla-closed), and it is under construction at Los Alamos (Fig. 4). It will be some years before it is completed and, ideally, it is expected to increase $n\tau$ by a factor of up to 50 from the present value of 2.5×10^{11}.

Alternatively, it is possible to increase confinement time by lengthening the present straight system. This certainly has the merit of simplicity and the magnetic field is uniform. To reach a value of $n\tau$ appropriate to a reactor by this method alone would, however, require a length of several km. But it may be possible to restrict the loss from the ends and a roughening procedure in the magnetic field has given some theoretical support for optimism, in essence giving the plasma flow Knudsen[10] characteristics. A loss reduction by a factor x reduces the reactor length by the same factor. Electron synchrotron radiation is likely to be much less of a problem in θ pinches because they are high n, high β devices. The straight θ pinch reactor has a simple geometry (Fig. 5) and involves the least extrapolation beyond present plasma physical knowledge and experience. The ends form a natural way of disposing of the plasma, and may even provide extra power by direct conversion or by a pulsed magnetohydrodynamic generator.

The great length of the reactor in its unstoppered form makes is impractical and, above all, the engineering problem of supplying economically the enormous pulse power (proportional to the length) is unresolved. Condensers are also too



POWER OUT AND TO PULSE GENERATOR
ELECTRIC GENERATOR
TURBINE
LENGTH DEPENDS ON EFFECTIVENESS OF END STOPPING.
LENGTH ~ 1,000 METERS FOR × 10 STOPPING.
POWER OUTPUT (ELECTRIC) ~ 1,000 MW/100 METERS AT 1 PULSE/s 1/40 DUTY CYCLE
Li-Be HEAT EXCHANGER-WATER BOILER
STEAM OUT
COOL Li+Be
TRITIUM RECOVERY
HOT Li+Be
1 METER
PLASMA 10 keV AT 2×10^16 ion/cm^3
EXHAUST PROCESSING AND POSSIBLY-MHD DIRECT ENERGY CONVERSION
CRYOGENIC MAGNETIC PULSE ENERGY STORAGE
MOTOR
PULSE POWER LEADS
Li-Be NEUTRON BLANKET AND SHIELD
REFRACTORY LINER
COMPRESSION COIL
D
He3
T
He4
RECYCLE
BYPRODUCT

Fig. 5 Scylla linear θ-pinch fusion reactor (schematic).

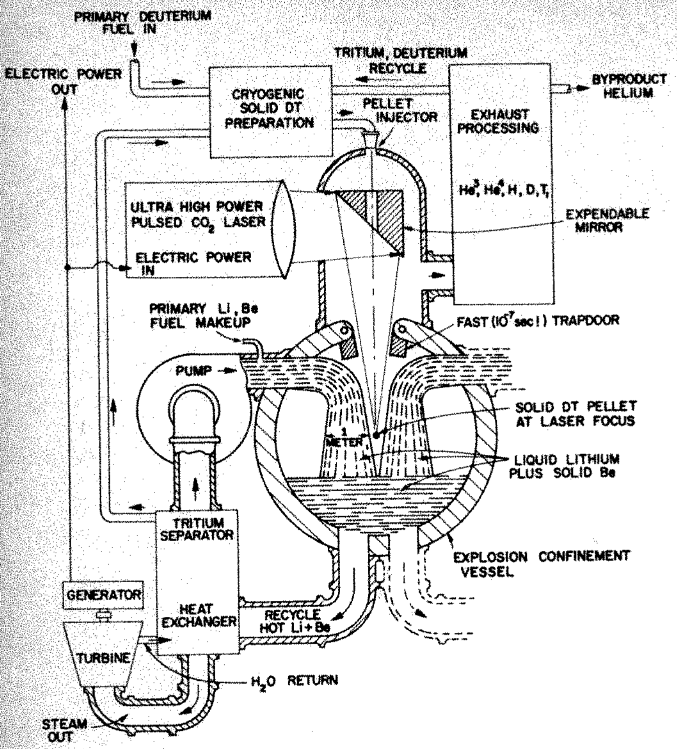

PRIMARY DEUTERIUM FUEL IN
ELECTRIC POWER OUT
TRITIUM, DEUTERIUM RECYCLE
BYPRODUCT HELIUM
CRYOGENIC SOLID DT PREPARATION
PELLET INJECTOR
EXHAUST PROCESSING
He^3, He^4, H, D, T,
ULTRA HIGH POWER PULSED CO_2 LASER
EXPENDABLE MIRROR
ELECTRIC POWER IN
PRIMARY Li, Be FUEL MAKEUP
FAST (10^{-7} sec!) TRAPDOOR
PUMP
SOLID DT PELLET AT LASER FOCUS
1 METER
LIQUID LITHIUM PLUS SOLID Be
TRITIUM SEPARATOR
EXPLOSION CONFINEMENT VESSEL
GENERATOR
HEAT EXCHANGER
RECYCLE HOT Li+Be
TURBINE
H_2O RETURN
STEAM OUT

Fig. 6 Conceptual (1969) laser DT pellet reactor.

expensive but rotating superconducting generators[11] have been proposed.

The toroidal Scyllac is more radical in concept and more liable to instability but has lower pulse power requirements. The serious problem of feeding plasma in and removing it without damage to the inner wall has not yet been tackled.

Laser Heated DT Pellet

The density of solid DT at 4 K is 6×10^{22} atom cm^{-3}. If a pellet of radius r could be heated instantaneously to 10 keV, a strong thermonuclear reaction during the rapid expansion (velocity $\sim 10^8$ cm s^{-1}) would take place. The effective confinement time will be $\sim r/10^8$ s.

From Fig. 1, it can be deduced that r would have to be about 1 mm to reach reactor status. Expressed alternatively, the input energy is proportional to the volume r^3, but the thermonuclear output energy is proportional to this volume and the time (one more factor of the radius) or r^4. Thus there has to be a value of the radius such that the output exceeds the input. According to elementary plasma theory, at the density of 6×10^{22}, radiation from known lasers would be below the plasma cut-off frequency for that density and would be reflected. It is far from clear whether this holds true at the radiation intensities existing at a laser focus. Further, a pre-preparation of the pellet surface density can be envisaged, which would increase the laser energy absorption at the surface. At first, in the mid-1960s, it seemed to many of us that such ideas were hopeless for power production, for the pumping efficiency of, say, Nd glass lasers was $<1\%$—which imposed a hundred-fold adverse energy factor at the outset. More recently, however, the CO_2 laser has achieved an efficiency $>20\%$ (theoretical limit $\sim 40\%$), which might just be acceptable.

The reactor that might develop from these ideas (Fig. 6) is the model of simplicity—no magnetic fields, no stability. In fact instability is an advantage, for it may help the heating. The pellet is dropped into a boiler which is strong enough to withstand the explosion and the inner walls of which consist of a rapidly flowing 1 m layer of liquid lithium, which acts as the tritium regenerator and carries off the heat to the exchanger. There should be no trouble about heating of the wall, a sensitive point in all other fusion reactors, as it is constantly renewed.

The energy input and output are sensitive functions of the pellet radius at break-even. If the laser energy input for positive energy balance turns out to be, say, 10^5 J—which is some distance beyond present laser practice but imaginable—and the energy (thermal) output is, say, 10^7 J—equivalent to a few pounds of TNT which could easily be confined, even repetitively in a boiler of reasonable dimensions—the reactor will be very attractive. But an increase in the break-even radius of, say, a factor of ten would increase these figures by a factor of 10^3; this would place the system far from reality and would necessitate a boiler which could withstand the equivalent of several tons of TNT exploding repetitively—it would be beyond present engineering practice. Clearly it is very advantageous to increase n because this diminishes the τ for break-even in inverse proportion, which in turn brings down the required input energy by the same proportion to the third power. Any way of slowing down the expansion rate pays off equally handsomely.

It should be obvious that there are very rewarding directions for the application of ingenuity to this problem. And there is even an alternative possibility to the laser for the heating source—a pinched-down beam of relativistic electrons.

There are two other interesting problems. The laser radiation must be admitted into the boiler through some aperture and, clearly, the explosion must not destroy the optics. There is very little time between the admission of the radiation and the impact of the explosion shock-wave on reaching the walls, so the manipulation of a trapdoor would be very difficult. Thin metallized plastic expendable mirrors spring to mind, however. Finally, factors of reactor economics common to all fusion reactors show up with great clarity in this case. One kWh (electric), $=3.6$ MJ, can be generated currently for 0.3 to 0.5 cents and therefore that is all that can be spent per shot (for the 10^7 J case above) to cover the laser pumping, plant, salaries, processing, DT and cryogenics. It will obviously have to be a very efficient operation. It is too early to guess how the DT pellet reactor will fare in the fusion reactor race. Superficially it looks very good—but this may be because it is also the newest. In France, this approach is being studied at Limeil and in the United States at Livermore, Los Alamos, and Oak Ridge. At the Lebedev Institute, Moscow, they are also thinking along similar lines.

All fusion reactor approaches have unsolved problems and their problems differ greatly in kind. Nonetheless, it seems to me very likely that out of the diversity, one at least will succeed. Nuclear energy is certainly essential for the future of mankind and, although fission is solving some of the energy problems, fusion will in many ways be far more satisfactory. But it will probably not become competitive for thirty years.

This work was performed under the auspices of the US Atomic Energy Commission.

[1] Brown, A., Bonner, J., and Weir, J., *The Next Hundred Years* (The Viking Press, 1957).
[2] Weinberg, A. M., *Physics Today*, 18 (1959).
[3] Tuck, J. L., in *Cosmology, Fusion, and Other Matters* (Colorado Associated University Press, in the press) and *Los Alamos Scientific Laboratory Preprint LADC*-9519.
[4] Robinson, E. S., *et al.*, *Los Alamos Scientific Laboratory Report LA*-4547 (1971).
[5] Artsimovich, L., *et al.*, *Proceedings of the Third Intern. Atoms for Peace Conference, Novosibirsk, 1968*, **1**, 157 (IAEA, Vienna, 1969).
[6] Rosenbluth, M. N., *Nuclear Fusion*, **10**, 341 (1970).
[7] Post, R. F., *University of California, Livermore Radiation Laboratory Report UCRL*-71753 (1969).
[8] Boyer, K., Elmore, W. E., Little, E. M., Quinn, W. E., and Tuck, J. L., *Phys. Rev.*, **119**, 857 (1960).
[9] Quinn, W. E., Ribe, F. L., Riesenfeld, W. B., Sawyer, G. A., and Tuck, J. L., *Los Alamos Scientific Laboratory Report LA*-3294-*MS* (1965).
[10] Tuck, J. L., *Proc. Third Internat. Conf., Novosibirsk, 1968*, **2**, 595 (IAEA, Vienna, 1969).
[11] Smith, P. F., *Synchrotron Power Supplies using Superconductive Energy Storage; Rutherford (UK) Laboratory Report* (1967).
[12] *Nuclear Engineering International*, **16**, 176/177 (1971).

Acetylcholine Receptors in Muscle Fibres

R. MILEDI & L. T. POTTER
Department of Biophysics, University College, London

There are calculated to be approximately 10^9 acetylcholine receptors in a frog muscle fibre. This has important implications for theories concerning the nerve to muscle transmission mechanism.

THE transmission of impulses from nerve to skeletal muscle fibres of vertebrates is mediated by the release of a transmitter—acetylcholine (ACh)—from the motor nerve endings. After diffusing across a narrow synaptic cleft, the ACh molecules react with a specific "receptor" in the muscle fibre membrane, and consequently the ionic permeability of the membrane is greatly increased. While the process of transmitter release has been relatively well studied[1], our knowledge of ACh-receptors is, so far, fragmentary.

Intracellular recording and micro-ionophoretic application of ACh have established that the ACh-sensitivity of normal muscle fibres is maximal in the region of neuromuscular contact and falls sharply therefrom[2]. Such experiments demonstrated the pattern of distribution of the ACh-receptors but not their number, which can apparently now be obtained with the use of snake toxins.

It has long been known that several snake venoms have a curare-like action[3]. Chang[4] showed clearly that the venom from a Formosan snake (*Bungarus multicinctus*) abolished neuromuscular transmission by blocking the action of ACh, apparently in an irreversible manner. Chang and Lee[5], and Lee, Tseng and Chiu[6], subsequently established that a subfraction of this venom blocked neuromuscular transmission by combining with a site on or near ACh-receptors. We have confirmed and extended their work.

Twelve pure proteins were isolated from the dried venom of *B. multicinctus* (details to be published elsewhere). The most prevalent of these (20–25% of the protein of the venom) is a basic protein with a molecular weight of 8,000; it probably corresponds to the "α-bungarotoxin" of Chang and Lee[5], and was used for our experiments.

ACh-receptors in Normal Muscles

When α-bungarotoxin (α-BuTX) was added to Ringer solution bathing frog or rat muscles, neuromuscular transmission was blocked. Nerve impulses continued to invade the nerve terminals and release ACh, but the action of ACh on the postsynaptic membrane was progressively reduced. Fig. 1 illustrates the effect of 10^{-6} g/ml. of α-BuTX on the amplitude of the end-plate potential (e.p.p.) which can be taken as an index of transmitter action on the muscle fibre membrane. At zero time the bath was changed to one containing the toxin and, when the microelectrode was reinserted into the fibre a few minutes later, the e.p.p. was already reduced. Subsequently the e.p.p. amplitude continued to fall, approximately exponentially, with a half time in this case of about 2.25 min. The sensitivity of the end-plate to ionophoretic or bath application of ACh is concomitantly reduced.

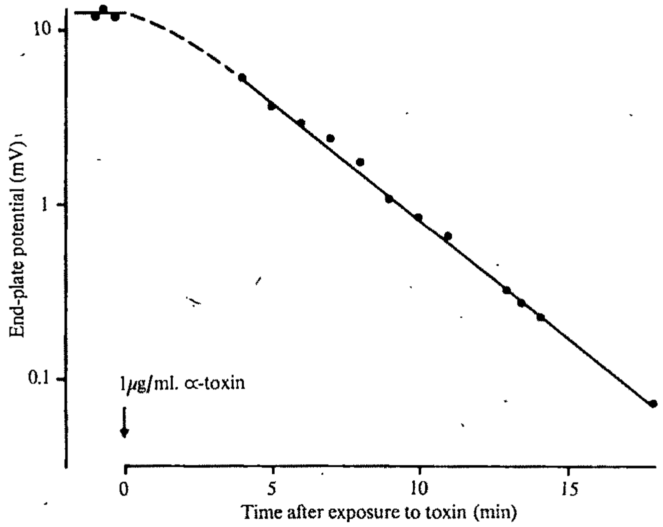


Fig. 1 Effect of α-BuTX on the amplitude of end-plate potentials from a surface end-plate in a frog sartorius muscle. Each point is the average of twelve potentials.

The time-course of blockade was concentration-dependent, and also varied appreciably from fibre to fibre within a muscle. End-plates deep within the muscle took longer to block than surface end-plates, but with 10^{-6} g/ml. α-BuTX all end-plates of frog sartorius muscles ceased to transmit impulses in 1–2 h. This variability is very likely due to limitations in the access of the toxin to the end-plates, either because of diffusion barriers or because the toxin reacts at other sites on its way to the end-plates. In some experiments α-BuTX was applied ionophoretically. This makes it possible to block only a small part of an end-plate, and the rate of block is then much faster than shown in Fig. 1.

Several muscles were exposed to 10^{-5} g/ml. of α-BuTX for about 2 h, and were then washed for several hours at room temperature, with no sign of recovery of ACh-sensitivity. Washing was then continued at 3–4° C and even more than 8 days later the muscle fibres remained excitable but there was still no recovery of either neuromuscular transmission or sensitivity

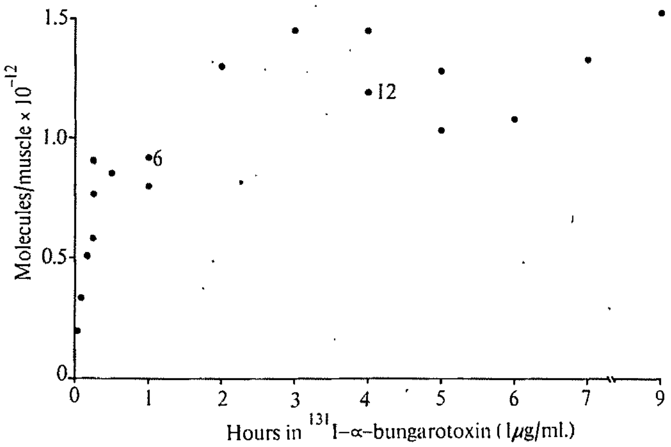


Fig. 2 Saturation of toxin-binding sites of frog sartorius muscles. Muscles were exposed to ^{131}I-α-BuTX for the periods indicated; then washed and assayed for radio-iodine (see text). Points represent single muscles or mean values of groups of muscles the number of which is shown.

to acetylcholine, which shows that blockage of ACh-receptors by α-BuTX is, for practical purposes, irreversible.

To study its binding to cholinoceptive tissues α-BuTX was labelled[7] with ^{131}I. Such labelling consistently gave an initial specific activity (of 23,000 Ci/mol) with no observable loss in the ACh-receptor blocking activity of the toxin. Muscles were exposed to the labelled toxin at 20° C for various times and then washed free of unbound toxin by repeated bath changes for about 24 h at 4° C. The tissue was then dissolved in an alkaline solvent ('Soluene', Packard), and the amount of ^{131}I was determined by liquid scintillation spectrometry. Corrections for isotopic decay were made by measuring the activity of the stock solution of ^{131}I α-BuTX at the same time as a series of muscle samples.

The time-course of toxin-binding to frog sartorius muscles is shown in Fig. 2. Binding increased during the first 2 h at a rate comparable with that of neuromuscular blockade, and then reached a plateau level of 1.2×10^{12} molecules of toxin per muscle (mean of twenty values obtained after 2 h). Similar experiments with diaphragms of rats and mice gave respective values of 4.7×10^{11} and 1.6×10^{11} toxin-binding sites per hemidiaphragm (means of twenty-eight and six respectively).

If the amount of bound toxin is to be used to estimate the number of ACh-receptors or to mark them for subsequent isolation, it is important to determine the specificity of the binding. One measure of the specificity of toxin-binding to receptors is its localization on the muscle fibres. Using autoradiography, Lee, Tseng and Chiu[6] have shown that in rat most of the label is in the end-plate region. To obtain a more quantitative estimate of the localization of binding, we have divided muscles into portions, some of which were practically free of end-plates. In the frog sartorius, "end-plate free" portions (amounting to about 20% of the wet weight of the muscles) had an average of 2% of the total counts (mean of twenty-four muscles); and even some of this binding is probably still associated with the ACh-receptors which are known to exist in "end-plate-free" regions[8,9]. In the rat diaphragm end-plate-free portions accounted for five-sixths of the muscle and had about 10–15% of the total radioactivity. All this shows that non-specific binding of the toxin is not a large complicating factor.

As most of the toxin is bound at end-plate regions, the plateau level of binding (Fig. 2) may be used to estimate the number of toxin-receptive sites per end-plate. In the frog sartorius muscle, which has about 500 muscle fibres and two to four end-plates per fibre[10,11], the number of toxin-receptive sites per end-plate is about 10^9. The corresponding number for the rat hemidiaphragm which has about 10^4 singly-innervated fibres[12] is about 4.7×10^7 sites per end-plate. Assuming the same number of fibres in the mouse hemidiaphragm gives a value of 1.6×10^7 sites. The last figure is several times larger than the number of curarine-binding sites found by Waser[13], perhaps partly because curarine, a bisquaternary ammonium compound, blocks only half of the toxin-binding sites (see below). In this context, it is interesting that Changeux, Kasai and Lee[14] have found roughly twice as many binding sites for α-BuTX as for decamethonium (another bisquaternary amine) in the electric tissue of eels.

ACh-receptors in Denervated Muscle

After denervation the entire muscle fibre membrane develops ACh-receptors with properties resembling in most respects those of the junctional receptors[8,11,15]. They are, however, less sensitive to curare[16], and it was therefore important to find if the new receptors are also blocked by α-BuTX. The ACh-sensitivity of muscle fibres was measured[11], some 6 mm away from the end-plates, in a muscle which had been denervated for 28 days. After 3 h in α-BuTX (10^{-6} g/ml.) the ACh-sensitivity had fallen by more than 1,000 times, a clear indication that α-BuTX also blocks the newly developed ACh-receptors. Accordingly, the amount of bound toxin was also increased after denervation. The number of toxin sites per muscle increases progressively (Fig. 3), reaches a peak and then starts to decrease, presumably because the muscle fibres atrophy and decrease in size and number on account of the prolonged denervation.

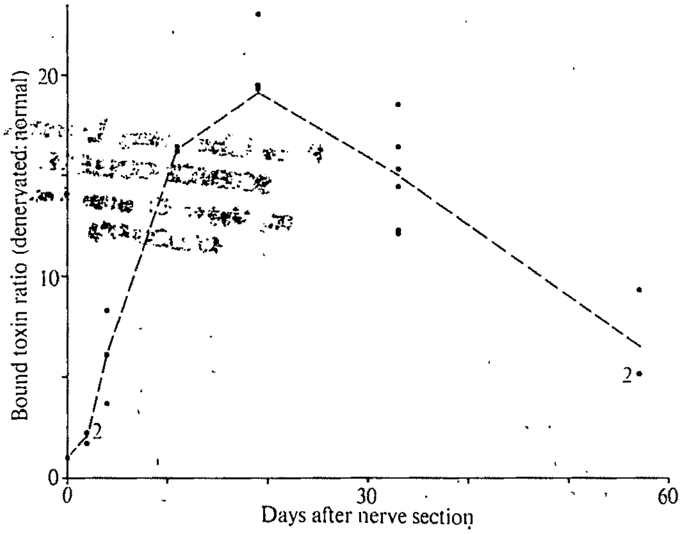
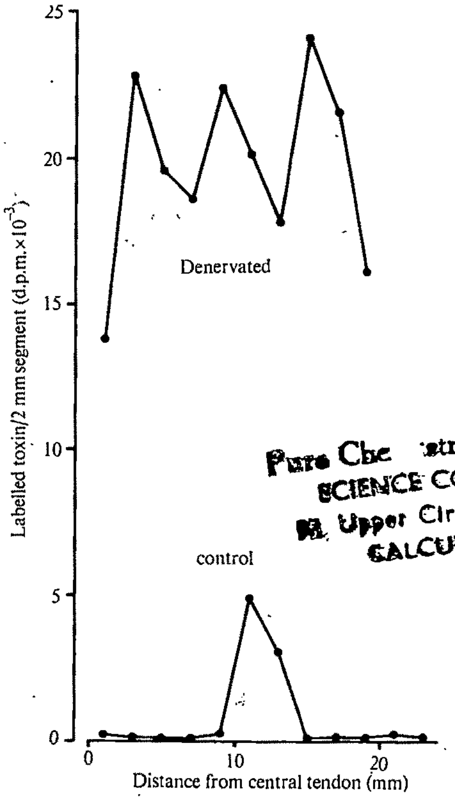


Fig. 3 Effect of denervation on number of binding sites. Left diaphragm muscles were denervated by section of the phrenic. At the indicated times after denervation, the muscles were exposed to labelled α-BuTX, 1 μg/ml., for 4 h at 20° C, then washed and assayed for radioactive iodine. The normally innervated right hemidiaphragms and one sham operated left hemidiaphragm (point at 0 days) bound a mean of 3.95×10^{11} toxin molecules/hemidiaphragm. The ratio of toxin-binding sites in denervated to normal hemidiaphragms is shown on the ordinates.

Just as the ACh-sensitivity spreads to the entire muscle fibres, so does the binding of α-BuTX[6]. This was shown by incubating strips from a denervated diaphragm and from its opposite innervated control muscle in α-BuTX. After washing, the strips were fixed in glutaraldehyde, as previous experiments showed that all the bound toxin was retained after various fixative procedures. Subsequently, the strips were divided into 2 mm segments and their labelled toxin content measured (Fig. 4). The increase in toxin bound to extra-junctional regions is large and, as there is no detectable ACh-sensitivity far from the end-plates in the normally innervated diaphragm[2], it seems likely that most of the small amount of toxin bound at these regions in normal muscles is unspecific. Nevertheless, after denervation the number of receptors in these regions increases by at least 200 times.

As extra-junctional regions develop a high ACh-sensitivity after denervation, and the ratio of total surface membrane to synaptic membrane areas is roughly 300, it is puzzling that the overall increase in toxin-receptive sites is only about twenty times. Perhaps the increase in receptors is not larger because, during the first weeks after denervation, the ACh-sensitivity of the extra-junctional region does not reach the level of sensitivity at the end-plate[8], while subsequently the muscle becomes atrophic. Also, the juxta-synaptic region already has ACh-receptors[2] so after denervation the increase in receptors in this region will be comparatively smaller.



Pure Chemistry Library
SCIENCE COLLEGE.
92, Upper Circular Road
CALCUTTA

Fig. 4 Distribution of bound toxin along normal and denervated muscle fibres from the rat diaphragm. The end-points in the denervated strip have less toxin bound because of tapering of the strips.

After regeneration of neuromuscular synapses the ACh-sensitivity of extra-junctional regions again becomes very low[17] and, as expected, the number of toxin-receptive sites is also decreased. For instance, two muscles were studied 71 days after crushing the phrenic nerve. With this procedure, the muscle fibres are denervated for approximately two weeks, and during that time they develop the generalized sensitivity to ACh, and are then rapidly reinnervated without allowing the muscle to become very atrophic. After exposure to toxin the reinnervated muscles had a mean of 5×10^{11} molecules of toxin per hemidiaphragm and a regenerated/normal ratio of 0.97, values similar to those observed in normal muscles.

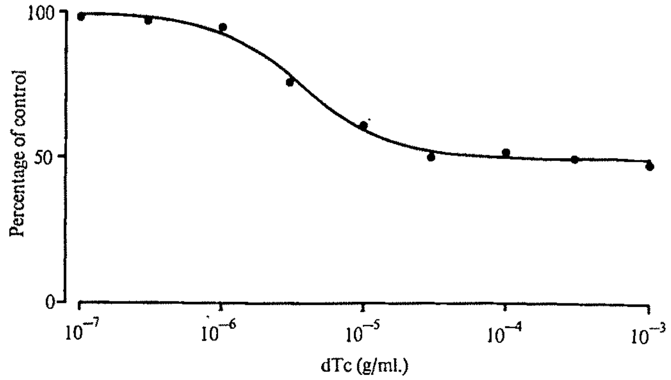


Fig. 5 Effect of *d*-tubocurarine (dTc) on binding of α-BuTX. One of each of nine paired frog sartorius muscles was equilibrated for 2 h in *d*-tubocurarine at the specified concentrations, and then exposed to α-BuTX while still in the dTc. All muscles were exposed to 1 μg/ml. ^{131}I-α-BuTX for 1 h and then washed for 1 h in 10^{-4} g/ml. dTc without toxin to reduce further binding of toxin during the washing period. Ordinates represent the amount of toxin bound by the muscle incubated in dTc as a percentage of that of its paired muscle incubated without prior or simultaneous exposure to dTc.

Protection of Toxin-binding Sites by Curare

Chang[4] and Chang and Lee[5] have shown that *d*-tubocurarine (dTc) protects muscles against the postsynaptic action of the venom from *B. multicinctus*, and dTc would therefore be expected to reduce the binding of α-BuTX to muscles. To estimate the degree of this protection, frog sartorius muscles were equilibrated in various concentrations of dTc and were then exposed in addition to labelled α-BuTX (10^{-6} g/ml. for 1 h). The paired control muscle were separately exposed to the toxin without previous treatment with dTc. Fig. 5 shows that dTc decreased the binding of α-BuTX, and protected the ACh-receptors. With dTc concentrations higher than 10^{-5} g/ml. some muscle fibres still gave action potentials to nerve stimulation after both the toxin and dTc had been washed out.

An unexpected finding was that toxin-binding was not reduced below 50%, even with the highest dTc concentration used (Fig. 5). There seem to be, therefore, two classes of toxin binding sites, only one of which can be blocked by dTc at the concentrations studied, but the reason for this is not yet entirely clear. It could be that half the toxin binding sites are not ACh-receptors. This does not, however, seem very likely because the unspecific binding outside the end-plate is negligible, and because the binding of α-BuTX can be reduced further by ACh itself. A more plausible explanation is that there are two different membrane molecules, both capable of reacting with ACh and α-BuTX, but only one with a high affinity for dTc. While the pharmacological competition between ACh and dTc suggests that both act on the same site, it is also conceivable that there is a dTc-receptive molecule which does not have ACh-reactive sites, but which blocks the action of ACh through allosteric interactions. Differences in the distribution of the dTc-receptive sites could explain the lower dTc-sensitivity of extra-junctional receptors after denervation[16] and also explain why the binding of radioactive curarine does not increase much after denervation[13].

Desensitized Receptors

During prolonged exposure to a drug, the responsiveness of many tissues to the same or similar drugs is greatly reduced. In the case of skeletal muscles, Thesleff[18] has shown that during continuous application of ACh, depolarization of muscle fibre membranes is not maintained (the resting potential gradually returns to its normal level, even though the ACh is still present). Thus muscle fibres become refractory to the action of ACh.

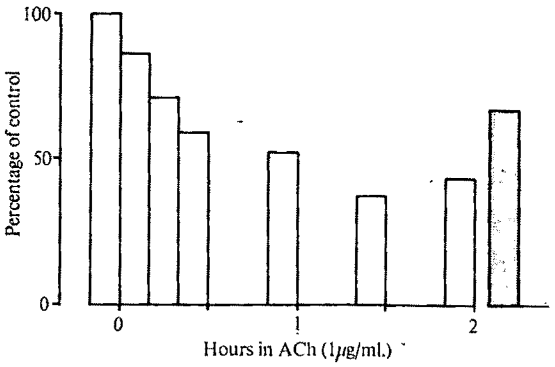


Fig. 6 Effect of desensitization of ACh-receptors on binding of α-BuTX. One of each pair of muscles was exposed to toxin (2 μg/ml. for 10 min) after being in ACh (AChCl+neostigmine HBr both 1 μg/ml.) for the indicated times. The other muscle from each pair was exposed to the toxin without prior treatment with ACh and served as control. All muscles were placed in ACh for a total of 3 h. One muscle (shaded bar) was removed from the ACh after 2 h and washed repeatedly for 5 min before being exposed to toxin, after which it was returned to ACh for 1 h.

This process of "desensitization" was later studied in more detail by Katz and Thesleff[19] and others, and to explain it several hypotheses have been proposed[19–23].

In principle, the loss of action of ACh during sustained drug application could occur at any step from the ACh+receptor combination to the development of the ionic current responsible for membrane depolarization. An irreversible antagonist such as α-BuTX might produce some clues to this problem. For instance, if desensitization were to involve changes in the later steps, subsequent to the combination of ACh with the receptors, then the binding of α-BuTX in desensitized muscles might be expected to be the same as in a normal muscle. Alternatively, the change might occur at the receptor level and perhaps reduce the binding of toxin.

To examine these possibilities, muscles were exposed to ACh (10^{-6} to 10^{-4} g/ml.) in the presence of neostigmine methylsulphate (10^{-6} g/ml. to prevent hydrolysis of ACh by cholinesterases). Acetylcholine caused a marked and immediate depolarization of the end-plates, followed by a gradual recovery of the resting potential. One to three hours after commencing the application of ACh, the muscle bath was rinsed several times with normal Ringer to wash out the ACh, and approximately 10 min later the muscle was exposed to α-BuTX (10^{-5}—3.6×10^{-5} g/ml.) for 5–10 min, and rinsed again several times. When these muscles had recovered from the desensitization caused by ACh, miniature end-plate potentials could be recorded from many surface fibres, and stimulation of the nerve evoked end-plate potentials which sometimes triggered muscle action potentials, in spite of having been exposed to the toxin. In contrast, muscles which had not been desensitized by ACh were blocked by the same treatment with α-BuTX. These experiments indicated clearly that desensitized muscles are less susceptible to the blocking action of α-BuTX, and further experiments were carried out to see if the binding of toxin was also reduced.

The binding of α-BuTX to muscles exposed to ACh for various times is progressively decreased as the muscles become desensitized (Fig. 6). During simultaneous exposure to ACh and α-BuTX, some competition for receptive sites may occur but this, however, would presumably have a faster time course than the decrease in binding illustrated in Fig. 6. A further indication that the decrease in binding is due to desensitization of receptors is that binding was still reduced 5 min after removing the ACh (shaded bar in Fig. 6). In other experiments it was found that a higher concentration of ACh (10^{-4} g/ml.) already reduced toxin binding maximally (81%) when applied simultaneously with the toxin, and that full recovery of binding occurred with a time course roughly parallel to re-sensitization of the muscle fibre membrane.

In their model of desensitization, Katz and Thesleff[19] proposed that receptors can exist in either an active or a refractory form. Their experimental results were best fitted to a scheme in which the affinity of the drugs for desensitized receptors was much higher than for resting receptors. More recently, Rang and Ritter[24] have found that certain bisquaternary drugs have a preferential affinity for desensitized receptors. It seems clear, however, that desensitization of the muscle fibre membrane by ACh reduces its ability to bind α-BuTX but it is not known how this is brought about. There are, of course, several possible explanations. For example, it could be that ACh molecules remain on the receptor for many seconds—instead of merely a fraction of a second—and that during that time receptors are unable to combine with the toxin. Another possibility is that activation of a receptor by ACh leads to a slowly-reversible change in the structure of the receptor, a change which prevents further combinations with ACh or toxin molecules.

Solubilization of the Toxin-binding Material

^{131}I-labelled α-BuTX saturates binding sites in washed fragments of membranes from *Torpedo* electric tissue, and the toxin-tagged material can be "solubilized" (brought into a form which does not sediment during ultracentrifugation for several hours) with the non-ionic detergent, 'Triton X-100' (ref. 27).

Preliminary studies of the toxin binding sites of frog sartorius muscles by the same procedures (Fig. 7) indicate that the labelled substances in muscles and electric tissue are very

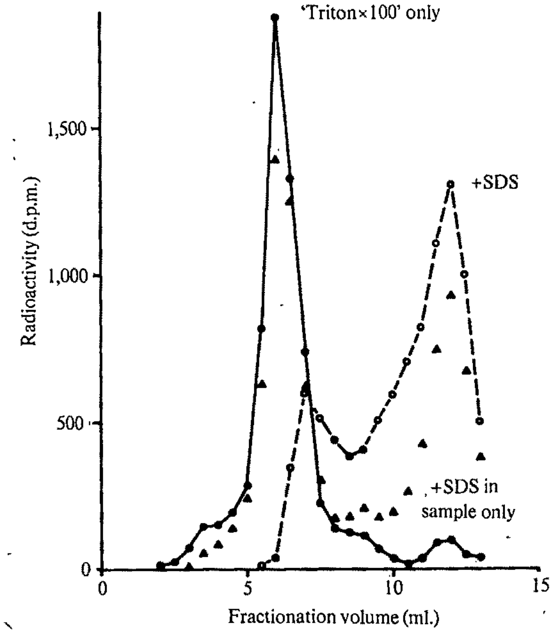


Fig. 7 Ultracentrifugation of toxin-binding material. Twelve frog sartorius muscles labelled with ^{131}I-α-BuTX were homogenized in fifteen times their weight of 20 mM sodium phosphate buffer, *p*H 7.5. Large particles including cell membranes were sedimented at 10^6g-min, and were extracted with 3 ml. of 1.5% 'Triton X-100' in 20 mM buffer for 2 h at 4° C. After recentrifugation, 0.5 ml. samples of the clear extract ±2.5 mg sodium dodecyl sulphate (SDS) were centrifuged over linear density gradients (5–25% sucrose in 20 mM buffer+1% 'Triton X-100' ±0.5% SDS; 12.5 ml. in Spinco SW 40 Ti tubes) for 22 h at 39,000 r.p.m. Results are given as d.p.m. ^{131}I per 0.5 ml. fraction; volumes are measured from the bottom of each centrifuge tube. The mobility of the toxin-binding material in 'Triton X-100', and its partially-reversible disaggregation in SDS, appear the same as that previously seen with toxin-binding material from *Torpedo* electric tissue[27].

similar. After toxin-labelled sartorius muscles were disintegrated with a high-speed blender in dilute buffer, 93% of the ^{131}I sedimented with cell particles during ultracentrifugation. Extraction of the sediment with 'Triton X-100' permitted quantitative recovery of the labelled substance in the supernatant fluid after recentrifugation. Examination of this material by density gradient centrifugation indicated that most of it sedimented in one peak, exactly as did similarly-treated toxin receptor from *Torpedo* electric tissue (compare Fig. 7 with Fig. 4*B* from ref. 27). In addition, SDS reversibly disaggregated the toxin-tagged material from muscles. These results indicate the feasibility of extensive purification and characterization of the muscle receptor for α-BuTX.

Toxin-receptive Sites and ACh-receptors

The correspondence between the pattern of ACh-sensitivity and the localization of bound toxin, in normal and denervated muscles, already indicates a strong similarity between ACh- and toxin-receptive sites; but confirmation that they are identical will have to await the elucidation of the detailed structure of the receptor. It should, however, be mentioned here that the effects of dTc, and in particular the experiments on muscles desensitized by ACh, suggest that ACh and α-BuTX act on the same site, or on sites close enough to allow interactions.

On the assumption that one toxin site is equivalent to one ACh-receptor, there are about 10^9 ACh-receptors in a frog muscle fibre. This is only a preliminary estimate, but already some interesting points emerge if considered in relation to the recent suggestion by Katz and Miledi[25,26] that the depolarizing action of ACh is built up of elementary events with a calculated amplitude of 0.22 μV.

The neural transmitter is released in quanta, each of which would evoke about 2,000 elementary events; and a nerve impulse, releasing say 300 quanta, would evoke 6×10^5 elementary events. The question is still open whether one elementary event is caused by the activity of one or more receptors; but assuming, for the time being, that one active receptor is equivalent to one elementary event, a nerve impulse would activate less than one thousandth of the available receptors. Thus there seems to be a large number of spare receptors, even if each elementary event were to require the activation of ten receptors, which is not likely.

During external application of ACh, about 10^7 elementary events are required per second to maintain a depolarization of 10 mV (compare ref. 25). There would, therefore be only sufficient receptors to maintain this depolarization for 100 s if each receptor were active only once. As such a depolarization can be maintained for longer, it follows that in this case some receptors are activated more than once. With larger doses of ACh a receptor might be active only once, or a few times, before the muscle is desensitized.

Another point of interest concerns the average density of receptors at an end-plate. Consider an average end-plate in the frog sartorius, with a total synaptic length of about 600 μm (unpublished results of R. M.). Assuming a 3 μm wide contact and 1,200 synaptic folds—each with 10 μm^2 of membrane—the total synaptic membrane would then be 1.4×10^4 μm^2. If 10^9 receptors occupied only the synaptic area, there would be in the order of 10^5 receptors per μm^2. The average separation between receptor centres would then be about 3.2 nm, which would make it difficult to accommodate all the receptors in a monolayer and still leave space to accommodate the ionophores and the cholinesterase molecules known to be at the end-plate. The existence of extra-junctional receptors, however, must also be taken into account. Their distribution on the muscle fibre membrane can be estimated from the spatial pattern of ACh-sensitivity, but for the moment let us say that the synaptic area contains one-fifth of the receptors. In such a case the inter-receptor distance would still be only about 8.4 nm. But the ionophore need not be a separate molecular arrangement, since the receptor macromolecule may provide the ionic "channel"; and the cholinesterase may not be located in the membrane matrix but instead in the basement membrane, as suggested by the recent experiments of Betz and Sakmann[28].

It is interesting that, as in eel[14] and *Torpedo*[27] electric tissue, the calculated numbers of active sites of cholinesterase in frog[29], rat[30] and mouse[31] muscle fibres seem to be within an order of magnitude of the number of toxin-binding sites. This correspondence is particularly interesting because the turnover number for the enzyme is very high (about 1–10 molecules per msec per active site[29]) whereas the receptors desensitize and recover slowly.

Finally, the activity of an ACh-receptor can be predicted from preliminary estimates of the number of "effective" ACh-molecules. On collision theory, the number of collisions on a surface is $Z = nS\sqrt{\dfrac{kT}{2\pi m}}$, where n is the number of molecules per ml., S is the surface area and m the mass of the molecule[32]. During application of, say, 10^{-7} g/ml. of ACh to a sartorius muscle, and assuming that the ACh-receptor presents a target area of 20 Å^2, and disregarding hydration, there would be 3.3×10^{13} collisions/s on the receptors (i.e., 3.3×10^4 collisions/s on each receptor). If the ACh depolarized the muscle by about 10 mV, requiring 10^7 elementary events/s, then only 3×10^{-7} of the collisions are effective. This may be so, not only because many collisions have a low energy, but also because in all likelihood there are restrictions on the orientation for effective collisions. If more than one active receptor is required to elicit an elementary event, or if the target area is smaller than assumed, the number of effective collisions would be greater than estimated; all of which tends to suggest that the ACh-receptor is a rather reactive molecule.

Received July 20, 1971.

[1] Katz, B., *The Release of Neural Transmitter Substances* (Liverpool University Press, 1969).
[2] Miledi, R., *J. Physiol.*, **151**, 24 (1960).
[3] Brunton T. L., and Fayrer, J., *Proc. Roy. Soc.*, B, **21**, 358 (1873); Brunton, T. L., and Fayrer, J., *Proc. Roy. Soc.*, B, **22**, 68 (1874).
[4] Chang, C. C., *J. Formosan Med. Assoc.*, **59**, 315 (1960); Chang, C. C., *J. Formosan Med. Assoc.*, **59**, 416 (1960).
[5] Chang, C. C., and Lee, C. Y., *Arch. Intern. Pharmacodyn.*, **144**, 241 (1963).
[6] Lee, C. Y., Tseng, L. F., and Chiu, T. H., *Nature*, **215**, 1177 (1967).
[7] Pressman, D., and Eisen, H. N., *J. Immunol.*, **64**, 273 (1950).
[8] Miledi, R., *Enzymes and Drug Action*, 220 (edit. by Monger, J. L., and de Reuck, A. W. S.) (Churchill, London, 1962).
[9] Katz, B., and Miledi, R., *J. Physiol.*, **170**, 379 (1964).
[10] Katz, B., and Kuffler, S. W., *J. Neurophysiol.*, **4**, 209 (1941).
[11] Miledi, R., *J. Physiol.*, **151**, 1 (1960).
[12] Krnjević, K., and Miledi, R., *J. Physiol.*, **140**, 472 (1958).
[13] Waser, P., in *Molecular Properties of Drug Receptors*, 59 (edit. by Porter, R., and O'Connor, M.) (Churchill, London, 1970).
[14] Changeux, J-P., Kasai, M., and Lee, C. H., *Proc. US Nat. Acad. Sci.*, **67**, 1241 (1970).
[15] Axelsson, J., and Thesleff, S., *J. Physiol.*, **147**, 178 (1959).
[16] Beranek, R., and Vyskocil, F., *J. Physiol.*, **188**, 53 (1967).
[17] Miledi, R., *J. Physiol.*, **154**, 190 (1960).
[18] Thesleff, S., *Acta Physiol. Scand.*, **34**, 218 (1955).
[19] Katz, B., and Thesleff, S., *J. Physiol.*, **138**, 63 (1957).
[20] Nastuk, W. L., Manthey, A. A., and Gissen, A. J., *Ann. NY Acad. Sci.*, **137**, 999 (1966).
[21] Nastuk, W. L., and Parsons, R. L., *J. Gen. Physiol.*, **56**, 218 (1970).
[22] Taylor, D. B., and Nedergaard, O. A., *Physiol. Rev.*, **45**, 523 (1965).
[23] Rang, H. P., and Ritter, J. M., *Mol. Pharmacol.*, **6**, 357 (1970).
[24] Rang, H. P., and Ritter, J. M., *Mol. Pharmacol.*, **6**, 383 (1970).
[25] Katz, B., and Miledi, R., *Nature*, **226**, 962 (1970).
[26] Katz, B., and Miledi, R., *Nature* (in the press).
[27] Miledi, R., Molinoff, P., and Potter, L. T., *Nature*, **229**, 554 (1971).
[28] Betz, W., and Sakmann, B., *Nature New Biology*, **232**, 94 (1971).
[29] Nachmansohn, D., *Chemical and Molecular Basis of Nerve Activity* (Academic Press, New York and London, 1959).
[30] Hall, S. W., and Kelly, R. B., *Nature*, **232**, 62 (1971).
[31] Barnard, E. A., and Rogers, A. W., *Ann. NY Acad. Sci.*, **144**, 584 (1967).
[32] Moelwyn-Hughes, E. A., *The Kinetics of Reactions in Solutions* (Clarendon Press, Oxford, 1947).

Molecular Evolution in the Descent of Man

MORRIS GOODMAN & JOHN BARNABAS

Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48207, and
Plymouth State Home and Training School, Northville, Michigan 48167

GENJI MATSUDA

Department of Biochemistry, Nagasaki University School of Medicine, Nagasaki

G. WILLIAM MOORE

Institute of Statistics, Biomathematics Program, North Carolina State University, Raleigh, North Carolina 27607

Three computer programs applied to data from phylogenetic trees and protein polymorphism can be used to find the rate of molecular evolution of different species. It can be shown that this is slower for higher primates than for other mammals.

It is likely that the level of molecular organization in higher animals is now more complicated than would have been possible in the first life forms of three billion years or so ago. Even after the emergence of the genetic code and the biochemical apparatus of translation, natural selection would have had to act over long stretches of evolutionary time to produce the sophisticated structures and functions of the multichained proteins now found in living organisms. To begin with, almost any point mutation in a gene coding for a polypeptide chain would have been tolerable; with the evolution of higher levels of molecular organization, however, increasingly stringent functional restraints would have markedly decreased the number of acceptable mutations.

The notion that the level of molecular organization places restraints on how fast genes evolve is implicit in Ingram's hypothesis[1] that the genes for alpha globin have changed more slowly than those for beta globins. The same alpha chain combines with different types of beta chains in the several tetrameric haemoglobins which are synthesized during the life of a mammal. Since there are more restraints on variations of the alpha chains than of the beta chains, fewer mutations would be tolerated at an alpha locus than at a beta locus and alpha genes would not evolve as rapidly as beta genes. In the same way, there may be differences in the rates of evolution of the globin genes at earlier and later evolutionary stages. The original single-chained globin would no doubt have been recognizable by its three-dimensional structure and haem binding site, but it would yet have had to acquire specific sites for subunit combination, for binding diphosphoglycerate, for allosteric modulation of structure to promote the Bohr effect and for combining with the plasma protein haptoglobin. With these functions, natural selection tolerated fewer mutations in the globin genes. From this it follows that the primordial globin genes evolved more rapidly than the later globin genes of vertebrates, consonant with an earlier hypothesis that molecular evolution at a number of gene loci has decelerated, especially in the lineage leading to man[2-4].

This hypothesis, followed by others[5,6], attributes divergent evolution in proteins to selectively neutral mutations but also implies that more mutations were neutral in primitive organisms than in descendant species. Thus biochemical adaptations in human antecedents must have increased with the development of new organs such as the placenta and the cerebral neocortex and the extra functional restraints would then decrease the chances for fresh mutations to accumulate. An immunological mechanism could have further restricted the degree of genetic variability in higher primate populations, for the haemochorial placenta (with its intimate apposition of foetal and maternal blood circulations) and the long gestation period would have increased the risk to the foetus from maternal immunizations to foetal allotypes.

We present evidence in this article that molecular evolution has indeed been slower in higher primates than in other mammals. In particular, from a detailed analysis of the phylogeny of globin genes, we support Ingram's hypothesis that the evolution of alpha globin genes has been slower than that of the beta genes. Available data on proteins also bear on the cladistic relationships of man and other mammals.

The most penetrating view of the molecular changes in individual genes during the descent of man is provided by comparing the amino-acid sequences of human proteins with homologous proteins in other organisms. We have followed the approach of Fitch and Margoliash[7] in constructing gene phylogenetic trees from amino-acid sequence data and for this purpose have used three computer programs. The first program (MMUTD) calculates mutation distances for every pairwise comparison of aligned amino-acid sequences using a matrix based on the genetic code which gives for each amino-acid pair the minimum number of nucleotides that would need to be changed to bring about such a mutation. The second program[6] (UWPGM) produces from a dissimilarity matrix of the mutation distances a dendrogram of the gene species in the set. This dendrogram is an initial approximation to a cladogram, that is a graph which depicts the order of ancestral branching in the gene species set. The UWPGM builds the tree from the smallest to the largest branches in a series of pairwise clustering cycles, each time grouping together the two members of the set (either singleton species or joined species from a previous cycle) with the smallest mutation distance between them. If all the species in the set had evolved at a uniform, or nearly uniform, rate in their descent from a common ancestor, the dendrogram produced by the UWPGM would be the true cladogram[9]. But neither gene species nor animal species necessarily evolve at uniform rates. Thus, with the third computer program (DENDR) we construct alternative dendrograms. The DENDR program calculates the patristic mutation length * for each pair of adjacent nodes in a dendrogram, sums the patristic lengths through the sequence of nodes between species to calculate a reconstructed mutation distance for each pair of species in the set, and then compares the reconstructed mutation distances to the given mutation distances in the original dissimilarity matrix to calculate a coefficient for the entire dendrogram called "average percentage standard deviation" (APSD)[7]. Among the alternative dendrograms the one with least deviation between original and reconstructed mutation distances (that is, the lowest APSD coefficient) is considered the closest approximation of those tried to a true phylogenetic tree. This approach to finding the best fitting tree has proved capable of capturing the cladistic relationships among gene species even when markedly dissimilar amounts of mutational change characterize their descent from a common ancestor[10].

Globin Phylogenetic Tree

Table 1 is the matrix of mutation values from all possible pairings of sixty-eight metozoan globin chain sequences[11-47]. These chains range in size from 135 to 153 amino-acid residues. They include, apart from carp alpha, chicken alpha, and mammalian alpha and non-alpha haemoglobin chains, a globin of lamprey, myoglobins of horse and sperm whale, and a globin of the insect chironomus. The chicken alpha chain is the first non-mammalian tetrapod haemoglobin chain to be sequenced[17]. It diverges less from human (and chimpanzee) alpha than from any other mammalian alpha (Table 1). This is not due to any special relationship between chicken and man, but, as will be shown, results from the marked conservativism of higher primate alpha chains.

The best tree so far obtained depicting the phylogeny of the globins is given in Fig. 1. Fig. 2 shows the best of forty-one alternatives tried for the restricted set of tetrapod alpha chains, and Fig. 3 the best tree of forty-five alternatives tried for the restricted set of mammalian non-alpha or beta-like globin chains. The APSD coefficient was lowered from 15.11 for the alpha tree constructed by the UWPGM to 11.14 for the tree in Fig. 2, and from 11.32 for the beta-like tree by the UWPGM to 8.40 for the tree in Fig. 3. The original tree for the sixty-eight globin species constructed by the UWPGM had an APSD coefficient of 18.52. This was lowered by several points on trying a dendrogram which followed the branching order shown in Figs. 2 and 3, but which otherwise maintained the topology of the UWPGM tree. Shifting (*a*) the carp alpha branch from its UWPGM union with other alphas to a union with the line ancestral to both mammalian beta-like globins and tetrapod alphas, and (*b*) the lamprey globin branch from its UWPGM union with the branch ancestral to all the globin chains in vertebrate haemoglobins over to union with the myoglobin branch, lowered the APSD coefficient to 12.27 and produced the tree shown in Fig. 1. So far eighteen alternative configurations for the major branches of the large globin tree have been tried.

Course of Globin Evolution

The recognizable homologies between insect haem binding protein and vertebrate myoglobins and haemoglobins in codon sequence and in three-dimensional structure and functional properties[47] place the primordial globin gene deep in the Pre-Cambrian epoch before the protovertebrates had embarked on an independent course of evolution. Fig. 1 reveals that when man and lamprey still had a common ancestor, the primitive vertebrate genome already had more than one gene locus coding for globin chains; it was then that a gene duplication separated the ancestor of myoglobin genes from that of haemoglobin genes. Almost immediately after the myoglobin–haemoglobin duplication, the line leading to the globin in lamprey split off from the myoglobin side as a separate branch. Myoglobins are single chained proteins. Similarly, lamprey globins, including the one which has been sequenced, are single chained, although in certain physico-chemical conditions these lamprey globin chains form larger aggregates[45].

In the early bony fishes ancestral to the land vertebrates a haemoglobin gene duplicated to produce the split between the alpha and beta-like chain genes. This is depicted in Fig. 1 where the tetrapod alpha and beta-like lines separate from each other immediately after their separation from the carp alpha line. Much later in the lineage leading to therian mammals a gamma–beta chain gene duplicated to produce, on the one hand, the first separate ancestor of the genes for the kangaroo non-alpha chain, the human gamma chain (found in the haemoglobin of the human foetus) and the lemuroid non-alpha chains and, on the other hand, the separate ancestor for the mammalian beta chain genes. The first separate ancestor of the lemuroid (lemur and sifaka) non-alpha globin chains may have been a gamma–beta hybrid produced by unequal homologous crossing over between originally linked gamma and beta genes. There are two residues (13 Ser, 21 Glu) in kangaroo, lemur and sifaka chains and three additional residues in the lemur and sifaka chains (9 Ala, 50 Ser, 52 Ser) which are homologous to human gamma chain. Furthermore, lemur and sifaka chains resemble more human gamma from the N-terminal end than human beta and resemble more human beta from the C-terminal end than human gamma.

At the nodes of Fig. 2 for the two most ancient branching points among mammalian alpha genes, ancestral rabbit and tree shrew genes diverged from each other immediately after their separation from the branch to all other mammalian alphas. Tree shrew alpha actually shows a greater mutation distance from rabbit, and rabbit from tree shrew, than either shows from other mammalian alpha chains (Table 1). This type of pattern, which resembles that of the human gamma, kangaroo and lemuroid non-alpha branch of the beta-like tree, suggests that alpha gene duplications had occurred in the early therian mammals producing ancestral rabbit and tree shrew genes and also the ancestor of other mammalian alphas. Recent duplications of alpha genes are known to occur in present day mammals, for example, in buffalo[50], horse[27], goat[52], macaques[54], mouse[56] and probably rabbit[18].

During eutherian phylogeny, beta chain genes duplicated

---

* The patristic mutation lengths associated with any node, N, on a dendrogram are calculated as follows. Any node N divides the species of which it is the common ancestor into two sets: set A and set B. All remaining species under study comprise set C. Let D_{ab} be the average of dissimilarity values for pairs of species such that the first species is a member of set A and the second species is a member of set B, and let D_{ac} and D_{bc} be similarly calculated. The length from set A to node N is called a; the length from set B to node N is called b; and the length from set C to node N is called c. These lengths are calculated by the following formulas:

$$a=\frac{D_{ab}+D_{ac}-D_{bc}}{2}$$

$$b=\frac{D_{ab}+D_{bc}-D_{ac}}{2}$$

$$c=\frac{D_{ac}+D_{bc}-D_{ab}}{2}$$

For the special case that N is the apex of the tree (that is, the common ancestor of all species under study): $a=b=\frac{D_{ab}}{2}$

c is undefined.

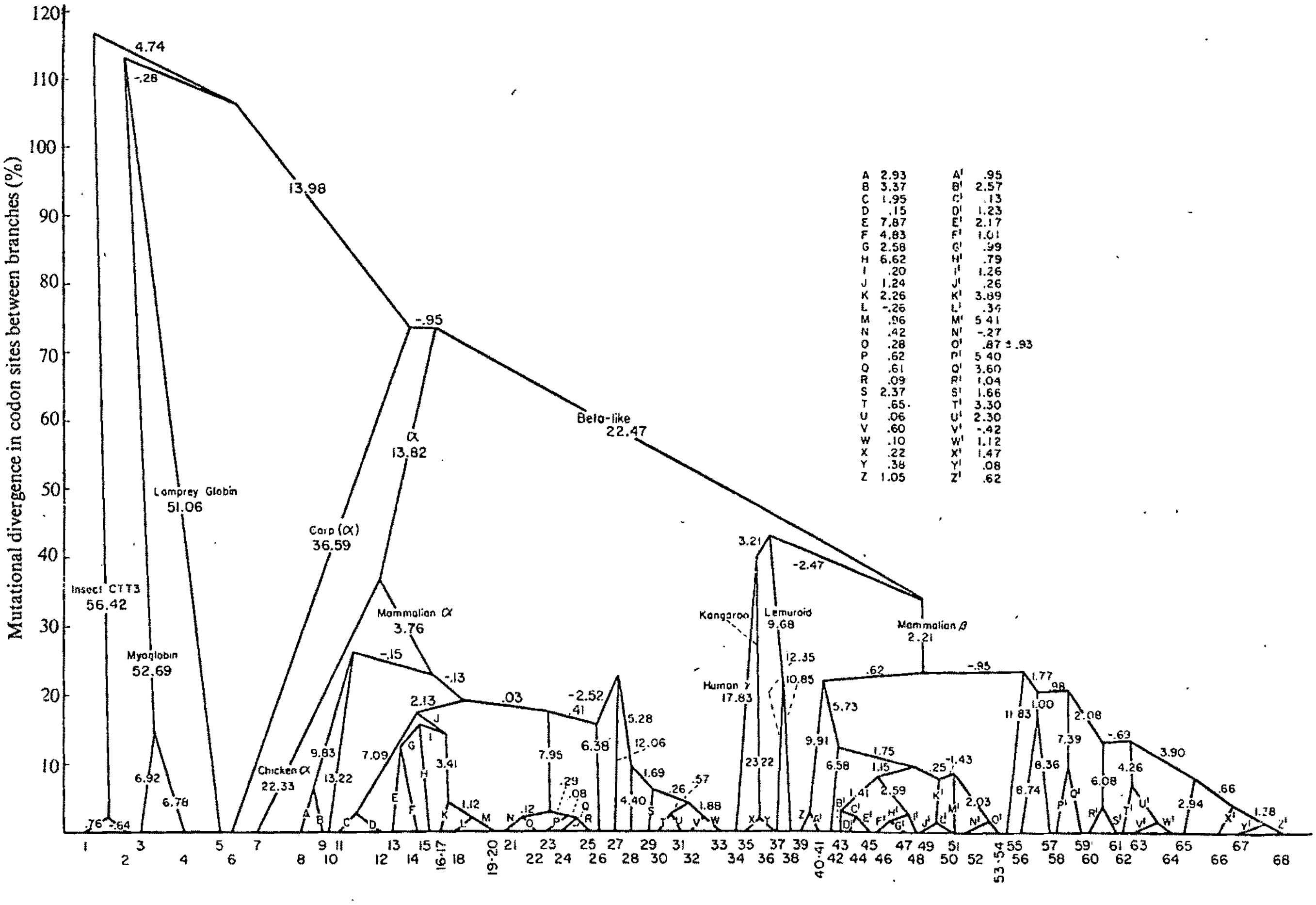


Fig. 1 Globin phylogenetic tree. 1, Insect CTT-3 (*Chironomus thummi*)[11]; 2, insect CTT-3 (sub)[11]—alternative amino-acids (39 Thr, 57 Ile) were used for ambiguous positions; 3, myoglobin sperm whale[13]; 4, myoglobin horse—sequence alignment as given in ref. 14; 5, globin lamprey[15]; 6, alpha carp[16]; 7, alpha chicken[17]; 8, alpha rabbit[18]; 9, alpha rabbit (sub)[18]—alternative amino-acids (29 Leu, 48 Leu, 70 Thr, 76 Val, 80 Ser, 113 His) were used for ambiguous positions; 10, alpha tree shrew[19]; 11, alpha mouse NB[20,21]; 12, alpha mouse C-57Bl[20,21]; 13, alpha sifaka (*Propithecus verreauxi*)[19]; 14, alpha lemur (*Lemur fulvus*)[19]; 15, alpha bush baby (*Galago crassicaudatus*)[19]; 16, alpha *Macaca mulatta*[22]; 17, alpha *Macaca fuscata* (G. Matsuda, unpublished); 18, alpha gorilla[23]; 19, alpha chimpanzee[24]; 20, alpha human[25,26]; 21, alpha donkey[27]; 22, alpha horse slow (24 Phe)[27]; 23, alpha horse fast (24 Phe)[27]; 24, alpha horse fast (24 Tyr)[27]; 25, alpha horse slow (24 Tyr)[28]; 26, alpha pig—sequence alignment as given in ref. 14; 27, alpha llama—sequence alignment as given in ref. 14; 28, alpha bovine[29]; 29, alpha goat II (non-allelic)[30]; 30, alpha goat B (allelic)[30]; 31, alpha goat A[30]; 32, alpha sheep D[31]; 33, alpha sheep A[32,33]; 34, gamma human[34]; 35, beta kangaroo[35]; 36, beta kangaroo-2 (2 Gln)[35]; 37, beta sifaka[19]; 38, beta lemur[19]; 39, beta mouse AKR[36]; 40, beta mouse Sec[36]; 41, beta mouse C-57Bl[36]; 42, beta rabbit[37]; 43, beta squirrel monkey (*Saimiri sciures*)[38]; 44, beta tamarin (*Saguinus nigricollis*)[38]; 45, beta spider monkey (*Ateles geoffroyi*)[38]; 46, delta squirrel monkey[38]; 47, delta tamarin[38]; 48, delta spider monkey[38]; 49, beta *Macaca fuscata*[39]; 50, beta *Macaca mulatta*[22]; 51, delta human[40]; 52, beta gorilla[23]; 53, beta chimpanzee[24]; 54, beta human[25]; 55, beta horse slow[42]; 56, beta pig—sequence alignment as given in ref. 14; 57, beta llama—sequence alignment as given in ref. 14; 58, beta bovine foetal[42]; 59, beta sheep foetal[43]; 60, beta bovine A[44]; 61, beta bovine B[44]; 62, beta sheep barbary C[45]; 63, beta goat C[47]; 64, beta sheep C[46]; 65, beta sheep B[46]; 66, beta sheep A[46]; 67, beta goat A[47]; 68, beta goat A′ (allelic)[47].

independently in separate mammalian lines and certain of the descending duplicated genes ultimately coded for new proteins. For example, postnatal human blood contains in addition to its major haemoglobin (A) a minor haemoglobin (A_2) in which a different beta chain, called delta chain[1,57], combines with alpha chain. Similarly, the ceboid monkeys have a minor haemoglobin[38,58] with a beta chain (again called delta) different from the beta chain of the principal ceboid haemoglobin. Fig. 3 depicts in the early Anthropoidea, shortly after the branching apart of ceboids and catarrhine primates, two independent beta gene duplications: one on the catarrhine side coincided with the hominoid–cercopithecoid split to give rise to hominoid delta, and the other on the ceboid side produced the ancestor of ceboid deltas. Independent beta gene duplications also occurred during the evolution of the ruminants. Before the splitting apart of bovines and caprines, a descendant gene from a beta duplication in the early ruminant line began to code for the bovid foetal beta chain. The caprines (but not the bovines) have, like hominoids and ceboids, a second adult haemoglobin[45–47,59] (in this case, called haemoglobin C) normally present in negligible quantities, but synthesized in large amounts in animals under severe anaemic stress. In this second adult caprine haemoglobin, the beta C chain is found rather than the regular beta chain of caprine haemoglobin A. The ancestral *A–C* gene duplicated in the caprine line to give rise to the linked *A* and *C* gene loci shortly after the ancestral separation of bovines and caprines (Fig. 3).

Later in caprine evolution, at some point before the ancestral separation of sheep and goat, a mutation at the A locus produced the first separate ancestor of the present day sheep beta B gene. The period of separate evolution of sheep betas B and A seems to be greater than that of sheep and goat beta As. Present day sheep beta B differs from sheep or goat beta A by seven point mutations, whereas sheep beta A differs from goat beta A by four point mutations (Table 1). The sheep A and B-beta results illustrate that typical allelic proteins within a mammalian population can differ by several or more amino-acids. Bovine A and B beta alleles provide another example of such allelic variation; they differ by four point mutations

Table 1 Mutation Values from Pairwise Comparisons of Vertebrate Globin Sequences

Full name of globin species given in legend to Fig. 1. The half matrix lists minimum numbers of mutations interrelating pairs of polypeptide chains. These mutational values are converted into percentage mutational divergence

$$\left(\frac{\text{No. of mutations}}{\text{No. of shared amino-acids}} \times 100\right)$$

values, the mutation distances used for constructing the phylogenetic tree in Fig. 1. Alpha llama, sheep foetal beta, and goat A′ beta are not completely sequenced. Hence their mutation values may not represent full mutation values.

	INSECT CTT3	INSECT CTT3 SUB	Mb. S. WHALE	Mb. HORSE	GLOBIN LAMPREY	α CARP	α CHICKEN	α RABBIT	α RABBIT SUB	α TREE SHREW	α HORSE SLOW	α HORSE FAST	α HORSE SLOW 24F	α HORSE FAST 24F	α DONKEY	α PIG	α LLAMA	α BOVINE	α GOAT II	α GOAT B	α GOAT A	α SHEEP D	α SHEEP A	α MOUSE NB	α MOUSE C-57	α SIFAKA	α LEMUR	α BUSH BABY	α MULATTA	α FUSCATA	α GORILLA	α CHIMP	α HUMAN	γ HUMAN	β KANGAROO	β KANGAROO -2	β SIFAKA	β LEMUR	β HORSE SLOW	β PIG	β LLAMA	β BOVINE F	β SHEEP F	β BOVINE A	β BOVINE B	β BARBARY C	β SHEEP C	β GOAT C	β SHEEP B	β SHEEP A	β GOAT A	β GOAT A′	β MOUSE SEC	β MOUSE C-57	β MOUSE AKR	β RABBIT	β SP. MONKEY	β TAMARIN	β SQ. MONKEY	δ SQ. MONKEY	δ TAMARIN	δ SP. MONKEY	β MULATTA	β FUSCATA	δ HUMAN	β GORILLA	β CHIMP	β HUMAN
INSECT CTT3	0																																																																		
INSECT CTT3 SUB	2	0																																																															
Mb. S. WHALE	160	159	0																																																													
Mb. HORSE	164	163	21	0																																																											
GLOBIN LAMPREY	145	145	149	152	0																																																									
α CARP	154	154	150	146	130	0																																																							
α CHICKEN	142	143	155	159	130	101	0																																																					
α RABBIT	145	145	153	153	123	94	54	0																																																				
α RABBIT SUB	143	143	154	154	127	96	53	9	0																																																		
α TREE SHREW	145	145	148	151	125	93	60	37	37	0																																																	
α HORSE SLOW	137	137	153	154	120	94	51	31	33	35	0																																																
α HORSE FAST	138	138	153	155	120	95	52	32	34	36	1	0																																														
α HORSE SLOW 24F	138	138	154	155	120	93	52	32	34	36	1	2	0																																													
α HORSE FAST 24F	139	139	154	156	120	94	53	33	35	37	2	1	1	0																																											
α DONKEY	138	138	154	155	119	92	53	33	35	36	2	3	1	2	0																																										
α PIG	138	138	146	148	119	88	56	28	29	31	21	20	21	20	22	0																																									
α LLAMA	130	130	140	139	107	75	48	28	28	28	21	22	22	23	23	24	0																																								
α BOVINE	141	142	143	146	120	92	50	29	31	29	23	23	24	24	25	18	26	0																																							
α GOAT II	138	139	140	144	116	89	55	30	30	25	24	23	25	24	24	22	27	11	0																																						
α GOAT B	138	139	141	145	116	89	53	31	33	25	21	20	22	21	21	20	27	10	5	0																																					
α GOAT A	137	138	143	146	116	88	52	30	32	25	20	19	21	20	20	19	26	10	4	1	0																																				
α SHEEP D	138	139	145	150	118	95	56	34	36	29	25	25	26	26	26	20	30	14	8	35	5	0																																		
α SHEEP A	139	140	145	149	118	95	56	34	36	28	24	24	25	25	25	19	29	13	7	4	4	1	0																																	
α MOUSE NB	143	144	149	150	119	91	54	34	32	28	28	29	29	30	30	29	26	24	27	27	26	31	30	0																																
α MOUSE C-57	143	144	148	149	118	88	51	31	29	26	27	28	28	29	29	26	23	21	24	24	23	28	27	3	0																															
α SIFAKA	148	148	154	156	120	96	57	34	33	33	37	38	38	39	39	32	32	34	34	36	35	38	38	30	27	0																														
α LEMUR	143	143	152	154	120	90	54	30	29	31	29	30	30	31	30	24	30	28	27	27	26	28	29	28	25	18	0																													
α BUSH BABY	144	144	153	156	118	93	49	32	31	30	27	28	28	29	29	25	26	25	24	24	23	27	27	25	22	25	19	0																												
α MULATTA	141	142	152	154	116	95	46	29	30	29	19	20	20	21	21	21	26	19	23	21	20	25	24	21	20	24	20	14	0																											
α FUSCATA	141	142	152	154	116	95	46	29	30	29	19	20	20	21	21	21	26	19	23	21	20	25	24	21	20	24	20	14	0	0																										
α GORILLA	137	138	146	148	109	89	45	28	29	31	21	22	22	23	23	20	22	20	23	22	21	26	25	22	19	24	18	13	6	6	0																									
α CHIMP	140	141	151	153	113	93	45	28	29	31	22	23	23	24	24	20	23	20	24	23	22	27	26	22	19	23	18	14	5	5	1	0																								
α HUMAN	140	141	151	153	113	93	45	28	29	31	22	23	23	24	24	20	23	20	24	23	22	27	26	22	19	23	18	14	5	5	1	0	0																							
γ HUMAN	165	164	159	157	138	105	109	105	105	108	99	100	99	100	99	98	90	101	102	100	100	102	101	104	101	106	105	106	106	106	100	105	105	0																						
β KANGAROO	166	165	160	155	144	114	114	113	113	116	109	109	109	109	109	105	102	106	107	105	105	109	108	114	111	114	113	115	112	112	108	111	111	60	0																					
β KANGAROO -2	166	165	160	155	144	114	114	113	113	116	109	109	109	109	109	105	102	106	107	105	105	109	108	114	111	114	113	115	112	112	108	111	111	61	1	0																				
β SIFAKA	164	163	161	157	144	110	113	114	115	119	106	107	105	106	105	27	98	109	109	106	105	107	107	115	113	115	111	115	112	112	107	112	112	51	72	72	0																			
β LEMUR	166	165	162	158	141	111	116	111	112	115	104	105	103	104	103	99	97	103	104	102	101	102	101	110	108	114	110	109	110	110	106	110	110	55	71	71	34	0																		
β HORSE SLOW	154	152	155	155	141	104	110	102	104	100	95	96	94	95	94	91	89	97	96	94	93	94	95	97	95	101	99	99	99	99	98	100	100	47	59	58	44	41	0																	
β PIG	152	152	146	143	135	107	107	103	104	101	95	96	94	95	94	90	89	94	94	92	91	93	92	100	98	106	101	102	99	99	98	100	100	50	52	53	43	39	26	0																
β LLAMA	149	149	155	153	142	107	107	102	102	98	95	95	94	94	94	90	88	92	92	90	89	90	89	96	93	103	99	97	98	98	98	98	98	48	54	53	49	44	29	25	0															
β BOVINE F	157	156	157	156	144	101	110	106	107	111	103	104	102	103	102	99	90	102	103	103	102	105	105	107	105	108	104	104	105	105	103	105	105	49	66	66	45	47	34	28	34	0														
β SHEEP F	138	139	141	142	135	89	95	94	95	97	86	87	85	86	85	86	78	89	91	89	88	89	89	93	91	96	92	92	89	89	89	90	90	46	55	55	43	43	38	31	37	12	0													
β BOVINE A	165	164	160	156	143	107	115	107	107	107	100	101	99	100	99	92	94	100	100	98	97	97	96	103	100	105	101	100	101	101	98	100	100	46	54	54	52	45	30	26	25	26	28	0												
β BOVINE B	168	167	161	158	142	107	114	107	107	108	98	99	97	98	97	92	95	100	100	98	97	97	96	104	101	105	102	101	102	102	99	101	101	48	56	56	51	45	31	26	29	27	27	4	0											
β BARBARY C	151	151	152	151	132	98	100	97	98	97	93	94	92	93	92	88	84	94	96	94	93	92	91	96	93	96	94	93	95	95	93	93	93	44	58	58	49	48	38	30	28	29	24	22	24	0										
β SHEEP C	157	156	158	157	137	101	103	98	100	98	92	93	91	92	91	87	83	92	93	91	90	89	88	96	93	96	95	94	95	95	92	93	93	45	60	60	48	48	35	32	24	34	28	23	25	9	0									
β GOAT C	154	154	157	156	132	98	98	95	97	94	89	90	88	89	88	85	81	89	91	89	88	86	85	93	90	93	92	91	92	92	90	90	90	41	57	57	46	45	34	31	25	32	26	23	25	8	1	0								
β SHEEP B	158	158	157	155	142	107	107	103	100	101	96	97	95	96	95	88	91	94	93	93	92	92	91	98	95	100	96	95	99	99	96	97	97	49	53	53	50	45	36	25	32	31	27	14	16	21	22	21	0							
β SHEEP A	157	157	156	154	134	103	105	100	99	97	94	95	93	94	93	87	86	90	89	91	90	89	88	94	91	96	93	92	95	95	92	93	93	50	57	57	50	45	37	30	32	30	28	17	19	19	18	16	7	0						
β GOAT A	158	158	153	151	135	102	104	101	98	98	95	96	94	95	94	88	87	91	90	92	91	90	89	95	92	97	93	92	96	96	93	94	94	48	54	54	51	45	39	30	33	30	27	14	16	18	19	17	7	4	0					
β GOAT A′	146	146	142	141	134	92	96	93	90	88	85	86	84	85	84	81	79	84	83	85	84	84	83	88	85	90	86	85	88	88	85	86	86	45	48	48	48	39	38	30	34	31	28	15	17	19	20	18	8	5	1	0				
β MOUSE SEC	149	148	150	148	133	100	103	99	100	101	94	95	94	95	94	87	87	94	93	90	89	91	90	98	95	102	99	97	98	98	94	96	96	44	58	59	49	47	38	36	39	44	40	37	39	37	39	37	39	39	39	36	0															
β MOUSE C-57	149	148	150	148	133	100	103	99	100	101	94	95	94	95	94	87	87	94	93	90	89	91	90	98	95	102	99	97	98	98	94	96	96	44	58	59	49	47	38	36	39	44	40	37	39	37	39	37	39	39	39	36	0	0													
β MOUSE AKR	153	152	149	147	132	99	102	97	98	100	93	94	93	94	93	88	86	93	92	89	88	90	89	97	94	101	98	96	97	97	93	95	95	45	57	58	49	47	40	38	41	44	41	38	40	39	41	39	40	40	41	38	3	3	0												
β RABBIT	160	159	163	161	143	109	110	104	105	109	101	102	100	101	100	97	95	101	97	95	94	97	96	105	103	110	104	102	102	102	100	103	103	46	51	52	42	39	30	27	25	37	36	30	32	33	37	34	31	32	32	30	32	32	33	0											
β SP. MONKEY	159	158	162	160	147	108	111	108	109	109	101	102	100	101	100	99	96	103	101	99	98	100	99	104	101	109	106	104	104	104	100	103	103	48	54	55	41	42	28	28	22	38	36	27	29	35	31	31	31	32	32	30	34	34	35	18	0										
β TAMARIN	156	155	162	160	143	109	106	104	105	105	97	98	96	97	96	96	93	99	98	96	95	96	95	100	97	105	102	100	100	100	97	99	99	44	53	54	39	39	28	30	25	40	37	30	32	36	33	32	32	34	34	32	35	35	36	16	5	0									
β SQ. MONKEY	157	156	161	159	145	108	112	107	108	109	100	101	99	100	99	97	94	102	101	99	98	100	99	105	102	109	105	104	104	104	99	102	102	47	58	59	40	42	30	30	26	39	37	28	30	35	31	31	32	33	33	31	35	35	36	20	6	7	0								
δ SQ. MONKEY	161	160	161	159	143	108	110	109	110	106	99	100	98	99	98	98	97	102	100	98	97	99	98	104	101	107	105	103	104	104	100	103	103	48	58	59	41	44	33	32	27	38	37	28	28	32	30	28	33	33	33	32	33	33	34	21	11	13	13	0							
δ TAMARIN	161	160	162	160	144	108	110	109	110	106	99	100	98	99	98	98	97	102	100	98	97	99	98	103	100	107	105	103	104	104	100	103	103	49	58	59	41	42	32	32	28	39	38	28	28	34	32	30	33	33	33	32	33	33	34	22	10	12	14	3	0						
δ SP. MONKEY	159	158	161	158	145	106	112	108	109	106	100	101	99	100	99	96	95	101	100	98	97	99	98	103	100	108	104	103	104	104	99	102	102	47	58	59	40	41	30	30	26	38	37	25	27	34	30	30	31	32	32	31	32	32	33	22	8	11	10	5	4	0					
β MULATTA	160	159	161	158	145	106	110	102	103	110	99	100	98	99	98	98	96	102	100	98	97	99	98	108	105	103	99	101	101	101	97	100	100	45	50	51	41	38	33	31	30	38	36	28	30	36	36	34	33	35	31	29	35	35	35	18	12	12	14	17	16	16	0				
β FUSCATA	160	159	161	158	145	106	110	103	104	111	100	101	99	100	99	99	97	103	101	99	98	100	99	107	104	104	100	102	102	102	98	101	101	44	50	51	40	37	32	30	29	37	35	27	29	35	35	33	32	33	30	28	34	34	34	17	11	11	13	16	15	15	1	0			
δ HUMAN	158	157	164	160	144	109	112	106	107	111	100	101	99	100	99	98	97	103	100	98	97	94	98	106	103	107	102	102	103	103	96	100	100	49	57	58	43	43	32	30	27	39	37	28	30	36	34	32	32	34	33	32	37	37	38	21	12	13	14	14	13	12	12	11	0		
β GORILLA	153	153	157	155	137	103	105	95	96	100	93	94	92	93	92	90	89	95	92	90	89	91	90	96	93	97	94	93	93	93	90	91	91	45	49	50	41	38	31	29	26	37	36	23	25	34	33	33	32	34	32	30	31	31	33	16	9	10	11	16	14	13	9	8	12	0		
β CHIMP	152	152	158	156	138	104	107	98	97	101	94	95	93	94	93	91	90	96	93	91	90	92	91	97	94	98	95	94	94	94	91	92	92	46	50	51	40	39	30	28	25	36	35	24	26	33	32	32	31	33	33	31	30	30	32	15	8	9	10	15	13	12	10	9	11	1	0	
β HUMAN	157	156	162	160	144	107	113	101	102	107	98	99	97	98	97	95	94	101	97	95	94	97	96	102	99	103	100	99	98	98	94	97	97	49	53	54	42	40	30	28	25	37	35	25	27	34	33	33	32	33	33	31	31	31	32	16	8	9	10	15	13	12	10	9	11	1	0	0

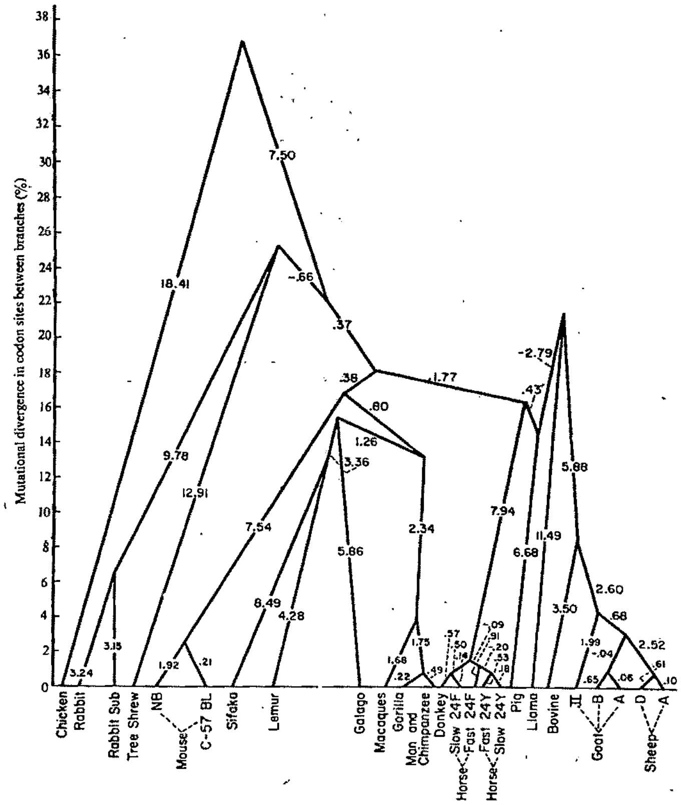


Fig. 2 Chicken and mammalian alpha globin phylogenetic tree.

(Table 1). Allelic human haemoglobins, on the other hand, differ from common human haemoglobin A mostly by only single point mutations.

The difference between bovid and human populations with respect to the kind of allelic variation expressed may be because the human foetus is at greater risk from maternal isoimmunizations than the bovid foetus. Bovids, like many mammals, have placentas of the epitheliochorial type[60] which separate the maternal and foetal vascular systems by several layers of tissue and thereby minimize the chance of maternal immune attacks on the foetus. In contrast the haemochorial placenta of the higher primates maximizes the opportunity for such attacks. Thus, the type of allelic beta chains with multiple differences in bovids, if found in man, could give rise to maternal immune reactions and be selected against. The first placentas to evolve in mammals were probably of the epitheliochorial type[60], so that the early mammals would not have been subjected to the immunological mechanism restricting genetic variability which we postulate for higher primate populations.

Rates of Globin Evolution

The patristic distance data, tabulated in Table 2, demonstrate that rates of evolution have varied markedly among vertebrate globin genes. The mammalian alpha genes diverge the least from the common vertebrate globin ancestor and the myoglobin genes; the group of non-alpha kangaroo, lemuroid, and human gamma genes diverge the most; lamprey globin, carp alpha, and chicken alpha each show more mutational change from this common ancestor than does any mammalian alpha (Fig. 1; Table 2, column 4).

While the patristic mutation lengths from Fig. 1 reveal that the proportion of codon positions showing mutations varied markedly among the principal branches of vertebrate globins, they do not show any especially marked variation among mammalian alpha genes, or among typical mammalian beta genes. But the patristic mutation lengths for the mammalian alpha portion of the globin tree from Fig. 2 and for the mammalian beta portion from Fig. 3 reveal that rates of evolution have indeed varied markedly both among mammalian alpha genes and among mammalian beta genes (Table 2, column 3). In both sets of mammalian genes, the most slowly evolving lines were those which led to man and other higher primates. In the large globin series (Fig. 1) the mutation distance between very anciently separated species, such as between any alpha and any beta globin chain, would be grossly underestimated due to uncounted multiple mutations at the same nucleotide sites. Thus, differences in rates of evolution among homologous mammalian genes, which are reflected in the patristic mutation lengths shown in Figs. 2 and 3, are smoothed out in the lengths shown in Fig. 1 where the apportioning of the mutation distances between the more recently separated genes is disproportionately affected by the mutation distance comparisons to the many, very anciently separated globin genes. Kimura[61] concluded that homologous genes evolved at uniform rates because about the same number of amino-acid substitutions separate any mammalian beta from any mammalian alpha and from carp alpha. But he failed to consider that the further in the past the separation of two lineages the larger the number of multiple mutations that must have occurred at the same nucleotide sites.

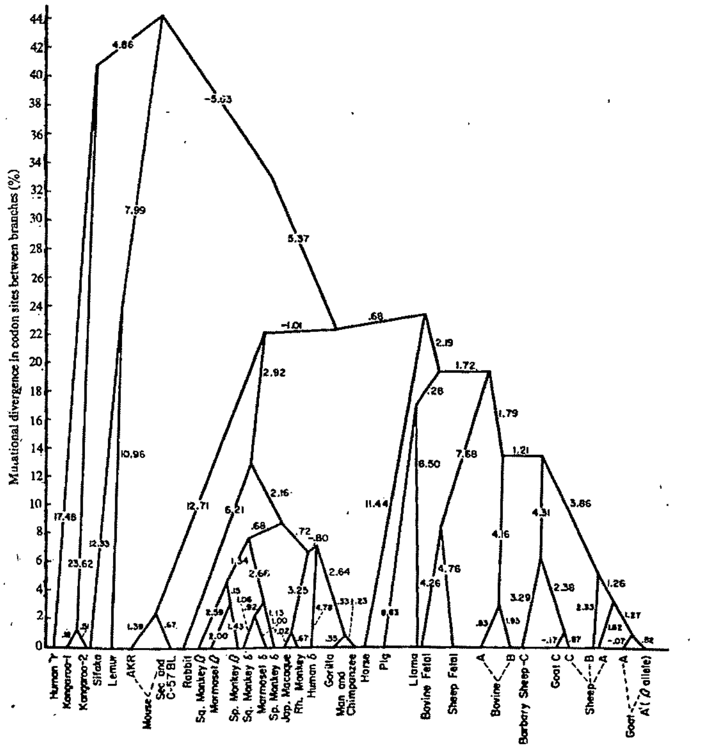


Fig. 3 Mammalian beta-like globin phylogenetic tree.

To detect a larger proportion of the accumulated mutations in a gene lineage, we constructed ancestral sequences (Table 3) for branching points of the globin phylogenetic tree and, using the MMUTD program, determined the mutation distances through the consecutive ancestors to the contemporary polypeptide chains. The mutational change in codon sites from the node for ancestral carp–tetrapod haemoglobin chains to the node for ancestral chicken–mammal alphas and from the latter to the ancestral node for mammalian alpha chains was 23.9 and 9.2% respectively. In the descent of the beta-like line, the mutational change from the ancestral carp–tetrapod node to the ancestral gamma–beta node and from this node to the ancestral node for typical mammalian beta chains was 53.2 and 2.0% respectively. The further mutational change in descent of each eutherian alpha and beta line is given in column 2 of Table 2. These results show

Table 2 Percentage Mutational Divergence of Vertebrate Globins during Descent

Globin species	From ancestral eutherian mammal MMUTD †	From ancestral eutherian mammal PML	From ancestral Hb–Mb PML
Human alpha	6.3	6.13	39.34
Chimp alpha *	6.3	6.13	39.34
Gorilla alpha *	7.0	5.86	38.12
Mulatta alpha	7.1	5.57	41.68
Fuscata alpha	7.1	5.57	41.68
Mouse C57 alpha	8.5	8.50	39.75
Mouse NB alpha	10.6	10.21	41.65
Goat A alpha	10.6	8.96	36.26
Lemur alpha *	11.3	10.45	41.46
Bush baby alpha *	11.3	8.67	40.67
Goat B alpha *	11.3	9.55	36.85
Goat II alpha	11.3	13.04	37.74
Bovine alpha *	11.3	9.16	38.08
Horse slow alpha	12.0	9.97	40.08
Horse fast alpha *	12.7	10.32	39.44
Sheep A alpha	12.7	11.56	37.61
Horse slow 24F alpha *	12.7	10.72	38.86
Horse fast 24F alpha *	13.4	10.99	39.37
Donkey alpha *	13.4	11.15	39.00
Sheep D alpha *	13.4	12.07	41.71
Pig alpha *	13.4	9.25	37.30
Rabbit alpha *	15.6	12.36	43.22
Rabbit (sub) alpha *	16.3	12.27	43.66
Sifaka alpha *	17.7	14.66	44.50
Tree shrew alpha *	17.7	12.25	43.68
Llama alpha *	19.1	11.27	40.46
Chicken alpha	—	—	49.18
Human beta	7.5	7.19	48.46
Chimp beta *	7.5	6.73	46.60
Squirrel monkey beta	7.5	8.68	50.94
Gorilla beta *	8.2	6.98	46.39
Fuscata beta *	8.2	8.06	50.21
Spider monkey beta	8.2	7.67	50.67
Tamarin beta	8.8	8.24	49.73
Mulatta beta	8.9	8.71	50.29
Rabbit beta	8.9	8.12	50.64
Human delta *	9.5	8.77	50.04
Tamarin delta	10.2	9.33	51.33
Spider monkey delta	10.2	8.54	50.81
Squirrel monkey delta	10.9	9.39	51.35
Pig beta *	14.2	11.78	48.27
Bovine A beta	16.4	12.47	48.71
Llama beta *	17.7	11.65	47.89
Bovine B beta	17.8	11.37	49.33
Sheep B beta	18.4	13.78	47.76
Horse beta	19.0	12.02	48.59
Goat A beta *	19.1	12.64	46.92
Sheep A beta	19.8	14.33	46.93
Bovine foetal beta	19.8	16.53	52.30
Goat C beta *	20.3	14.11	47.46
Mouse AKR beta *	20.4	13.09	49.29
Mouse Sec and C-57 beta *	20.7	12.37	49.19
Goat A′ beta *	21.2	13.23	47.46
Sheep C beta	21.7	15.15	48.58
Sheep Barb. C beta *	21.7	15.19	48.46
Sheep foetal beta *	24.2	17.03	50.50
Kangaroo-1 beta	—	—	59.68
Kangaroo-2 beta	—	—	59.84
Human gamma	—	—	54.07
Lemur beta *	—	—	53.56
Sifaka beta *	—	—	55.06
Carp alpha	—	—	50.57
Lamprey globin	—	—	50.78
Mb–horse	—	—	59.47
Mb–sperm whale	—	—	59.33
Insect CTT-3	—	—	—
Insect CTT-3 (sub)	—	—	—

* Sequence in part or wholly by composition and homology.

† Values of rabbit alpha, rabbit (sub) alpha, tree shrew alpha, mouse NB alpha and mouse C-57 alpha are directly from ancestral mammalian alpha. Values of prosimian alphas are through ancestral primate alpha node while those of catarrhine alphas are through both ancestral primate alpha node and ancestral catarrhine alpha node. Values of mouse AKR beta, mouse Sec and C-57 betas and rabbit beta are directly from ancestral mammalian beta while those of ungulate betas and primate betas are through ancestral ungulate beta node and ancestral Anthropoidea beta node, respectively.

that after the alpha–beta globin gene duplication in early vertebrate history, after the ancestral separation 380 million years ago of teleosts and tetrapods, many more mutations accumulated during the descent towards mammals in the beta-like line than in the alpha line. Moreover, during eutherian phylogeny, both alpha and beta chain evolution was much slower (on the average, almost by half) in higher primates than in other mammals. Furthermore, from the ancestral carp–tetrapod node about as much mutational change accumulated up to the ancestral chicken–mammalian alpha branching point as after this point in the descending mammalian alpha line. Yet one has to go back about 280 million years ago to the early reptiles to find the most recent common ancestor of birds and mammals. Thus the evidence suggests that the descending alpha genes evolved at a more rapid rate in earlier less advanced vertebrates than in later higher vertebrates.

The patristic distance data on beta genes show that ceboid delta, bovid foetal beta, and caprine C beta initially evolved more rapidly than the genes from which they were duplicated (Fig. 3), but later in descent their rates of evolution slowed. Note (Table 1) that ceboid deltas actually diverge less from each other than ceboid betas, that sheep and bovine foetal betas diverge less from each other than sheep and bovine adult betas, and that sheep and goat C betas diverge less than sheep and goat A betas. Presumably mutations had accumulated freely at the duplicated loci until certain fortuitous mutations resulted in the emergence of new proteins with useful functions. Then natural selection not only caused these particular advantageous mutant genes to spread rapidly through the populations in which they occurred but also drastically slowed their further evolution.

Following the gamma–beta gene duplication, more mutational change occurred in the descent of human gamma than in most typical eutherian beta chains. Nevertheless amino-acid composition data[62] indicate that slightly less divergence exists between gamma chains of man and baboon than between either beta or alpha chains of these animals. Thus when sequence data become available on gamma chains of different higher primates, the pattern of decelerating evolution will probably be found again. This pattern is presented by the myoglobin genes, for the divergence between horse and sperm whale myoglobins (Fig. 1 and Table 1) is no more than that between any two alpha chains belonging to different eutherian orders; yet the overall mutational change in the descent of the myoglobin branch is larger than in the branch containing the various chains of vertebrate haemoglobin (Table 2, column 4). Ohno[63] describes early vertebrate history as a time when tetraploidization and bursts of gene duplication were occurring in our ancestors; at the later, mammalian stage specialized functions were found for pre-existing redundant genes, but the level of genome organization was such that viable tetraploidization was no longer possible. If mutations accumulate more readily in silent genes than in functional ones, this is equivalent to the hypothesis that molecular evolution decelerated during the descent of man.

In lemurs and other prosimians, foetal haemoglobin seems to be the same as adult haemoglobin[64]. But in the lineage leading to higher primates a specialized function, coding for the non-alpha chain of foetal haemoglobin, was found for the gamma locus. A further advance took place when from certain beta duplications of the early Anthropoidea functioning delta genes and a new haemoglobin type emerged in later Ceboidea and Hominoidea. The new level of organization imposed additional functional restraints, which increased the likelihood that new mutations would be detrimental, thereby slowing the further evolution of the haemoglobin genes. While the alpha and beta chains of man are among the most slowly evolved globins, those of the kangaroo and lemuroids are among the most rapidly evolved. As previously discussed, an additional selective factor, maternal isoimmunizations to foetal antigens, might have also acted to decelerate molecular

Table 3 Probable Ancestral Residues for Vertebrate Globin Chains at Different Nodes during Descent

Residue position	11	13	14	15	16	18	19	21	22	23	24	25	27	28	29	30	31	32	34	36	38	40	42	43	44	45	46	48	50	51	52	54
β-Mammal	His	Thr	Ala	Glu	Glu	Ala	Ala	Thr	Ala	Leu	Trp	Gly	Val	Asx	—	—	Val	Asp	Val	Gly	Ala	Gly	Leu	Leu	Val	Val	Tyr	Trp	Gln	Arg	Arg	Glu
γ-β-Mammal	His	Thr	Ala	Glu	Glu	Ala	Ala	Thr	Ala	Leu	Trp	Gly	Val	Asn	—	—	Val	Asp	Val	Gly	Ala	Gly	Leu	Leu	Val	Val	Tyr	Trp	Gln	Arg	Arg	Glu
α-Mammal	—	Ser	Pro	Ala	Asp	Thr	Asn	Lys	Ala	Ala	Trp	Gly	Ile	Gly	Gly	His	Ala	Gly	Tyr	Ala	Ala	Glu	Met	Phe	Leu	Ser	Phe	Thr	Lys	Thr	Tyr	Pro
α-Chicken— Mammal	—	Ser	Ala	Ala	Asp	Ala	Asn	Lys	Ala	Ala	Trp	Ala	Ile	Gly	Gly	His	Ala	Glu	Tyr	Ala	Ala	Glu	Met	Phe	Ile	Gly	Phe	Thr	Lys	Thr	Tyr	Pro
α-β-Carp	—	Ser	Ala	Ala	Asp	Ala	Ala	Lys	Ala	Ala	Trp	Ala	Ile	Gly	Gly	His	Ala	Asp	Tyr	Gly	Ala	Gly	Met	Phe	Ile	Gly	Tyr	Thr	Lys	Thr	Thr	Ala

	55	57	61	62	63	64	65	66	68	69	70	71	73	79	81	83	84	85	86	88	90	92	93	94	97	98	99	100	103	106	107	110
β-Mammal	Ser	Gly	Ser	Ala	Asx	Ala	Val	Met	Asn	Pro	Lys	Val	Ala	Leu	Asx	Phe	Ser	Asp	Gly	Asx	Leu	Asn	Leu	Lys	Thr	Phe	Ala	Gln	Glu	Cys	Asp	His
γ-β-Mammal	Ser	Gly	Ser	Ala	Ser	Ala	Val	Met	Asn	Pro	Lys	Val	Ala	Leu	Asx	Phe	Ser	Asp	Gly	Asx	Leu	Asn	Leu	Lys	Thr	Phe	Ala	Gln	Glu	Cys	Asp	His
α-Mammal	His	—	His	—	—	—	—	—	Ser	Ala	Gln	Val	Gly	Ala	Asp	Leu	Thr	Asn	Ala	Gly	Leu	Asp	Leu	Pro	Ala	Leu	Ser	Ala	Asp	Ala	His	Arg
α-Chicken— Mammal	His	—	His	—	—	—	—	—	Ser	Ala	Gln	Val	Gly	Ala	Asp	Leu	Thr	Asn	Ala	Gly	Leu	Asp	Leu	Pro	Ala	Leu	Ser	Ala	Asp	Ala	His	Arg
α-β-Carp	His	Gly	Pro	—	—	—	—	—	Ser	Ala	Gln	Val	Gly	Ile	Asp	Val	Ser	Asp	Ala	Gly	Leu	Asp	Leu	Glu	Ala	Leu	Ala	Ala	Glu	Ala	His	Arg

	114	117	120	121	122	123	124	125	126	128	129	131	132	133	134	135	136	138	140	142	143	144	147	148	149	151	152	153	155	156	159
β-Mammal	Glu	Arg	Gly	Asn	Val	Leu	Val	Ile	Val	Ala	His	Phe	Gly	Lys	Glu	Phe	Thr	Glx	Gln	Ala	Tyr	Gln	Val	Ala	Gly	Ala	Asn	Ala	Ala	His	His
γ-β-Mammal	Glu	Arg	Gly	Asn	Val	Leu	Val	Ile	Val	Ala	His	Phe	Gly	Lys	Glu	Phe	Thr	Glx	Gln	Ala	Phe	Gln	Val	Ala	Gly	Ala	Asn	Ala	Ala	His	His
α-Mammal	Val	Lys	Ser	His	Cys	Leu	Leu	Val	Thr	Ala	Ala	Leu	Pro	Ala	Asp	Phe	Thr	Ala	His	Ser	Leu	Asp	Leu	Ala	Ser	Ser	Thr	Val	Thr	Ser	Arg
α-Chicken— Mammal	Val	Lys	Ser	His	Cys	Leu	Leu	Val	Thr	Ala	Ser	Leu	Pro	Ala	Glu	Phe	Thr	Glu	His	Ser	Leu	Asp	Leu	Ala	Ala	Ser	Thr	Val	Thr	Ser	Arg
α-β-Carp	Glu	Lys	Ser	Asn	Ala	Leu	Val	Val	Val	Ala	Ser	Leu	Pro	Ala	Glu	Phe	Thr	Glu	His	Ser	Val	Asp	Leu	Ala	Ala	Ala	Asn	Ala	Thr	Ser	Arg

Amino-acid residues of vertebrate globins are aligned so as to maximize homology by introducing the necessary gaps (refs. 14 and 15). The residue positions are numbered from 1 to 165 in order to accommodate lamprey globin. In this alignment carp alpha, tetrapod alphas, and mammalian betas begin at position 10 and end at position 159, with a gap at position 96. Carp alpha and tetrapod alphas have in addition gaps at position 11 and at position 62 through 66. Further gaps introduced are: tetrapod alphas at positions 57 and 78; mammalian betas at positions 29, 30, and 78; carp alpha at position 73. Residue positions omitted are the same as chicken[17] when aligned as described. Probable ancestral residues of mammalian alpha and beta chains at branching points within the span of eutherian phylogeny are given in the previous investigation[10]. Ancestral sequences were reconstructed by approximation after the rules described by Fitch and Margoliash[7]. In brief, an ancestral residue is selected if it gives fewer mutations in the lines of descent than any other residue which could be selected and, where more than one residue meets this criterion, distributes the mutations as equally as possible between the lines of descent.

evolution in the higher primates. The opportunity for such attacks would be virtually absent in kangaroo, and minimal in lemuroid primates which have epitheliochorial type placentas[65] rather than the haemochorial type of higher primates.

Cladistic Relationships of Man and Other Mammals

The phylogenetic trees constructed from amino-acid sequence data not only reveal differences in relative rates of evolution among homologous genes but also provide evidence on the cladistic relationships of the animal species in which the genes occur. The branching topologies of alpha chain genes and beta chain genes depict a very close relationship, with no detectable mutational divergence, between man and chimpanzee, and between this man–chimpanzee branch and gorilla, in which the divergence is only one point mutation per chain. A beta phylogenetic tree, which includes the partially sequenced gibbon beta chain, joins gibbon to the branch leading to chimpanzee, man, and gorilla, and then joins cercopithecoid (macaques) and hominoid branches[10]. Orang-utan haemoglobin chains have not been sequenced. Amino-acid composition data, however, show orang-utan diverging from the branch of African apes and man[66]. In the beta phylogenetic tree (Fig. 3), the ceboids branch away from the line leading to cercopithecoids and hominoids just before these two catarrhine groups branch apart. The phylogenetic tree of alpha genes (Fig. 2) joins the prosimians (galago together with the lemuroids, lemur and sifaka) to the line leading to higher primates, next joins the mouse branch to the base of the primate branch, and then joins the ancestral mouse–primate lineage with that of the ungulates. Both alpha and beta trees join caprines to bovines, llama and pig lines to the bovids, the horse branch with these artiodactyls, and then these ungulates with the lineage descending towards primates. Lemuroids do not produce a globin chain directly homologous to mammalian beta chains, so the phylogenetic tree of beta genes cannot provide cladistic evidence on the lemuroids. Otherwise the topology of the beta tree is similar to the alpha, except that rabbit beta joins primate betas before mouse.

Sequence data have also been obtained on mammalian fibrinopeptides A and B. They contain no more than forty amino-acid residues, all of which, with the exception of one or two invariant sites, have accepted amino-acid substitutions without harm to the function of fibrinogen. Thus these peptide sequences have been able to undergo such extensive divergent evolution that they are capable of depicting cladistic relationships among present day mammals. A matrix of mutation values for thirty-six mammalian combined A and B fibrinopeptide sequences[67–75] is given in Table 4, and the best tree of thirty alternatives tried portraying the phylogeny of these sequences is shown in Fig. 4. The APSD coefficient of this tree is 10.80 compared with 14.63 for the UWPGM tree.

The branching arrangement among ungulate fibrinopeptides is essentially the same as that for ungulates in the alpha and beta globin phylogenetic trees, except that more ungulate species are represented. Pronghorn and caprines join bovines and then these bovoids join cervids (reindeer and various deers). Yet caprines and cervids on the average show less mutation distance from each other than from bovines (Table 4). Doolittle and Blombäck[76] discussed the possibility that caprines might have a more recent common ancestor with cervids than with bovines, and, indeed, the tree produced in our study by the UWPGM algorithm depicts such an ancestry relationship. Nevertheless, the best arrangements of artiodactyl branches with respect to lowering the APSD coefficient placed the caprines closer to bovines than to cervids in agreement with morphological evidence on ruminant relationships.

The joining of carnivores with ungulates before they join the Primates fits established ideas on mammalian phylogeny, for the Palaeocene Condylartha and Carnivora (from which the two ungulate orders, Perissodactyla and Artiodactyla, descended) are traced to late Cretaceous palaeoryctoid insectivores[77]. The marked divergence of the rabbit line from all other mammals is also in agreement with fossil data on mammalian phylogeny[77]. Although joining rat to rabbit at the apex of the tree is not in accord with the idea[77] from palaeontological evidence that rodents evolved out of the basal Primates, it may be noted that rat fibrinopeptides show less mutational divergence from primate fibrinopeptides than from those of other mammals (Table 4). Furthermore, the globin phylogenetic trees joined mouse to the base of the Primates. A finding at variance with our general knowledge of mammalian phylogeny is the grouping of kangaroo, a marsupial, with the carnivore branch. Fibrinopeptides from additional marsupials need to be sequenced, however, if we are to construct a phylogenetic tree for the relationship between marsupial and placental fibrinopeptides.

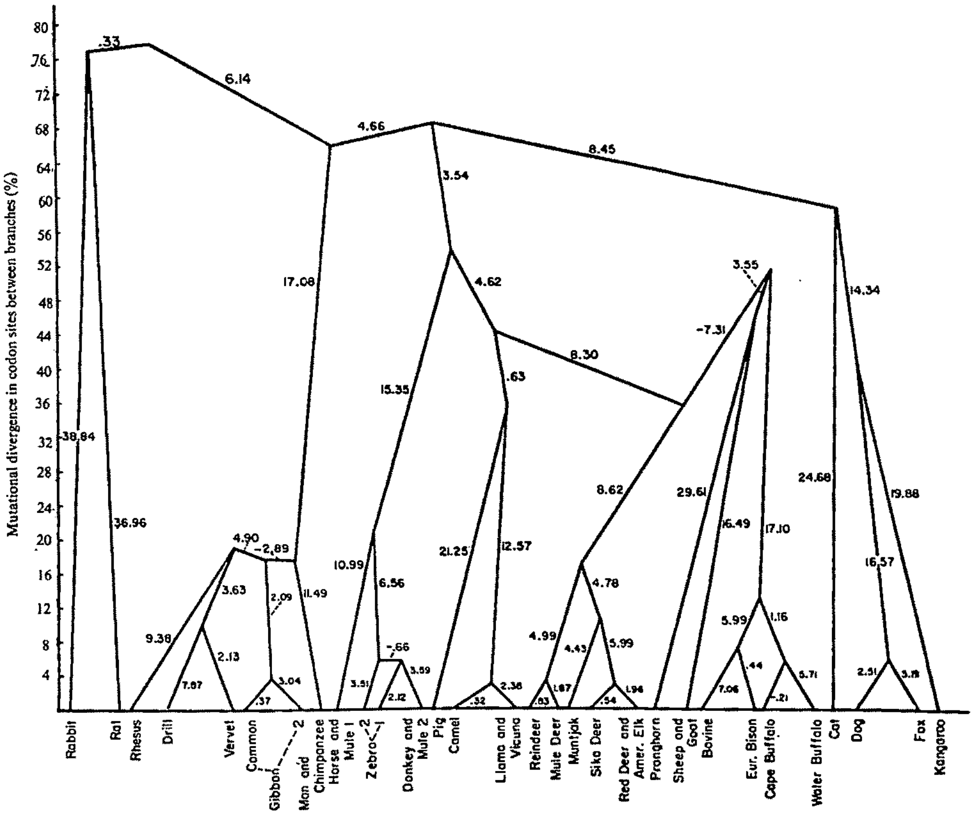


Fig. 4 Mammalian fibrinopeptide phylogenetic tree. 1, Rabbit[67,68]; 2, rat[67]; 3, *Macaca mulatta*[69]; 4, drill (*Mandrillus leucophaeus*)[70]; 5, vervet (*Cercopithecus aethiops*)[69]; 6, gibbon-1 (3 Gly–numbered from right to left)[71]; 7, gibbon-2 (3 Ser)[71]; 8, man[69]; 9, chimpanzee[72]; 10, horse[67,68]; 11, mule-1[67,68]; 12, zebra-2[73]; 13, zebra-1[73]; 14, donkey[67]; 15, mule-[67,68]; 16, pig[67,68]; 17, camel[74,75]; 18, llama[67,68]; 19, vicuna (*Vicugna vicugna*)[74,75]; 20, reindeer (*Rangifer tarandus*)[74,75]; 21, mule deer (*Odocoileus hemionus hemionus*)[74]; 22, muntjak (*Muntiacus muntjak*)[74,75]; 23, sika deer (*Cervus nippon*)[67,68]; 24, red deer (*Cervus elaphus*)[67,68]; 25, American elk (*Cervus canadensis*)[74,75]; 26, pronghorn (*Antilocapra americana*)[74,75]; 27, sheep[67,68]; 28, goat[67,68]; 29, bovine[67,68]; 30, European bison[67,68]; 31, Cape buffalo[67,68]; 32, water buffalo[67,68]; 33, cat[67,68]; 34, dog[67,68]; 35, fox[67,68]; 36, kangaroo[73].

In the descent of primate fibrinopeptides, where hominoids (chimpanzee and man) and cercopithecoids (macaque, vervet, drill) branch apart, the gibbons split off as a distinct lineage from the base of the cercopithecoids. No splitting occurs in the terminal descent of man and chimpanzee: their fibrinopeptides are identical. Furthermore, the mutational divergence among the catarrhine primates is not nearly as marked as in other comparable taxonomic groups. In this connexion, the fibrinopeptides of the catarrhine primates are among the least rapidly evolving ones in the class Mammalia as revealed by the patristic mutation lengths in Fig. 4.

In addition to the amino-acid sequence data on haemoglobin chains and fibrinopeptides, there are partial sequence data on the erythrocyte enzyme carbonic anhydrase B which can be used to depict cladistic relationships among catarrhine primates[78]. Of the 133 homologous residues examined, chimpanzee and man differ at only one site, whereas orang utan differs from chimpanzee at three sites and from man at four sites. These hominoids differ from cercopithecoids (vervet, irus and rhesus macaques, and baboon) at four to six sites. Thus again the very close genetic relationship between man and chimpanzee is highlighted. The findings from the various sets of amino-acid sequence data support the conclusion drawn from extensive comparative immunodiffusion studies[79–82] on primate proteins that in the descent of the hominoids, after the early divergence of gibbon and orang utan lines, there was still a common ancestor for man, chimpanzee, and gorilla. The cladistic relationships depicted by the protein sequences support a classification of the Hominoidea which places *Gorilla* and *Pan* with *Homo* in the family Hominidae[80–82]. This agrees with the view of Darwin[83] that man originated in Africa from the same ancestral stock as chimpanzee and gorilla.

Four to five million year old fossil remains of man's immediate generic ancestor, *Australopithecus*, have recently been found in Pliocene deposits of Ethiopia[84], and fourteen million year old remains of *Ramapithecus*[86] (*Kenyapithecus*[87]), interpreted as a lineal ancestor of *Australopithecus*, date from the terminal Miocene of Kenya. Another Kenya hominoid, an eighteen million year old *Dryopithecus* ape, is considered an ancestor of *Gorilla* and to have even then separated from the line to *Ramapithecus* and *Homo*[88]. The hypothesis that

Table 4 Mutation Values from Pairwise Comparisons of Mammalian Fibrinopeptide A and B Sequences

	Rabbit	Rat	Kulatta	Drill	Vervet	Gibbon-1	Gibbon-2	Man	Chimp	Horse	Mule-1	Zebra-2	Zebra-1	Donkey	Mule-2	Pig	Camel	Llama	Vicuna	Reindeer	Mule Deer	Muntjak	Sika Deer	Red Deer	Am. Elk	Pronghorn	Sheep	Goat	Bovine	Eur. Bison	Cape Buffalo	Water Buffalo	Cat	Dog	Fox	Kangaroo
Rabbit	0																																			
Rat	22	0																																		
Kulatta	20	16	0																																	
Drill	18	15	6	0																																
Vervet	17	15	3	3	0																															
Gibbon-1	16	12	5	6	3	0																														
Gibbon-2	17	11	4	7	4	1	0																													
Man	18	14	5	8	5	4	3	0																												
Chimp	18	14	5	8	5	4	3	0	0																											
Horse	20	19	15	18	16	14	15	19	19	0																										
Mule-1	20	19	15	18	16	14	15	19	19	0	0																									
Zebra-2	22	22	18	19	19	17	18	22	22	8	8	0																								
Zebra-1	22	22	17	19	18	16	17	21	21	7	7	2	0																							
Donkey	24	22	18	19	19	17	18	22	22	6	6	2	2	0																						
Mule-2	24	22	18	19	19	17	18	22	22	6	6	2	2	0	0																					
Pig	24	26	19	22	19	16	17	22	22	15	15	15	14	14	14	0																				
Camel	19	21	15	19	16	12	13	17	17	14	14	14	13	14	14	13	0																			
Llama	20	20	16	20	17	13	14	18	18	15	15	15	14	15	15	12	1	0																		
Vicuna	20	20	16	20	17	13	14	18	18	15	15	15	14	15	15	12	1	0	0																	
Reindeer	21	26	15	20	18	14	15	19	19	17	17	17	16	17	17	17	14	15	15	0																
Mule Deer	21	26	15	20	18	14	15	19	19	16	16	18	17	18	18	18	12	13	13	1	0															
Muntjak	23	24	17	20	20	16	17	21	21	18	18	18	18	18	18	16	14	14	14	4	5	0														
Sika Deer	21	22	17	23	21	17	18	22	22	19	19	19	18	19	19	17	14	14	14	6	7	4	0													
Red Deer	21	23	16	22	20	18	19	21	21	20	20	20	19	20	20	18	15	15	15	7	8	5	1	0												
Am. Elk	21	23	16	22	20	18	19	21	21	20	20	20	19	20	20	18	15	15	15	7	8	5	1	0	0											
Pronghorn	19	31	22	26	25	21	22	26	26	21	21	20	22	22	22	25	20	21	21	14	15	15	16	17	17	0										
Sheep	22	26	14	21	19	14	15	20	20	14	14	16	15	16	16	19	16	17	17	11	9	12	14	15	15	18	0									
Goat	22	26	14	21	19	14	15	20	20	14	14	16	15	16	16	19	16	17	17	11	9	12	14	15	15	18	0	0								
Bovine	26	24	19	20	20	17	18	20	20	20	20	22	22	21	21	18	18	18	18	16	16	13	16	15	15	24	22	22	0							
Eur. Bison	24	22	17	19	17	14	15	17	17	19	19	20	19	19	19	17	15	15	15	13	13	12	13	12	12	22	19	19	3	0						
Cape Buffalo	23	19	12	16	14	9	10	14	14	16	16	15	14	14	14	11	12	12	12	11	11	11	12	13	13	19	16	16	5	3	0					
Water Buffalo	25	23	16	21	19	14	15	19	19	18	18	20	19	19	19	17	14	15	15	13	13	14	15	16	16	20	17	17	7	6	2	0				
Cat	23	22	18	19	18	14	15	18	18	23	23	22	21	22	22	17	19	20	20	21	22	22	21	22	22	29	23	23	23	22	15	21	0			
Dog	25	23	18	26	25	22	23	25	25	22	22	20	19	18	18	23	21	22	22	23	24	25	24	25	25	27	23	23	29	27	18	25	19	0		
Fox	25	23	18	26	25	22	23	25	25	23	23	21	20	19	19	24	21	22	22	22	23	25	24	25	25	27	24	24	29	27	18	25	20	2	0	
Kangaroo	20	30	14	18	18	16	17	21	21	25	25	20	19	20	20	24	22	23	23	24	25	27	27	27	27	27	25	25	29	27	20	26	22	13	13	0

The half matrix lists minimum numbers of mutations interrelating pairs of fibrinopeptides. Numbers of shared amino-acids vary considerably in mammalian fibrinopeptides. For example, human has thirty amino-acid residues and rhesus has twenty-five residues, all the positions of which are shared by human. The mutation value for the human–rhesus pair presented in the table is obtained from the twenty-five shared amino-acid positions. Cape buffalo has thirty-six residues, only twenty-six positions of which are shared by human. Thus the mutation value for the human–Cape buffalo pair is obtained from these twenty-six shared positions. The alignments for most of the fibrinopeptide sequences can be found in ref. 14. The mutation values are converted into % mutational divergence values for constructing the phylogenetic tree in Fig. 4.

molecular evolution decelerated in higher primates reconciles such palaeontological views with protein findings on the close genetic relationship of the African apes to man. DNA reassociation experiments[89,90] on nonrepeating polynucleotide sequences (the DNA fraction best suited for cladistic comparisons) further demonstrate the close relationship between *Pan* and *Homo*. The DNA findings emphasize the possibility that steadily increasing generation lengths may have been an important parameter[79,89,90] in slowing molecular evolution during the past sixty-five million years in the descent of man.

We thank Mr Walter Farris for assistance, Miss Joan Bechtold and Mrs Geraldine Fockler for drawings, and the staff of the Wayne State University Computing Center for help. This work was supported by grants from the US National Science Foundation Systematic Biology and US–Japan Cooperative Science Programs.

Received February 17; revised March 18, 1971.

[1] Ingram, V. M., *Nature*, **189**, 704 (1961).
[2] Goodman, M., *Human Biol.*, **33**, 131 (1961).
[3] Goodman, M., in *Classification and Human Evolution*, 203 (Aldine Press, 1963).
[4] Goodman, M., in *Protides of Biological Fluids*, 70 (Elsevier, Amsterdam, 1965).
[5] Kimura, M., *Nature*, **217**, 624 (1968).
[6] King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).
[7] Fitch, W. M., and Margoliash, E., *Science*, **155**, 279 (1967).
[8] Sokal, R., and Sneath, P. H. A., *Principles of Numerical Taxonomy*, 309 (W. H. Freeman, San Francisco, 1963).
[9] Moore, G. W., thesis, Univ. North Carolina (1970).
[10] Barnabas, J., Goodman, M., and Moore, G. W., *Comp. Physiol. Biochem.*, **39B**, 455 (1971).
[11] Braun, V., Crichton, R. R., and Braunitzer, G., *Z. Physiol. Chem.*, **349**, 45 (1968).
[12] Braunitzer, G., Buse, G., and Braig, S., *Z. Physiol. Chem.*, **350**, 1477 (1969).
[13] Edmundson, A. B., *Nature*, **205**, 883 (1965).
[14] Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **4** (1969).
[15] Rudloff, V., Zelenik, M., and Braunitzer, G., *Z. Physiol. Chem.*, **344**, 284 (1966).
[16] Hilse, K., and Braunitzer, G., *Z. Physiol. Chem.*, **349**, 433 (1968).
[17] Matsuda, G., Takei, H., Wu, K. C., Mizuno, K., and Shiozawa, T., *Eighth Intern. Cong. Biochemistry*, Abstract, 4 (1970).
[18] Von Ehrenstein, G., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 705 (1966).
[19] Hill, R. L., unpublished data taken from *Handbook of Biochemistry* (The Chemical Rubber Company, 1968).
[20] Rifkin, D. B., Hirsch, D. I., Rifkin, M. R., and Konigsberg, W., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 715 (1966).
[21] Popp, R. A., *J. Mol. Biol.*, **27**, 9 (1967).
[22] Matsuda, G., Maita, T., Takei, H., Ota, H., Yamaguchi, M., Miyanchi, T., and Migita, M., *J. Biochem.* (Japan), **64**, 279 (1968).
[23] Zuckerkandl, E., and Schroeder, W. A., *Nature*, **192**, 984 (1961).
[24] Rifkin, D. B., and Konigsberg, W., *Biochim. Biophys. Acta*, **104**, 457 (1965).
[25] Braunitzer, G., Gehring-Mueller, R., Hilschmann, N., Hilse, K., Hobom, G., Rudloff, Y., and Wittman-Liebold, B., *Z. Physiol. Chem.*, **325**, 283 (1961).
[26] Schroeder, W. A., Shelton, J. R., Shelton, J. B., and Cormick, J., *Biochemistry*, **2**, 1353 (1963).
[27] Kilmartin, J. V., and Clegg, J. B., *Nature*, **213**, 269 (1967).
[28] Matsuda, G., Gehring-Mueller, R., and Braunitzer, G., *Biochem. Z.*, **338**, 669 (1963).
[29] Schroeder, W. A., Shelton, J. R., Shelton, J. B., Robberson, B., and Babin, D. R., *Arch. Biochem. Biophys.*, **120**, 1 (1967).
[30] Huisman, T. H. J., Brandt, G., and Wilson, J. B., *J. Biol. Chem.*, **243**, 3637 (1968).
[31] Huisman, T. H. J., Dozy, A. M., Wilson, J. B., Effremov, G. D., and Vaskov, B., *Biochim. Biophys. Acta*, **160**, 467 (1968).
[32] Beale, D., Lehman, H., Drury, A., and Tucker, E. M., *Nature*, **209**, 1099 (1966).
[33] Wilson, J. B., Brandt, G., and Huisman, T. H. J., *J. Biol. Chem.*, **243**, 3687 (1968).
[34] Schroeder, W. A., Shelton, J. R., Shelton, J. B., Cormick, J., and Jones, R. T., *Biochemistry*, **2**, 992 (1963).
[35] Air, G. M., and Thomson, E. O. P., *Austral. J. Biol. Sci.*, **22**, 1437 (1969).
[36] Rifkin, D. B., Rifkin, M. R., and Konigsberg, W., *Proc. US Nat. Acad. Sci.*, **55**, 586 (1966).
[37] Braunitzer, G., Best, J. S., Flamm, U., and Schrank, B., *Z. Physiol. Chem.*, **347**, 207 (1966).
[38] Boyer, S. H., Crosby, E. F., Thurmon, T. F., Noyes, A. N., Fuller, G. F., Leslie, S. E., and Sheppard, M. K., *Science*, **116**, 1428 (1969).
[39] Matsuda, G., Maita, T., Ota, H., Tachiwaka, I., Tanaka, Y., Araya, A., and Nakashima, Y., *Intern. J. Protein Res.*, **3**, 41 (1971).
[40] Ingram, V. M., and Stretton, A. O. W., *Biochim. Biophys. Acta*, **63**, 20 (1962).
[41] Smith, D. B., *Canad. J. Biochem.*, **46**, 825 (1968).
[42] Babin, D. R., Schroeder, W. A., Shelton, J. R., Shelton, J. B., and Robberson, B., *Biochemistry*, **5**, 1297 (1966).
[43] Wilson, J. B., Edwards, W. C., McDaniel, M., Dobbs, M. M., and Huisman, T. H. J., *Arch. Biochem. Biophys.*, **115**, 385 (1966).
[44] Schroeder, W. A., Shelton, J. R., Shelton, J. B., Robberson, B., and Babin, D. R., *Arch. Biochem. Biophys.*, **120**, 124 (1967).
[45] Huisman, T. H. J., Dasher, G. A., Moretz, W. H., Dozy, A. M., and Wilson, J. B., *Biochem. J.*, **107**, 745 (1968).
[46] Boyer, S. H., Hathaway, P., Pascasio, F., Orton, C., Bordley, J., and Naughton, M. A., *Science*, **153**, 1539 (1966).
[47] Huisman, T. H. J., Adams, H. R., Dimock, M. O., Edwards, W. E., and Wilson, J. B., *J. Biol. Chem.*, **242**, 2534 (1967).
[48] Huber, R., Epp, O., and Formanck, H., *J. Mol. Biol.*, **42**, 59 (1969).
[49] Briehl, R. W., *J. Biol. Chem.*, **238**, 2361 (1963).
[50] Balani, A. S., and Barnabas, J., *Nature*, **205**, 1019 (1965).
[51] Balani, A. S., Ranjekar, P. K., and Barnabas, J., *Comp. Physiol. Biochem.*, **24**, 809 (1968).
[52] Huisman, T. H. J., Wilson, J. B., and Adams, H. R., *Arch. Biochem. Biophys.*, **121**, 528 (1967).
[53] Ranjekar, P. K., and Barnabas, J., *Indian J. Biochem.*, **6**, 1 (1969).
[54] Barnicot, N. A., and Huehns, E. R., *Nature*, **215**, 1485 (1967); Wade, P. T., Skinner, A. F., and Barnicot, N. A., *Protides in Biological Fluids*, 263 (Pergamon, London, 1970).
[55] Oliver, E., and Kitchen, H., *Biochim. Biophys. Acta*, *Res. Commun.*, **31**, 749 (1968).
[56] Hilse, K., and Popp, R. A., *Proc. US Nat. Acad. Sci.*, **61**, 930 (1968).
[57] Kunkel, H. G., and Wallenius, G., *Science*, **122**, 288 (1955).
[58] Kunkel, H. G., Ceppellini, R., Mueller-Eberhard, V., and Wolf, J., *J. Clin. Invest.*, **36**, 1615 (1957).
[59] Huisman, T. H. J., Reynolds, C. A., Dozy, A. M., and Wilson, J. B., *J. Biol. Chem.*, **240**, 2455 (1965).
[60] Hamilton, W. J., Boyd, J. D., and Mossman, H. W., *Human Embryology* (Williams and Wilkins, Baltimore, 1952).
[61] Kimura, M., *Proc. US Nat. Acad. Sci.*, **63**, 1181 (1969).
[62] Buettner-Janusch, J., and Buettner-Janusch, V., *Amer. J. Phys. Anthrop.*, **33**, 73 (1970).
[63] Ohno, S., *Evolution by Gene Duplication* (Springer, Berlin, 1970).
[64] Buettner-Janusch, J., and Hill, R. W., in *Evolving Genes and Proteins*, 167 (Academic Press, London and New York, 1965).
[65] LeGros Clark, W. E., *The Antecedents of Man* (Edinburgh University Press, Edinburgh, 1959).
[66] Buettner-Janusch, J., Buettner-Janusch, V., and Mason, G. A., *Arch. Biochem. Biophys.*, **133**, 164 (1969).
[67] Blombäck, B., Blombäck, M., and Grondahl, N. J., *Acta Chem. Scand.*, **19**, 1789 (1965).
[68] Blombäck, B., Blombäck, M., Grondahl, N. J., and Holmberg, E., *Arkiv. Kemi.*, **25**, 411 (1966).
[69] Blombäck, B., Blombäck, M., Grondahl, N. J., Guthrie, C., and Hinton, M., *Acta Chem. Scand.*, **19**, 1788 (1965); Blombäck, B., Blombäck, M., and Edman, B., *Biochim. Biophys. Acta*, **115**, 371 (1966).
[70] Doolittle, R. F., Glasgow, C., and Mross G. A., *Biochim. Biophys. Acta*, **175**, 217 (1969).
[71] Mross, G. A., Doolittle, R. F., and Roberts, B. F., *Science*, **170**, 468 (1970).
[72] Doolittle, R. F., and Mross, G. A., *Nature*, **225**, 643 (1970).
[73] Blombäck, B., and Blombäck, M., in *Chemotaxonomy and Serotaxonomy*, 3 (Academic Press, London and New York, 1968).
[74] Doolittle, R. F., Schubert, D., and Schwartz, S. A., *Arch. Biochem. Biophys.*, **118**, 456 (1967).
[75] Mross, G. A., and Doolittle, R. F., *Arch. Biochem. Biophys.*, **122**, 674 (1967).
[76] Doolittle, R. F., and Blombäck, B., *Nature*, **202**, 147 (1964).
[77] McKenna, M. C., *Ann. NY Acad. Sci.*, **167**, 217 (1969).
[78] Tashian, R. E., and Stroup, S. R., *Biochem. Biophys. Res. Commun.*, **41**, 1457 (1970).
[79] Goodman, M., *Human Biol.*, **34**, 104 (1962).
[80] Goodman, M., *Ann. NY Acad. Sci.*, **102**, 219 (1962).
[81] Goodman, M., *Human Biol.*, **35**, 377 (1963).
[82] Goodman, M., *Primates in Med.*, **1**, 10 (1968).
[83] Darwin, C., *The Descent of Man and Selection in Relation to Sex* (Appleton, New York, 1871).
[84] Patterson, B., Behrensmeyer, A. K., and Sill, W. D., *Nature*, **226**, 918 (1970).
[85] Howell, F. C., *Nature*, **223**, 1234 (1969).
[86] Simons, E. L., *Nature*, **221**, 448 (1969).
[87] Leakey, L. S. B., *Nature*, **213**, 155 (1967).
[88] Pilbeam, D. R., *Peabody Mus. Bull. Yale*, **31** (1969).
[89] Kohne, D. E., *Quart. Rev. Biophys.*, **3**, 327 (1970).
[90] Kohne, D. E., Chison, J. A., and Hoyer, B. H., *Carnegie Inst. Yr Book*, **69**, 488 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Krypton-85 in the Troposphere

KRYPTON-85 produced by fission in nuclear reactors is at present the most abundant man-made radioisotope in the troposphere. Its activity in air was 33 d.p.m. m^{-3} air by the end of 1969 and it continues to increase. In this communication we report recent measurements of the ^{85}Kr activity in samples from air liquefaction plants at Oberhausen and Duisburg in West Germany.

The raw krypton samples were purified by removal of the oxygen in a magnesium furnace, and their activity was measured in a small gas proportional counter, of sensitive volume 11 cm^3, surrounded by a lead shield 10 cm thick; a plastic scintillator was used as an anticoincidence counter. The counting efficiency was determined from other measurements on three ^{85}Kr samples in ^{14}C and T counters whose efficiencies were known. All samples were analysed more than four months after collection and the contributions of ^{133}Xe and ^{222}Rn (half-lives of 5.65 days and 3.82 days respectively) could be neglected. The amount of Xe in the purified krypton samples (~10%) was measured mass spectrometrically and the appropriate correction made. The only other correction was that for radioactive decay since collection. The mean standard deviation of the ^{85}Kr measurement is ±2.5%.

The data are presented in Fig. 1, together with data obtained previously in our laboratory by Schölch and already published in part[1,2]. The earlier values had to be lowered by about 5 to 9% to correct a calibration error and to compensate for radioactive decay. The increase of the atmospheric ^{85}Kr activity during the past decade is apparently linear and a least squares fit of a straight line gives an average net increase of 55 d.p.m. mmol^{-1} (Kr) yr^{-1}. The actual annual production of ^{85}Kr is, of course, larger than this because of the radioactive decay. Because the total tropospheric content of ^{85}Kr and thus the loss by radioactive decay increased linearly, the actual release of ^{85}Kr activity must have increased linearly with time by $(1+t/\tau)$, from 64.5 d.p.m. mmol^{-1} (Kr) yr^{-1} in 1959 to 99.5 d.p.m. mmol (Kr) yr^{-1} in 1969. t is the time counted from late 1956 and τ is the decay time of ^{85}Kr, 15.53 yr.

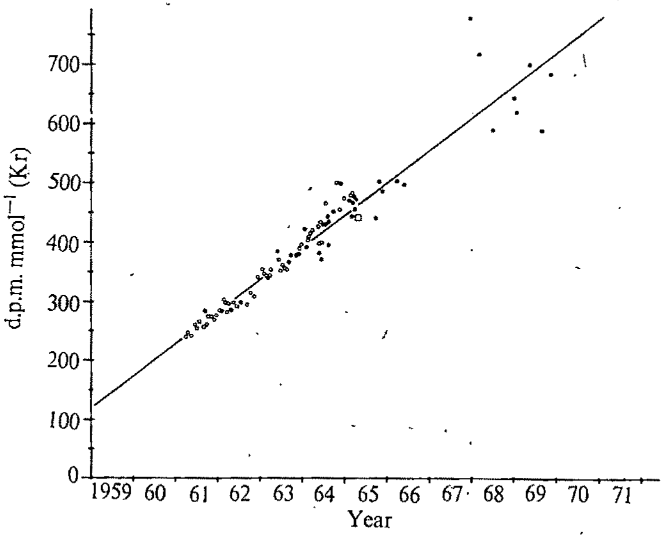


Fig. 1 ^{85}Kr activity in surface air at Duisburg and Oberhausen, Ruhr valley. ●, Recent measurements; ○, previous measurements from our laboratory; □, a sample distributed by the plant in Munich but probably collected at Oberhausen and measured by Shuping *et al.*[3]. The straight line is a least squares fit to the experimental data.

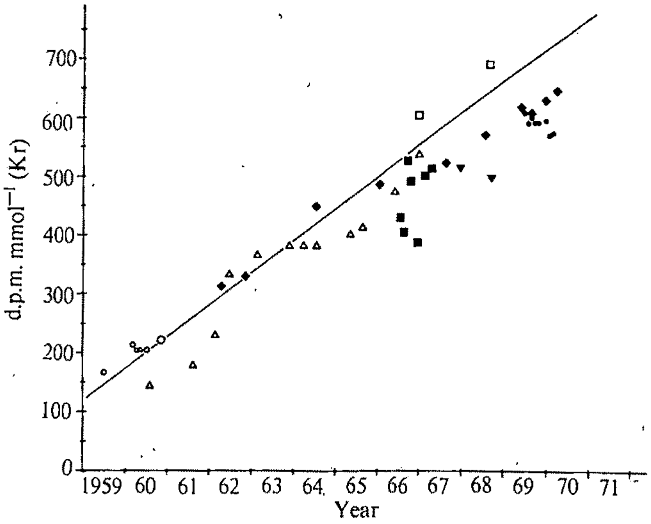


Fig. 2 ^{85}Kr activities reported at various locations by various authors. ○, Data from West Germany[7]; △, data from France[5]; □, data from Lyon, France[3]. The solid symbols represent measurements from North America[3,4]: ◆, Cleveland, Ohio; ■, Essington, Pennsylvania; ▲, Ontario; ●, Chester, West Virginia.

In Fig. 2 the ^{85}Kr measurements of other authors are compared with the fitted line. Although the individual data show a considerable scatter, even where monthly samples have been collected[2,3], the overall agreement is reasonably good and indicates that systematic differences between the various laboratories due to calibration errors are not too serious. The observed variations in the atmospheric ^{85}Kr concentration are probably real and are presumably caused by the discontinuous release of ^{85}Kr from fuel reprocessing plants and variations in horizontal and vertical mixing. A seasonal variation of the ^{85}Kr activity, which would be expected if ^{85}Kr were produced chiefly by nuclear explosions, cannot be detected above the noise level.

The data of Shuping *et al.*[3,4] from various stations of the North American continent in the years 1967 to 1969 fall below the fitted line of Fig. 2. As data from Lyon, France, measured by Shuping during the same period fall above the line, the apparent displacement cannot be explained by differences in calibration between the laboratories. In fact the average ^{85}Kr activity during 1968 and 1969 (including December 1967) was 587+9.2 for the North American stations and 668±29 for the Oberhausen and Duisburg samples, including the one sample from Lyon[3]. The mean standard deviations were calculated taking into account the systematic increase of 55 d.p.m. mmol^{-1} (Kr) yr^{-1}. The difference is 81±30, which seems significant.

The higher ^{85}Kr concentrations in the Ruhr valley could be derived from local sources. The large scatter of the samples from the Ruhr valley in 1968 and 1969, which is much larger than that of previous years (Fig. 1), also suggests contamination by local sources.

Finally the tropospheric burden of ^{85}Kr can be estimated from the present data. Using Pannetier's data[5] on meridional and height distribution of ^{85}Kr we obtain 9.65×10^{26} atoms ^{85}Kr by 1969. Assuming a linear increase of the tropospheric ^{85}Kr activity starting from zero at the end of 1956, this amount corresponds to a total ^{85}Kr production of 1.36×10^{27} atoms ^{85}Kr, which would correspond to the fission of 182 tons of ^{235}U (fission yield for thermal neutrons 0.293%, ref. 6).

The samples were provided by W. Stich of the Linde AG, Munich. The National Center for Atmospheric Research is sponsored by the National Science Foundation.

J. SCHRÖDER
K. O. MÜNNICH

II Physikalisches Institut,
C-14 Laboratorium,
Universität Heidelberg

D. H. EHHALT

National Center for Atmospheric Research,
Boulder, Colorado 80302

Received August 16, 1971.

[1] Ehhalt, D. H., Münnich, K. O., Roether, W., Schölch, J., and Stich, W., *J. Geophys. Res.*, **68**, 3817 (1963).

[2] Ehhalt, D. H., Münnich, K. O., Roether, W., Schölch, J., and Stich, W., *Third United Nations International Conference on the Peaceful Uses of Atomic Energy* (A/CONF, 28/P/541, May 1964).

[3] Shuping, R. E., Phillips, C. R., and Moghissi, A. A., *Anal. Chem.*, **41**, 2082 (1969).

[4] Shuping, R. E., Phillips, C. R., and Moghissi, A. A., *Radiolog. Health Data and Repts*, **11**, 671 (1970).

[5] Pannetier, R., *Rapport CEA-R-3591* (Saclay, 1968).

[6] Katcoff, S., *Nucleonics, Data Sheet No. 24*, **16**, 78 (1958).

[7] Griesser, O., and Sittkus, A., *Z. Naturforsch.*, **16**, 620 (1961).

Amino-acid Synthesis from Gases detected in Interstellar Space

Fox and Windsor[1] have synthesized amino-acids by heating a solution of formaldehyde, ammonia and water to 185° C and they interpreted their results in a cosmochemical context. We now report that reaction products from different combinations of ammonia, methanol vapour, formic acid vapour and formaldehyde react to produce amino-acids when exposed to ultraviolet radiation. Because ammonia[2], methanol[3], formic acid[4] and formaldehyde[5] have been identified in interstellar space, our finding suggests that amino-acids can be formed without water in interstellar space. Also, in spite of the absence of conclusive evidence of the presence of any biomolecules indigenous to the lunar surface, our findings suggest that amino-acids can be formed on the Moon. Although the conditions of our experiments differed from those in interstellar space and on the Moon, we believe that our findings have implications for interstellar chemistry.

The combinations of gases used in our experiments were ammonia, methanol and formaldehyde, and ammonia, methanol and formic acid. The ammonia was obtained from a cylinder of the compressed gas and we generated the formaldehyde gas by heating paraformaldehyde in a flask; the methanol and formic acid vapours were formed by heating the liquids in flasks. Glass tubes led the gases from the generating flasks to a 200 ml. 'Kimax' spherical flask in which the gases reacted.

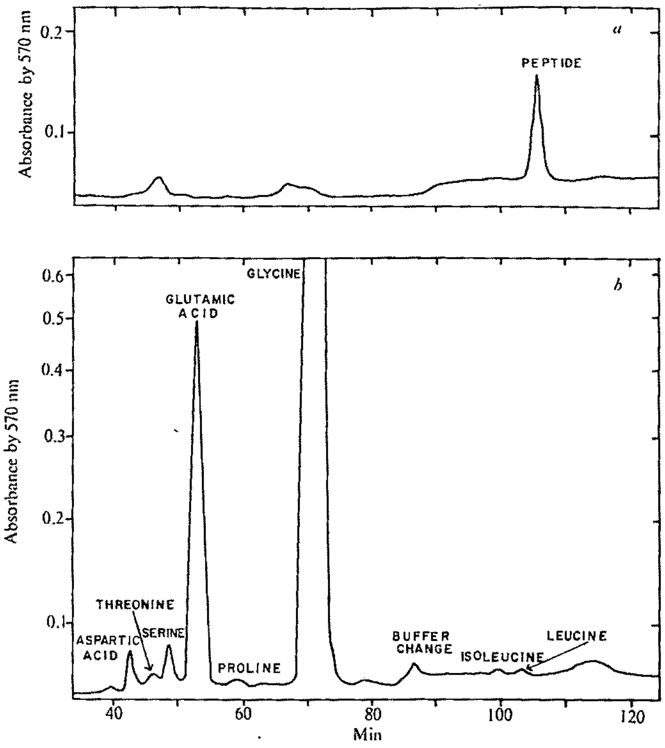


Fig. 1 Amino-acid analysis ion exchange chromatograms. *a*, Unhydrolysed, and *b*, hydrolysed samples (K) of a radiation product of ammonia, methanol vapour and formaldehyde.

All chemicals used were anhydrous because we intended to conduct the experiments without water. We also flushed out the mixing flask with ammonia before the two experimental runs to eliminate any residual water vapour. To prevent condensation of unreacted methanol and formic acid during mixing of the gases, we maintained the temperature of the mixing flask at about 115° C by directing four sunlamps on it (each 275 W) during both runs.

The gases were slowly introduced into the mixing flask and after 45 min the gas flows were stopped and the mixing flask was immediately sealed with a glass stopper.

The ammonia, methanol and formaldehyde reacted to yield 14 g of a pale yellow liquid, and the ammonia, methanol and formic acid yielded 19 g of a colourless liquid. We made no analyses of the reaction products immediately after mixing because our earlier experiments indicated that the formation of amino-acids from reaction products required exposure to ultraviolet radiation.

The flasks containing the reaction products were exposed to ultraviolet radiation from a 275 W sunlamp situated 60 cm below the flasks. The temperature at the bottom of the flasks remained at 35° C.

The liquid produced by mixing ammonia, methanol and formaldehyde started to crystallize after exposure to ultraviolet radiation for 2 days. The other reaction product started to crystallize after 7 days.

Negative tests for amino-acids indicated that the substance produced by mixing ammonia, methanol and formic acid had to be exposed to ultraviolet radiation for 25 days before amino-acids formed. The product yielded by mixing ammonia, methanol and formaldehyde was first analysed for amino-acids after exposure to ultraviolet radiation for 3 days and the analysis was positive.

The analyses for amino-acids were carried out by the method described by Moore *et al.*[6], Spackman *et al.*[7], Moore and Stein[8], and Granberg *et al.*[9]. The instrument used was a Beckman–Spinco model 120B amino-acid analyser. The samples were hydrolysed by treatment with 6 N HCl at 110° C under vacuum in sealed tubes for 24 h. Hydrolysis of the radiation reaction product of ammonia, methanol and formaldehyde yielded chiefly glycine and glutamic acid (Fig. 1). Relatively small amounts of aspartic acid, threonine, serine, proline, isoleucine and leucine were, however, also present.

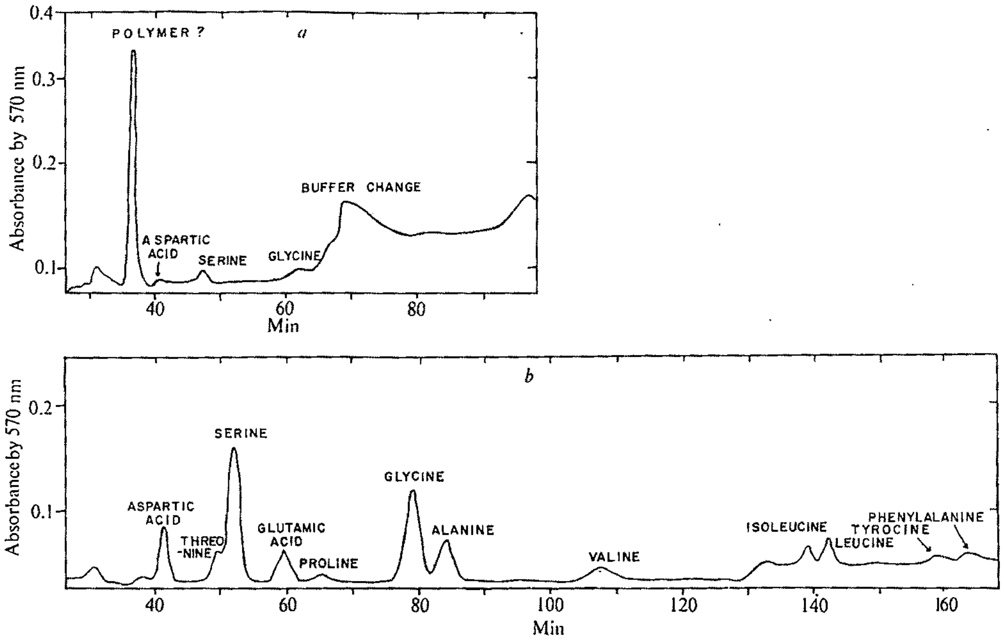


Fig. 2 Amino-acid analysis ion exchange chromatograms. *a*, Unhydrolysed, and *b*, hydrolysed samples (M) of a radiation reaction product of ammonia, methanol vapour and formic acid vapour.

The radiation reaction product of ammonia, methanol and formic acid yielded a larger number of amino-acids after hydrolysis. These included aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine (Fig. 2).

On hydrolysis, 50 mg of the radiation reaction product of ammonia, methanol and formaldehyde produced 0.135 mg of amino-acids—a yield of 0.27%. On the other hand, 50 mg of the radiation reaction product of ammonia, methanol and formic acid gave 0.07 mg of amino-acids—a yield of 0.14%.

The amino-acids produced by the ammonia–methanol–formic acid system are similar to those produced by Fox and Windsor[1] on heating formaldehyde, ammonia and water, and to those produced by Pavlovskaya and Pasynski[10] by ultraviolet radiation of ammonium chloride, formaldehyde and water.

The experimental procedure eliminated the possibility of accidental introduction of amino-acids as contaminants. The ammonia was provided from a cylinder of the compressed gas and thus it could not contain amino-acids—which are either non-volatile or sublime at temperatures greater than that at which the ammonia was drawn from the cylinder. By heating the paraformaldehyde, formic acid and methanol in separate flasks and introducing only the vapours into the mixing flask we avoided contamination by the non-volatile amino-acids. On the other hand, the relatively low temperature (115° C) at which the flasks were maintained precluded the introduction of those amino-acids which sublime, because they sublime at fairly high temperatures. We also tested each of the substances from which the vapours were generated; no amino-acids were found.

The chromatogram of the unhydrolysed sample (Fig. 1) indicates that ultraviolet radiation of the reaction product of ammonia, methanol and formaldehyde produced a peptide. The high peak shown in the chromatogram (Fig. 2) of the unhydrolysed sample of the other radiation reaction product indicates that a polymer might have formed. In this chromatogram as well as in the chromatogram of the hydrolysed sample shown in Fig. 1, the artefact peak due to a change in the eluting buffer is clearly marked.

A microbiuret test for peptides was positive for the radiation reaction product of ammonia, methanol and formaldehyde. The test of the other radiation reaction product was negative, suggesting that the polymer is the "HCN polymer" described by Labadie *et al.*[11], rather than polyamino-acid.

As established by mass spectrographic analyses the radiation reaction product of ammonia, methanol and formaldehyde yielded hexamethylenetetramine, but the other reaction product did not. The production of hexamethylenetetramine indicates that some water was formed during the reaction.

The facts that one of the reactions yielding amino-acids took place in the absence of water and that we only used gases suggest that perhaps some rethinking is needed about the kind of chemical reactions possible in interstellar conditions. The absence of water in our experiments strongly suggests that compounds which play an important role in the origin of life may form in interstellar space and that such compounds as amino-acids may form on the Moon.

Richard Granberg carried out the amino-acid analyses. We thank Kenneth King who suggested these experiments and M. Ewing, S. Moore, J. Oró, P. B. Hamilton, G. M. Sharma, S. J. Rasool, P. Thaddeus, D. Horn, N. T. Lui, B. Horn and B. Ericson-Moody for help. We also thank Janet Wollin for assistance. The research was supported by the US National Science Foundation.

Goesta Wollin
David B. Ericson

Lamont-Doherty Geological Observatory,
Columbia University,
Palisades, New York 10964

Received May 28; revised Spetember 14, 1971.

[1] Fox, S. W., and Windsor, C. R., *Science*, **170**, 984 (1970).
[2] Cheung, A. C., Rank, D. M., Townes, C. H., Thornton, D. D., and Welch, W. J., *Phys. Rev. Lett.*, **21**, 1701 (1968).
[3] Gottlieb, C. A., Ball, J. H., and Lilley, A. E., *Astrophys. J.* (in the press).
[4] Gottlieb, C. A., Ball, J. H., Lilley, A. E., and Zuckerman, B., *Astrophys. J.* (in the press).
[5] Snyder, L. E., Buhl, D., Zuckerman, B., and Palmer, P., *Phys. Rev. Lett.*, **22**, 679 (1969).
[6] Moore, S., Spackman, D. H., and Stein, W. H., *Ann. Chem.*, **30**, 1185 (1958).
[7] Spackman, D. H., Stein, W. H., and Moore, S., *Ann. Chem.*, **30**, 1190 (1958).
[8] Moore, S., and Stein, W. H., *Amino Acid Analysis, Methods in Enzymology*, **6**, 819 (1963).
[9] Granberg, R., Walsh, K. W., and Bradshaw, R. A., *Anal. Biochem.*, **30**, 454 (1969).
[10] Pavlovskaya, T. E., and Pasynski, A. G., in *The Origin of Life on the Earth* (edit. by Oparin, A. I., Pasynski, A. G., Braunstein, A. E., and Pavlovskaya, T. E.), 151 (Pergamon, London, 1959).
[11] Labadie, M., Jensen, R., and Neuzil, E., *Biochim. Biophys. Acta*, **165**, 525 (1968).

High Precision Charged Particle Detector using Noble Liquids

WE agree wholeheartedly with P. Rice-Evans[1] and the leading article in *Nature*[2] that there is a need for higher resolution particle detectors at the new synchrotrons. Following a suggestion by one of us[3], we are working towards the development of a thin multi-conductor chamber filled with a noble liquid. Our initial efforts with liquid argon have been described in several NAL Summer Study reports[4] and the successful operation of a liquid xenon proportional counter is the subject of a recent article[5].

We shall briefly summarize what has been learned so far.

First, a liquid xenon proportional chamber with an 8 mm diameter cathode and a single 4 μm anode detects charged particles with an efficiency of nearly 100%. The pulse rises in less than 150 ns and is typically 0.15 pCi in size. (Using liquid argon, all our chambers have been sensitive only at scattered points along the wire.)

Second, the electric field necessary for electron avalanche is ∼2 MV cm^{-1}. (Consequently the central wire must be the anode in order to avoid field emission.)

Third, for the detection of ionization pulses (liquid gain = 1) produced by a movable collimated source of alpha particles, a 700 μm thick chamber with five wires spaced 25 μm apart has a spatial accuracy better than 15 μm r.m.s.

Fourth, a series of parallel conducting strips mounted on a substrate is capable of inducing electron multiplication.

Fifth, severe electronegative contamination of liquid xenon (due to unknown impurities) can occur even when the content of oxygen and nitrogen is held below 0.1 p.p.m.[6]. Many of our purification techniques are described in ref. 6.

We are now making efforts to improve our control over electronegative impurities, to show that a series of substrate mounted conductors has high precision in the avalanche mode and to develop a practical readout scheme.

Although the liquid xenon multi-conductor chamber provides accurate spatial information in its "thin" form, it also provides efficient, rapid X-ray and γ-ray detection in its "thick" form. The thin form should prove invaluable in the field of high-energy and cosmic-ray physics, and the thick form holds equally great promise in the field of radiology.

This work was done under the auspices of the US Atomic Energy Commission.

STEPHEN E. DERENZO
GERARD SMADJA
ROBERT G. SMITS
HAIM ZAKLAD
LUIS W. ALVAREZ

Lawrence Berkeley Laboratory,
University of California,
Berkeley, California 94720

RICHARD A. MULLER

Space Science Laboratory,
University of California,
Berkeley, California 94720

Received October 1, 1971.

[1] Rice-Evans, P., *Nature*, **232**, 625 (1971).
[2] *Nature*, **232**, 599 (1971).
[3] Alvarez, L. W., *Group A Physics Note 672* (University of California Lawrence Radiation Laboratory, 1968).
[4] Derenzo, S. E., *et al.*, *National Accelerator Laboratory, Batavia, Illinois, Summer Study reports*, SS-154 (1969) and SS-181 (1970).
[5] Muller, R. A., Derenzo, S. E., Smadja, G., Smith, D. B., Smits, R. G., Zaklad, H., and Alvarez, L. W., *Phys. Rev. Lett.*, **27**, 532 (1971).
[6] Zaklad, H., thesis, (Lawrence Radiation Laboratory Report, UCRL-20690 (1971).

Mechanism of Oil Droplet Fragmentation in High Pressure Homogenizers

THE process of subdividing the relatively large polydisperse oil globules of a coarse oil-in-water emulsion into a large number of smaller globules of narrow size range is termed "homogenization". In a well homogenized emulsion the globules are so small that they have little tendency to rise and separate out; this is a desirable property of many emulsions of technical importance.

One of the most effective types of homogenizing machine utilizes a high pressure pump and a valve of the drop and lift type (Fig. 1*a*); pumping the coarse primary emulsion to the centre of the seating lifts the spring loaded valve and the emulsion escapes radially through the slit aperture to emerge with its oil phase finely dispersed. Machines with triple-piston pumps to minimize cyclic pressure fluctuations are used extensively in, for example, the dairy industry, for homogenizing milk, cream, ice-cream mix and other products[1]. A pressure drop of up to 250 kg cm^{-2} (3,500 pound inch^{-2}) or more may be applied across the valve and, the higher the pressure is, the smaller are the resulting oil globules. The fat globules in whole milk, which form a phase volume of about 0.035, have diameters up to about 10 μm with an average near 3.0 μm. Their subdivision into globules no larger than 1 μm may be accomplished readily with the homogenizer provided that the processing temperature is such that the fat is molten.

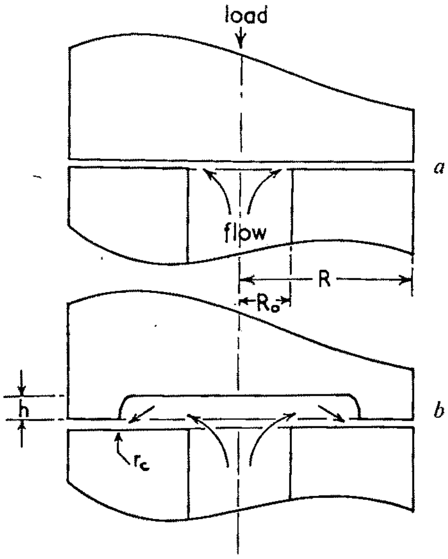


Fig. 1 *a*, Cross-section of simple flat-faced valve and seating; *b*, modified valve, to reduce the Reynolds number at r_c, the critical region for globule break-up.

In spite of the effectiveness of the high pressure machine the physical principles underlying the comminution process are obscure. I wish to report experimental data which favour one particular mechanism of droplet fragmentation. Past studies of the action of the piston homogenizer arose chiefly from the interest in homogenizing milk and my discussion is in the same context.

The lack of experimental evidence has precluded the acceptance of any one of the several theories[2] purporting to explain the physical principles underlying the homogenization of milk. Globule disruption within the valve has been variously thought to result from several effects or combinations of them. These are a shearing action within the milk itself, and between the milk and faces of the valve and its seat[3,4]; impact of the milk against the side walls of the valve[5,6]; changes in the milk velocity coupled with turbulence[7]; forces of cavitation[8–10] and, more recently, principally turbulence within the milk[11].

Some of these possibilities are questionable in the light of experimental observations. The finding that homogenization is almost complete soon after the milk enters the valve slit and before traversing the chief part of the valve radius[9] is evidence against the effectiveness of both impact forces and shearing between valve faces. Also claims that cavitational forces are responsible are not in accord with experiments which showed that effective homogenization still occurred when calculations indicated that cavitation was unlikely to be taking place in the valve[12].

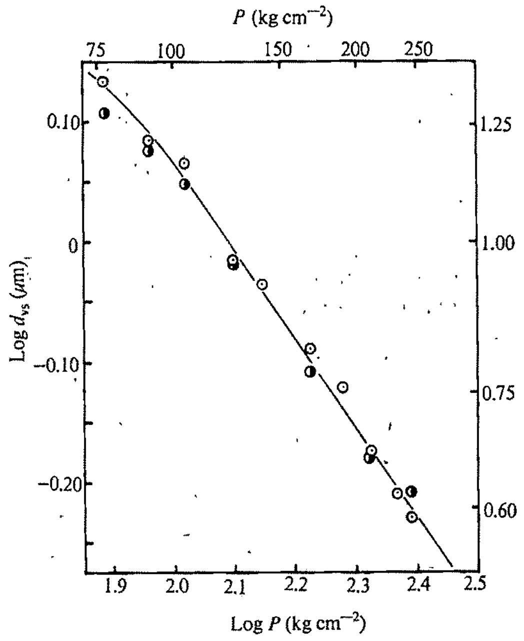


Fig. 2 The relationship between the mean fat globule diameter d_{vs} and the homogenizing pressure P at a processing temperature of 50° C. ○, Milk, normal flow rate of 100 l. h^{-1}, simple flat-faced valve (Fig. 1*a*), turbulent flow; ◑, milk/glycerol mixture, flow rate 29.9 l. h^{-1}, valve with recessed face (Fig. 1*b*), laminar flow at the critical radius for globule break-up.

The results from some of my own recent experiments support the conclusion that, in particular, shattering effects, shearing between valve faces and cavitational forces, all of which can occur to varying degree in a valve, have no significant effect on fat globule break-up. Of the remaining possibilities the effects of turbulence are of considerable interest because it has often been supposed that a turbulent flow aids homogenization. Recently, heavy turbulence has been reputed to be the principal effect involved[11] because an experimental correlation between the homogenizing pressures used and the mean globule sizes produced seemed to be explained by invoking Kolmogoroff's statistical theories of turbulence. If this were so, it would be expected that laminar flow, rather than the customary turbulent flow, through the valve would result in little or no fragmentation of globules. This has been investigated using a small capacity 3-piston machine (100 l. h^{-1}) (Rannie, Copenhagen, Denmark) in experiments with milk. A temperature of 50° C was chosen for the processing to ensure that the milk fat (melting point $\simeq 40°$ C) would be molten. The degree of homogenization or globule dispersion was assessed by turbidimetric determinations[13,14] of the mean volume–surface diameter $d_{vs}(=\Sigma nd^3/\Sigma nd^2)$ of the homogenized milk-fat globules.

Homogenizing milk at different pressures with the simple full-faced valve (Fig. 1*a*) showed that d_{vs} values, and hence the kinetics of the globule fragmentation, were reproducible. Typical results are plotted in Fig. 2*a* and show an approximately linear log/log relation[13,14]. The effect of increasing the pressure P (which produces smaller globules) and keeping the flow rate Q constant is to produce an increased emulsion velocity through the slit by a reduction in the valve lift, t. Clearly, the degree of globule dispersion is a function of this velocity. Calculations using approximate friction coefficients indicate that, for this machine, t decreases from 50 to 20 or 15 μm as P increases from roughly 10 to 300 kg cm^{-2}. The velocity at the slit entrance can be high; taking $t=20$ μm, $Q=100$ l. h^{-1}, $r=0.15$ cm, equation (1) gives $\nu=147$ m s^{-1}.

For a given flow rate Q and viscosity ν of the emulsion, the Reynolds number *Re* at any radius r of the valve face is independent of both the total pressure drop P across the valve and the width t of the slit between the valve faces. This follows from the form of the equation of continuity which, taking ν_r as the mean fluid velocity, is

$$Q=2\pi rt\nu_r \qquad (1)$$

and which combined with $Re=\nu_r t/\mathrm{v}$ gives the relation

$$Re=\frac{Q}{2\pi r\mathrm{v}} \qquad (2)$$

At the entrance to the slit, where $r=R_0=0.15$ cm for the valve in question, the *Re* defined by equation (2) is at a maximum (about 3,000) for milk at 50° C flowing at the normal rate of 100 l. h^{-1}, falling to about 800 at $R=0.55$ cm, the exit radius. The results of recent experiments (L. W. P., to be published) indicate that flow conditions would certainly be regarded as turbulent for these values of *Re*, but values of $Re \lesssim 250$ would be needed for laminar flow to be established within the valve slit.

Laminar conditions were achieved experimentally as follows. A circular recess of radius 0.4 cm was machined in the upper valve surface (Fig. 1*b*), leaving only an outer annulus of metal 0.15 cm wide to bear on the seat. The depth h of the recess was calculated to be such that the emulsion would suffer virtually no change in velocity immediately upon turning into the recess from the central conduit. This was assured by making $h=R_0/2=0.075$ cm. Radial flow of the emulsion with diminishing velocity would ensue up to the leading edge of the annular ridge, and it would then accelerate into the slit, to an extent depending on the valve lift, before finally emerging. This valve geometry produced a degree of homogenization of milk ($Q=100$ l. h^{-1}) equal to that attained at the same pressure with wholly plane surfaces. Levelling-off the annular ridge but maintaining a separation as large as h would be equivalent to operating a completely flat valve at $P\simeq 0$, and no homogenization would take place. It is certain, therefore, that globule break-up occurred at the ridge where, at the rate of flow in question, *Re* was about 1,125 ($r=0.4$ cm). A lowering of the value of *Re* at this critical radius was then achieved by reducing Q to 28.9 l. h^{-1} (that is, to about one-third of the usual rate), thus producing a transitional state of flow ($Re\simeq 320$) in the slit entrance. Such a change in conditions did not, however, impair the homogenizing effect of the valve and results were obtained for milk in close agreement with those plotted in Fig. 2.

A laminar flow at the critical radius was obtained finally by mixing glycerol with the milk to increase the bulk viscosity ν to 3.2 cS. The glycerol diluted the milk, but the degree of homogenization measured at a given pressure has been found to be insensitive to a reduction in the oil phase volume of the emulsion when this is low[14]; the presence of the glycerol was shown to have no influence on the spectroturbidimetric size analyses.

The combined effects of modifying the valve, reducing Q and increasing ν were calculated to give a value of *Re* at the entrance to the recess of approximately 270, decreasing with increasing radius to a value of 100 in the entrance to the valve slit (the critical region). In addition, the flow would be laminar in the central supply conduit and cavitation-free across the valve. The values of d_{vs} obtained at different homogenizing pressures under these conditions are plotted in Fig. 2*b*. Contrary to any expectation of poorer globule dispersion, it may be seen that values at corresponding pressures agree remarkably well with those achieved with the customary turbulent flow.

These results clearly show that turbulence does not play an important role in the fragmentation process. The possibility that two different mechanisms operate—one appropriate to laminar and one to turbulent flow—to produce identical results is hard to believe.

The degree of homogenization for a given pressure is relatively insensitive to quite large changes in Q, ν and the valve geometry; presumably this is a consequence of automatic, compensating changes in valve lift.

Experiment has shown that globules must fragment almost immediately after entering the valve slit. It has been suggested[7] that, at the slit entrance, globules are drawn into threads before they disintegrate and this seems the most likely possibility. In this region, the flow converges rapidly into the minute crevice and there is a steep velocity gradient. Such a gradient along the line of flow is to be found in a plane hyperbolic flow field and its distorting effect on globules at the slit entrance could be quite similar to the effects that have been observed in the laboratory on fluid drops in that type of field[15,17]. In those experiments, deformation and extension of a drop in the flow direction occurred; at a sufficiently high shear rate—depending on the fluid system—the drop could be drawn into a cylindrical thread which subsequently disintegrates into several small drops, particularly if the generating flow was abruptly retarded or stopped. In this type of flow threads can be formed even if the ratio of the viscosity of the emulsified drop to that of the suspending medium, η_1/η_0, is as high as 20 (ref. 15) or, as we have found by experiment, even 50 or more. The high value of 26 in the case of milk would, therefore, seem unlikely to impose any restrictions on the disintegration of molten milk fat globules by this mechanism. By contrast, it seems that in simple laminar shear flow drops do not disintegrate when $\eta_1/\eta_0 > 4$ but merely distort and twist in the flow[16]. It follows that, in the experiment above with laminar flow of the milk/glycerol mixture, the fact that η_1/η_0 was 6.4 is incompatible with a simple shear mechanism of break-up; this accords with experiment.

Calculations[17] of the minimum shear rate or velocity gradient G needed to deform a milk fat globule in hyperbolic flow give some idea of the local gradient that would be necessary to do this at the entrance to the valve slit. Thus taking an average globule diameter of 3 μm, a viscosity of 0.01 P for the continuous phase and 5 dyne cm^{-1} for the interfacial tension[18], a value of $2.5 \times 10^6\ \text{s}^{-1}$ is found. For a fairly high pressure drop across the valve the average velocity at the entrance to the slit can be $\sim 10^4\ \text{cm s}^{-1}$; the small upstream approach velocity may be neglected in comparison. Gradients of $G \sim 10^6\ \text{s}^{-1}$ would be possible, therefore, provided that the velocity change occurred over distances of the order of 10^{-2} cm. The rapidly decelerating radial flow of the bulk emulsion across the valve, which succeeds its acceleration into the slit, would then appear to be an appropriate condition for disintegration of the thread-like particles.

The degree of elongation and break-up of a globule probably depends on its precise trajectory into the slit because the velocity gradient across the width of the slit at the entrance would produce a corresponding variation in G over the cross-section. The greatest rupturing effect would be expected in the centre of the stream where the velocity is greatest and G is a maximum, though the length of the disrupting zone may still not favour the greatest break-up effect possible. Thus a single passage of the emulsion through the valve could not produce the maximum dispersive effect; this accords with the fact that repeated homogenization of the same emulsion at constant pressure progressively increases globule dispersion.

Speculating on the somewhat improved dispersion that can be achieved with breaker rings[4] and corrugated valve surfaces[7,19], it seems likely that this arises from the introduction, by these modifications, of additional regions where accelerated flow can occur.

I thank Professor J. N. Hunt, Applied Mathematics Department, University of Reading, for his interest and Miss S. Higgs and Mrs V. Bines for technical assistance.

L. W. PHIPPS

National Institute for Research in Dairying,
Shinfield,
Reading,
Berkshire

Received May 10; revised September 15, 1971.

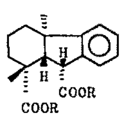
[1] Cronshaw, H. B., *Dairy Information* (Dairy Industries Ltd, London, 1947).
[2] Trout, G. M., *Homogenized Milk* (Michigan State College Press, East Lansing, 1950).
[3] Doan, F. J., *Rep. Pa. Assoc. Dairy Milk Insp.*, **7**, 70 (1931).
[4] Farrall, A. W., *Dairy Engineering* (Wiley, New York, 1942).
[5] Doan, F. J., and Minster, C. H., *Pa. Agr. Exp. Sta. Tech. Bull.*, 287 (1933).
[6] Sommer, H. H., *The Theory and Practice of Ice Cream Making*, third ed., 119 (published by the author, Madison, Wisconsin, 1938).
[7] Wittig, A. B., *Mechanics of the Homogenizing Process and the Influence of Emulsion Change Functions on Milk* (edit. by Norsk Meieriteknisk, Forening, 1950).
[8] Loo, C. C., Slatter, W. L., and Powell, R. W., *J. Dairy Sci.*, **33**, 692 (1950).
[9] Loo, C. C., and Carleton, W. M., *J. Dairy Sci.*, **36**, 64 (1953).
[10] Rees, L. H., *J. Soc. Dairy Tech.*, **21**, 172 (1968).
[11] Walstra, P., *Neth. Milk Dairy J.*, **23**, 290 (1969).
[12] McKillop, A. A., Dunkley, W. L., Brockmeyer, R. L., and Perry, R. L., *J. Dairy Sci.*, **38**, 273 (1955).
[13] Goulden, J. D. S., and Phipps, L. W., *Proc. Third Intern. Cong. Surface Activity*, C3, 190 (1960).
[14] Goulden, J. D. S., and Phipps, L. W., *J. Dairy Res.*, **31**, 195 (1964).
[15] Taylor, G. I., *Proc. Roy. Soc.*, **146**, 501 (1934).
[16] Karam, H. J., and Bellinger, J. C., *Ind. Eng. Chem.*, **7**, 576 (1968).
[17] Rumscheidt, F. D., and Mason, S. G., *J. Colloid Sci.*, **16**, 238 (1961).
[18] Horwitz, C., Fremlin, J. H., and Farr, R. F., *Phys. Med. Biol.*, **10**, 385 (1965).
[19] Goulden, J. D. S., and Phipps, L. W., *Proc. First Intern. Cong. Food Sci. Tech.*, London (1962).

BIOLOGICAL SCIENCES

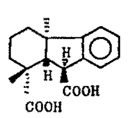
New Type of Compound with Strong Sweetness

CHEMISTS have long been interested in sweet substances to be used in place of sugar for medical and dietary purposes, and several such substances have been found in natural sources or as synthetic compounds. Sodium saccharin, sodium cyclohexylsulphamate, glycyrrhizin[1] (V), stevioside[2] (VI), naringin dihydrochalcone[3] (VII), neohesperidine dihydrochalcone[3] (VIII), and L-aspartyl-L-phenylalanine methyl ester[4] (IX) are well known as sweeteners, some of which have been put to practical use. We wish to report a new type of sweet compound with a different structure.

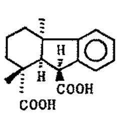
Studies on chemical conversion of pine tree rosin to biogenetically active compounds have been carried out in our laboratory. Recently four stereoisomers (I, II, III, and IV) of 4β, 10α-dimethyl-1, 2, 3, 4, 5, 10-hexahydrofluorene-4α, 6-



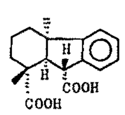
(I) a, R=H
b, R=Na



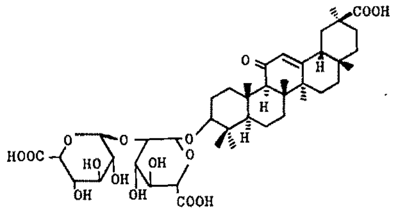
(II)



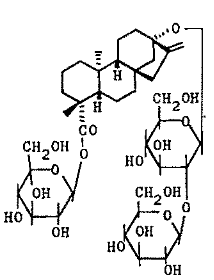
(III)



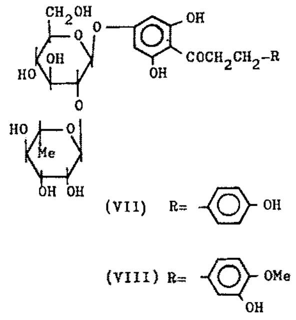
(IV)



(V)

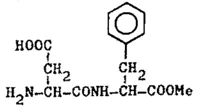


(VI)



(VII) R=

(VIII) R=



(IX)

dicarboxylic acid have been synthesized from pine tree rosin[5], one of them (I) had a remarkably sweet taste although the others had no taste. The taste of this compound had some bitterness besides the sweetness (Table 1). It is unique in that its structure is that of a simple diterpenoid. Though glycyrrhizin (V) and stevioside (VI) have a similar terpenoid skeleton, they have a glycoside formula as a whole. Correlations between sweetness and structure have been sought by many researchers, and the fact that only one out of four stereoisomers had a sweet taste should make a significant contribution. We are now working on its large scale preparation and on toxicity tests (acute, subacute, and chronic).

Table 1 Comparison of Taste

Compound	Relative sweetness (threshold value for sweetness; conc. % in H_2O solution)
Sucrose[6]	1
(Ia)	1,300~1,800 (0.0006%)
Na salt of I (Ib)	1,600~2,000 (0.0007%)
Sodium saccharin[6]	200~700
Sodium cyclohexylsulphamate[6]	30~ 80
Glycyrrhizin[6] (V)	50
Stevioside[6] (VI)	300
Naringin dihydrochalcone[6] (VII)	300
Neohesperidine dihydrochalcone[6] (VIII)	2,000
L-Aspartyl-L-phenylalanine methyl ester[6] (IX)	100~200
Compound	**Relative bitterness**
Caffein	1
(Ia)	12~18
Na salt of I (Ib)	12~13

We thank Dr Yasuo Abe and the staff of Takeda Chemical Industries Ltd for examining the sweetness and bitterness of compound (I).

AKIRA TAHARA
TADASHI NAKATA
YASUO OHTSUKA

Rikagaku Kenkyusho,
(The Institute of Physical and Chemical Research),
Wako-shi,
Saitama-ken

Received June 7, 1971.

[1] Beck, K. M., in *Encyclopedia of Chemical Technology*, second ed., **19** (edit. by Mark, H. F., Mcketta, jun., J. J., and Othmer, D. F.), 602 (Wiley, New York, 1969).
[2] Wood, jun., H. B., Allerton, R., Diehl, H. W., and Fletcher, jun., H. G., *J. Org. Chem.*, **20**, 875 (1955); Vis, E., and Fletcher, jun., H. G., *J. Amer. Chem. Soc.*, **78**, 4709 (1956).
[3] Horowitz, R. M., and Gentili, B., *J. Agric. Food Chem.*, **17**, 696 (1969).
[4] Mazur, R. H., Schlatter, J. M., and Goldkamp, A. H., *J. Amer. Chem. Soc.*, **91**, 2684 (1969).
[5] Tahara, A., and Ohtsuka, Y., *Chem. Pharm. Bull. (Tokyo)*, **18**, 859 (1970).
[6] Beck, K. M., in *Encyclopedia of Chemical Technology*, **19** (edit. by Mark, H. F., Mcketta, jun., J. J., and Othmer, D. F.), 594 (Wiley, New York, 1969).

Lesions due to Complement in Lipid Membranes

WHEN cells are lysed by antibody and complement (C), uniform bubbles or "holes" revealed by negative staining in the electron microscope appear at the surface. These lesions are probably due to micelle formation in the surface lipid layer (evidence reviewed in ref. 1). C activated by antibody through C8 has also been shown to damage liposomes containing Forssman antigen in their lipid membranes[2,3], though not to make holes in them[4]. C activated in the "reactive lysis" system (involving attachment to erythrocyte membranes or other hydrophobic surfaces of an unstable $\overline{\text{C567}}$ complex which in turn interacts with C8 and C9[5]) causes typical lesions in erythrocyte membranes[6] and in lecithin liposomes[7].

Although the detailed mechanism remains to be elucidated, the evidence indicates that the terminal components of C act on a lipid substrate to form micelles and that this is responsible

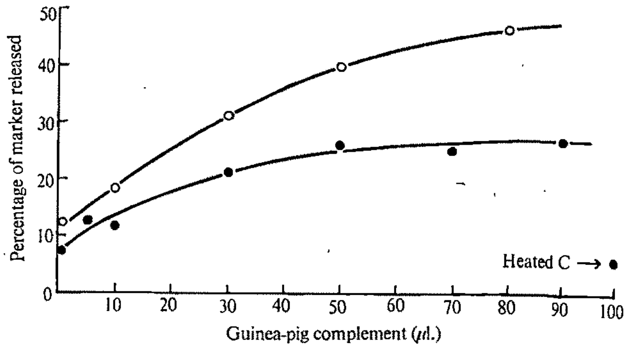


Fig. 1 Release of glucose marker from liposomes (made from extracted sheep erythrocyte lipids), as a function of the amount of guinea-pig C added. 200 µl. total reaction mixture was incubated for 1 h at 37° C. Anti-Forssman antibody dilution 1 : 10, ○; 1 : 25, ●.

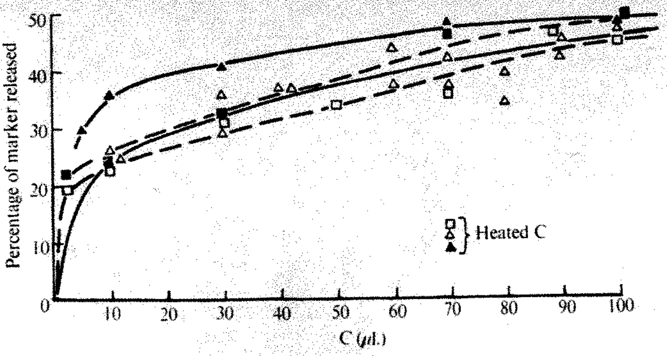


Fig. 2 Glucose marker release with human and rabbit C. Incubation conditions were as in Fig. 1. □, Human C; ■, human C+1 : 25 antibody; △, rabbit C; ▲, rabbit C+1 : 25 antibody.

for the irreversible cell damage. The only contrary evidence is a report that holes are formed by the action of C5, before cell damage is apparent[8], and the failure to demonstrate holes in liposomes damaged by C activated by antigen-antibody combination. We wish to report that typical holes are formed when liposomes are damaged by C in both the conventional antibody-mediated and the reactive lysis systems but that no lesions are detectable when C6-deficient rabbit serum is used as the source of C.

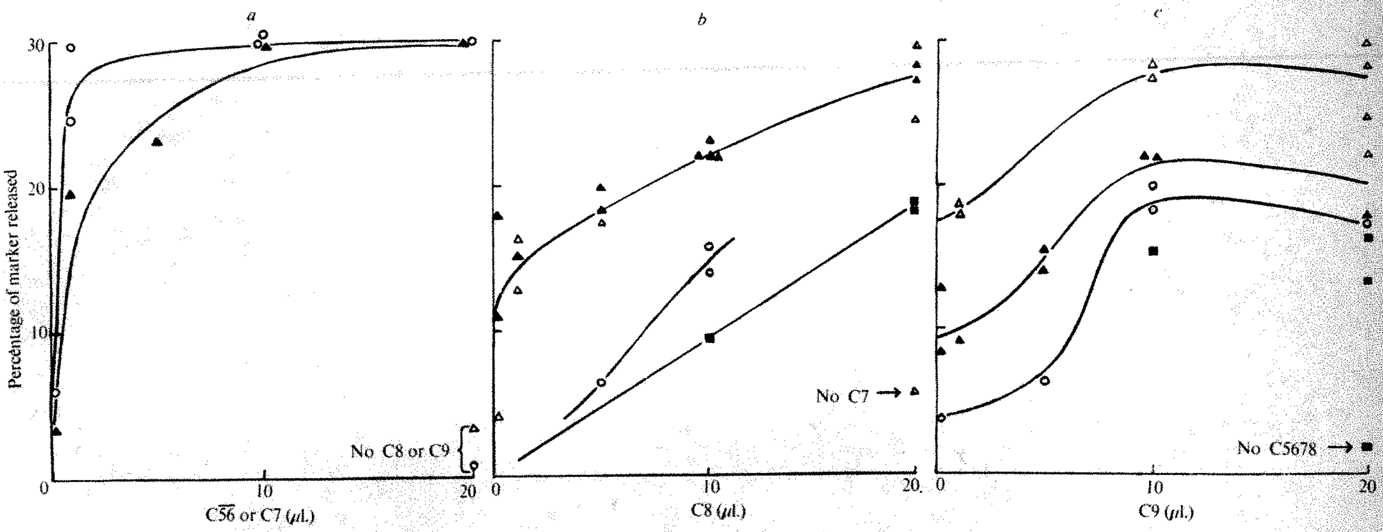


Fig. 3 Marker release from liposomes in the reactive lysis system. Conditions as for Fig. 1. *a*, ▲, 20 μl. C8+20 μl. C9+10 μl. C7+variable C$\overline{56}$; ○, 20 μl. C8+20 μl. C9+10 μl. C$\overline{56}$+variable C7. *b*, △, 10 μl. C$\overline{56}$+1 μl. C7+variable C8+ :−20 μl. C9; ▲, 10 μl. C9; ○, 5 μl. C9; ■, 1 μl. C9. *c*, △, 10 μl. C$\overline{56}$+1 μl. C7+variable C9 + :−20 μl. C8; ▲, 10 μl. C8; ○, 5 μl. C8 ; ■, 1 μl. C8.

Liposomes (aqueous dispersions of vesicles with walls of one or more stable bimolecular lipid layers containing Forssman antigen) were prepared from the lipid mixture extracted from sheep erythrocyte membranes (or whole washed erythrocytes) with 2 : 1 chloroform : methanol, and evaporated to dryness under a stream of dry nitrogen.

Samples of some preparations were dissolved in diethyl ether and added to 0.3 M aqueous glucose, and the ether was removed by evacuation in a rotary evaporator for about 30 min. In other samples dried lipids were allowed to swell in 0.3 M glucose under nitrogen without agitation for at least 2 h. Both methods yielded turbid suspensions containing 5–15 μmol total lipid per ml. based on the mean composition for sheep erythrocyte lipids[9]. After standing overnight liposomes were separated from most untrapped glucose by passage through a column (1.8 × 50 cm) of 'Sephadex G-25' (coarse), using for elution either the barbitone buffer (*p*H 7.2) (referred to hereafter as VBS) containing optimal amounts of Mg and Ca described by Mayer[10], or a lower ionic strength buffer (0.06 M NaCl, 0.18 M lactose, 4.87 mM Na barbitone, 1 mM $MgCl_2$, 0.15 mM $CaCl_2$ adjusted to *p*H 7.2). Preparations were stable for at least 3 weeks when stored under N_2 at 3° C. The presence and availability of Forssman antigen ware checked by showing that 1 ml. of the liposome preparation would neutralize about 97% of the haemolytic activity in an equal volume of a rabbit anti-Forssman antibody containing 200,000 haemolytic units per ml.

Release of glucose marker was measured by the change in absorbance at 340 nm due to reduction of TPN in the presence of hexokinase, glucose-6-phosphate dehydrogenase and ATP[11]. Both C and antiserum were dialysed beforehand in the cold against the appropriate buffer to remove pre-existing glucose, and a correction was made for any remaining traces. In most experiments 100 μl. of liposome suspension was mixed with diluted rabbit antibody followed by varying amounts of human, guinea-pig or rabbit serum as a source of C, and the volume was made up to 200 μl. with VBS. A suspension of liposomes in buffer was included as a control for untrapped glucose. After 1 h at 37° C the glucose released was measured, and the total glucose present was determined after complete lysis of the liposomes by adding 'Triton X-100' in chloroform at a final concentration of 2%. Release of glucose did not exceed about 5% with Forssman antibody over a range of concentrations 10^{-2} to 10^{-5} with or without heated C (56° C for 60 min) up to a concentration of 1 : 2.5. With antibody and active C, however, as much as 50% of the glucose was released (the amount varied with different liposome preparations, but was reproducible with a given preparation). Fig. 1 shows that the release by different amounts of guinea-pig C in the presence of 1 : 10 or 1 : 25 dilutions of antiserum (approximately 200,000 haemolytic U/ml.) reached plateau values of about 45 and 25% at 1 : 2.5 and 1 : 4 concentrations of C respectively. Fig. 2 shows similar curves for human and normal rabbit C (both of which contained "natural" Forssman antibody), with and without 1 : 25 added rabbit antibody. The lowest concentration of human C tested (1 : 100) caused about half maximal release

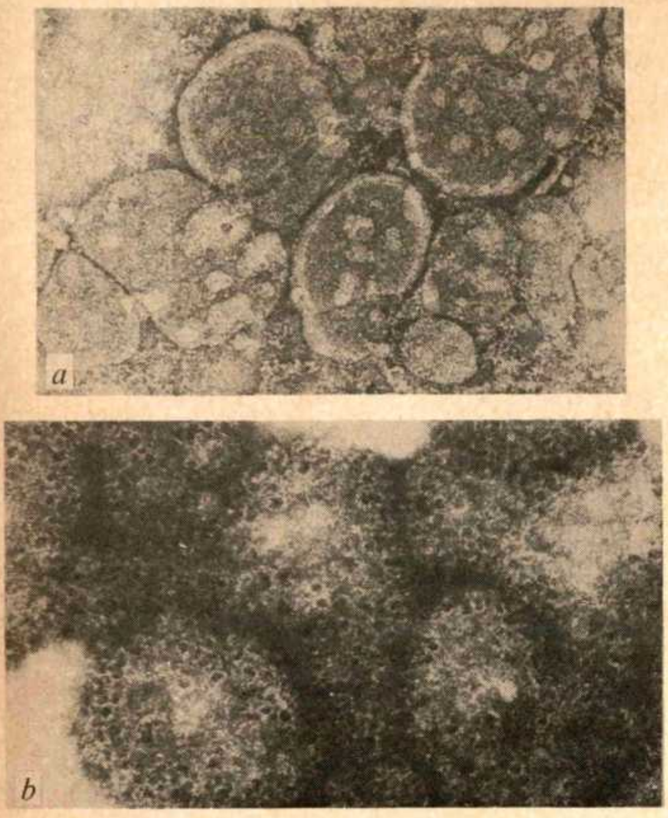


Fig. 4 Electron micrographs (negative staining) of grids coated with liposomes, treated with Forssman antibody and incubated (*a*) with heat-inactivated human C; (*b*) with unheated human C (× 120,000).

of glucose, even without additional rabbit antibody, and 1 : 40 rabbit C with added antibody released even more.

When C6 deficient rabbit serum[12] was tested as the source of C for the same range of concentrations no glucose was released in excess of the heated C control. That the C6 deficient serum was not inhibitory was checked by showing that addition of 1 : 10 normal rabbit C produced the expected degree of release even in the presence of three times as much C6 deficient serum.

The effect of different components of the reactive lysis system on liposomes was studied using human reagents prepared as described before[5,6]. 100 μl. of liposome suspension was stirred for 2 min at room temperature with a preparation of C$\overline{56}$; purified C7 was next added and stirring was continued for 5 min at 37° C. Purified C8 and C9 were added last and the mixture was stirred and incubated at 37° C for 1 h. Fig. 3*a* shows the effect of varying C$\overline{56}$ or C7 in the presence of an excess of the other reagents; glucose release increased as more C$\overline{56}$ was added up to 1/10 of the total volume, whereas maximal release occurred with minimal amounts of C7. When the concentration of C$\overline{567}$ was constant and the concentrations of C8 and C9 were varied, glucose release was maximal at about 1/10 C9 (Fig. 1*b*) and 1/10 C8 (Fig. 1*c*). Considerable release occurred at the highest concentrations of C8 in the absence of added C9, but the inference that C9 is unnecessary for lysis is weakened by uncertainty whether the C8 preparation was completely free of C9. Marker release in the presence of C$\overline{56}$, C7, C8 and C9 was increased by up to 7% by 0.01 M EDTA. In the absence of any single component, other than C9, it was minimal.

Attempts to demonstrate typical C lesions on liposomes by negative staining in either system were unsuccessful when carbon coated electron microscope grids were floated on or dipped into the liposome preparation at the end of the incubation period, when damage was expected. Even when the final suspension was prefixed by adding osmium tetroxide, damaged liposomes were not seen. Since it was possible that damaged liposomes might not attach to the grids, we tried allowing the liposomes to adsorb to the grids before damage occurred. Grids coated with 'Parlodion' and carbon were floated at room temperature on the original liposome suspension when using the Forssman antibody system, or on liposomes treated with C$\overline{56}$ and C7 for the reactive lysis system. After 10 min the grids with liposomes attached were rinsed in VBS and floated on diluted antibody followed by C or on C8 plus C9 respectively, and incubated at 37° C for 1 h. After incubation grids were washed in VBS (*p*H 7.2), negatively stained with 2% sodium silicotungstate and dried. Electron micrographs of grids coated with liposomes in this way, and then treated with IgM antibody and heated human C, showed liposome spherules about 0.1 to 1 μm in diameter covered with small filamentous particles which might be molecules of IgM. The appearance of grids coated similarly with liposomes, but treated with

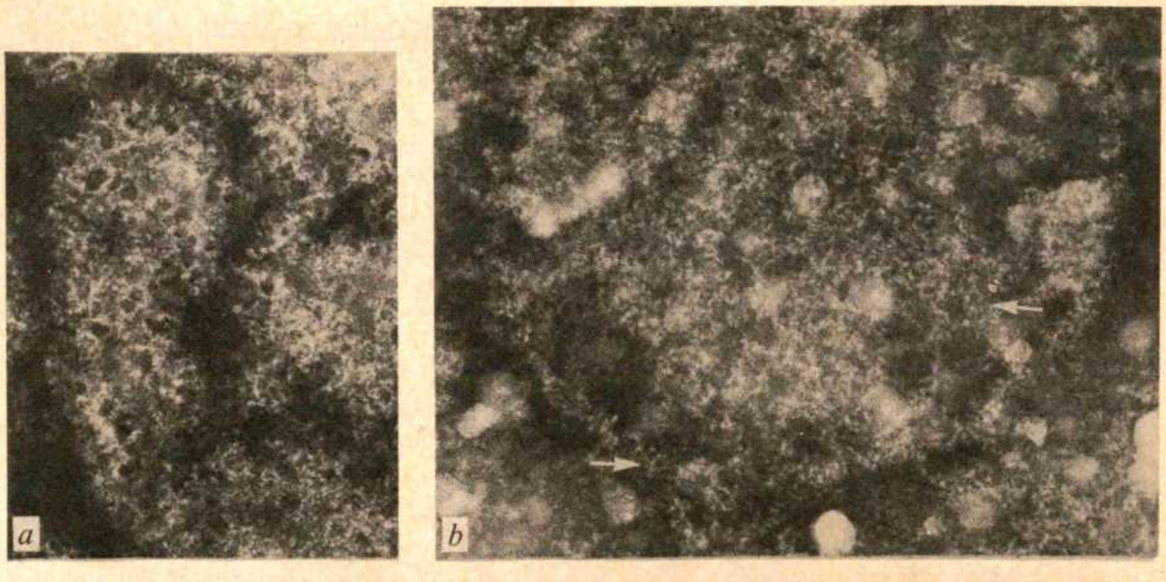


Fig. 5 Liposomes prepared as in Fig. 4, treated with Forssman antibody and *a*, normal rabbit C; *b*, C6 deficient rabbit C. Arrows point to ring-like structures (× 192,000).

antibody and unheated human C, differed in that most liposomes had either disappeared or were less prominent, and that both the remaining liposomes and the surrounding surface were covered with typical C holes (Fig. 4). These had a central dark area about 10 nm in diameter, similar to those produced by human C on *E. coli* lipopolysaccharide[1], though the external ring seemed to be narrower. Grids treated with antibody and normal rabbit C had similar holes. With C6 deficient serum "holes" were not evident, but some smaller ring-like structures could be discerned which may correspond to the membrane alterations reported after the action of C1-5[8] (Fig. 5). Typical lesions were obtained with the complete reactive lysis system, but when C8 and C9 or pretreatment with $\overline{C567}$ were omitted none were detected.

These experiments confirm evidence[1] that C-mediated damage to membranes, sufficient to cause them to leak, is correlated with disruption of the membranes by formation of characteristic micellar structures, visualized as holes within them. It is probable that holes were only visualized regularly when liposomes were adsorbed on the grids before disruption because the constituents of the liposomes, which are quite fluid structures, reassemble into smaller fragments when liposomes are disrupted in suspension. No lesions were apparent before the addition of C8 and C9 in the reactive lysis system, nor with C6 deficient serum in the conventional system; therefore the lesions must presumably be the final cause of damage and not incidental byproducts. Since the liposomes were composed only of lipids, the substrate upon which the terminal components of C act is presumably lipid, although it has not been formally excluded that some surface active substance, for example, a peculiar peptide, might be formed from one of the later C components and inserted into the lipid membrane. The report of Lachmann *et al.*[7] that holes could be produced by the reactive lysis system on pure lecithin liposomes indicates that the substrate is likely to be phospholipid, especially since lipid analysis revealed only a trace of cholesterol in any of the reagents which they used. The extent of any chemical changes may be very small, however, because none have yet been detected either by Inoue and Kinsky[13] who studied the action of guinea-pig C on liposomes containing Forssman antigen, or by one of us (S. N. P., unpublished) in a system involving human C and *E. coli* cell walls.

T. R. Hesketh
R. R. Dourmashkin
Sheila N. Payne
J. H. Humphrey
P. J. Lachmann

National Institute for Medical Research,
Mill Hill, London, NW7, and
Department of Pathology,
University of Cambridge

Received January 28; revised July 5, 1971.

[1] Humphrey, J. H., and Dourmashkin, R. R., *Adv. Immunol.*, **11**, 75 (1969).
[2] Haxby, J. A., Gotze, O., Muller-Eberhard, H. J., and Kinsky, S. C., *Proc. US Nat. Acad. Sci.*, **64**, 290 (1969).
[3] Kinsky, S. C., Haxby, J. A., Zopf, D. A., Alving, C. R., and Kinsky, C. B., *Biochemistry*, **8**, 4149 (1969).
[4] Knudson, K. C., Bing, D. H., and Kater, L., *J. Immunol.*, **106**, 258 (1971).
[5] Thompson, R. A., and Lachmann, P. J., *J. Exp. Med.*, **131**, 629 (1970).
[6] Lachmann, P. J., and Thompson, R. A., *J. Exp. Med.*, **131**, 643 (1970).
[7] Lachmann, P. J., Munn, E. A., and Weissmann, G., *Immunology*, **19**, 983 (1970).
[8] Polley, M. J., *J. Exp. Med.*, **133**, No. 1 (1971).
[9] Nelson, G., *Biochem. Biophys. Acta*, **144**, 221 (1967).
[10] Mayer, M. M., in *Experimental Immunochemistry* second ed. (edit. by Kabat, E. A., and Mayer, M. M.), 148 (Thomas, Springfield, Illinois, 1961).
[11] Slein, M. W., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. U.), 117 (1963).
[12] Lachmann, P. J., in *Protides of the Biological Fluids* (edit. by Peeters, H.), **17** (Elsevier, Amsterdam, 1970).
[13] Inoue, K., and Kinsky, S. C., *Biochemistry*, **9**, 4767 (1970).

Luminescent System in a Myctophid Fish, *Diaphus elucens* Brauer

The myctophids, or lantern fishes, which migrate diurnally to the surface at dusk, are among the most common and widely distributed deep-sea fishes known. They emit a blue light from tiny dermal photophores[1] arranged in species-specific patterns over the ventral and lateral surfaces of the body. Certain species also possess a large nasal or caudal organ. Although hydrogen peroxide stimulates the photophores to luminesce[2], nothing is known about the molecular mechanism involved. We report that photophore extracts of the myctophid, *Diaphus elucens* Brauer, cross-react to give light with highly purified luciferin (substrate) and luciferase (enzyme) of the marine ostracod crustacean, *Cypridina hilgendorfii*. The *Cypridina* luminescence reaction is due to the oxidation of luciferin by molecular oxygen, catalysed by luciferase.

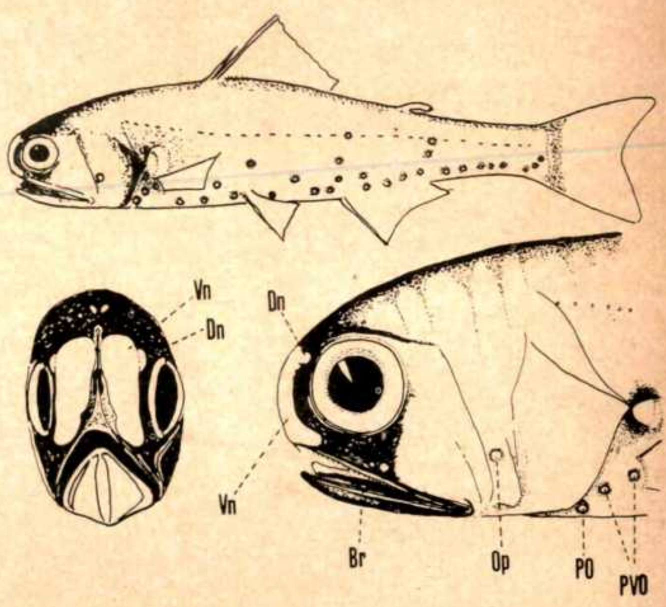


Fig. 1 *Diaphus elucens*, showing luminous organs on trunk and head. Note the large ventro-nasal organ (Vn) and the dorso-nasal organ (Dn). Other luminous organs: branchiostegal (Br), opercular (Op), pectoral (PO), and subpectoral (PVO). Drawn from actual specimen. Terminology of the photophores is according to Bolin[3].

Specimens of *D. elucens* were netted at night off Yui, Suruga Bay, Japan, at depths of 20–30 m as they migrated toward the surface from areas of the Bay, 1,000–1,600 m deep. They were preserved at −35° C. Specimens were 102–140 mm long from tip of snout to tail base and 24–31 mm deep. The distribution of the luminous organs is shown in Fig. 1. Cell-free extracts were prepared by grinding 100 dermal photophores in 10 ml. of 0.05 M Tris (pH 8.0) in a glass homogenizer chilled in an ice bath. The homogenate was centrifuged at 12,500g for 10 min at 4° C. The extract was dialysed overnight at 4° C against distilled water. *Cypridina* luciferin (in 0.1 N HCl and stored under argon in an ice bath to prevent autoxidation) and luciferase (1.73×10^{-6} mg/ml., in water) were prepared as previously described[4,5]. Light intensities were measured in arbitrary light units with a MacNichol photomultiplier (RCA 1P21) photometer[6].

Fig. 2 shows the result of mixing a highly active *Diaphus* extract with *Cypridina* luciferin. The light intensities (directly proportional to reaction rates) are plotted against time according to the equation for a first order reaction. The rate constants were for curve *a*, 4.59×10^{-2} s^{-1}; curve *b*, 2.15×10^{-2} s^{-1}; and curve *c*, 1.99×10^{-2} s^{-1}. They are very nearly proportional to extract concentration (curves *a*, *b*) and independent of luciferin concentration (curves *b*, *c*). The luminescent reaction therefore is first order and resembles the *Cypridina* reaction[4]. The reason for the high initial rate is unknown, but it also occurs in the *Cypridina* reaction[4].

The emission spectra of the reaction between *Diaphus* extract and *Cypridina* luciferin, and between *Cypridina* luciferin and luciferase, were measured with an Aminco-Bowman spectrophotofluorometer, using a Moseley 7035A (Hewlett-Packard) X-Y recorder. The *Diaphus* extract and *Cypridina* luciferase (+0.7 ml. 0.2 M Tris (*p*H 8.0)) were each injected into *Cypridina* luciferin diluted with 2 ml. of 0.2 M Tris (*p*H 8.0). The spectra are virtually identical (Fig. 3) and run from about 400 nm to about 600 nm, with a maximum at around 456 nm and half-intensity width of about 70 nm.

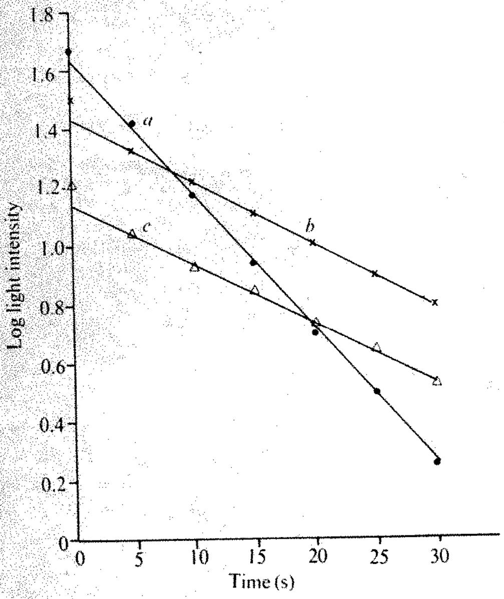


Fig. 2 Luminescence decay curves obtained by injecting *Diaphus* photophore extract into buffered *Cypridina* luciferin. Curve *a*, 1 ml. extract, 0.2 ml. luciferin, 2 ml. of 0.2 M Tris (*p*H 8.0); curve *b*, 0.5 ml. extract, 0.2 ml. luciferin, 2.5 ml. of 0.2 M Tris (*p*H 8.0); and curve *c*, 0.5 ml. extract, 0.1 ml. luciferin, 2.6 ml. of 0.2 M Tris (*p*H 8.0).

Diaphus extract mixed with *Cypridina* luciferin in anaerobic conditions did not luminesce until air was admitted. The activity of the extract was inhibited by immunoglobulin G isolated from rabbit antisera against *Cypridina* luciferase[7]. The inhibition of *Cypridina* luciferase by specific antisera but not by normal sera[8] indicates that the extract contains an enzyme similar to *Cypridina* luciferase. *Diaphus* extract (undialysed) also gave light with *Cypridina* luciferase. Only a slight stimulation of light emission occurred when *Diaphus* extract was mixed with 0.5% H_2O_2 or 0.5% H_2O_2 with either crystalline beef liver catalase (Worthington, 1 mg/ml., in water) or horseradish peroxidase (Worthington, 0.5 mg/ml., in water). *Cypridina* luciferin mixed with 0.5% H_2O_2 produced a brighter light. Nasal organ (Fig. 1, Vn, Dn) extracts gave results similar to dermal photophore extracts. Light was emitted when muscle and stomach extracts were mixed with *Cypridina* luciferin and luciferase. Purified luciferin and luciferase (details of which will be reported elsewhere) of the deep-sea decapod shrimp, *Hoplophorus gracilorostris*, also produced light with *Diaphus* extracts and *Cypridina* luciferin and luciferase.

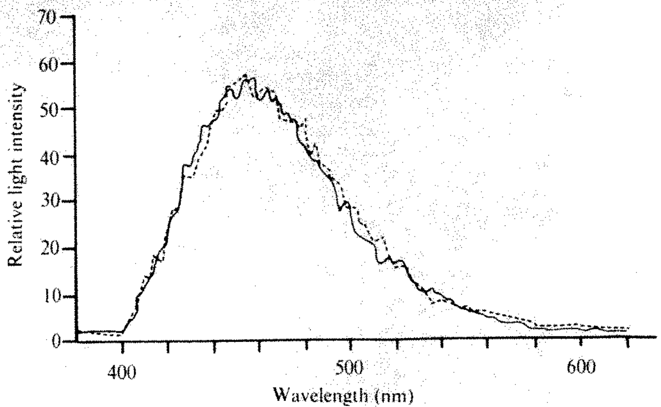


Fig. 3 Emission spectra of *Diaphus* extract–*Cypridina* luciferin and *Cypridina* luciferin–luciferase reactions. *Diaphus* spectrum (——): 1 ml. dialysed *Diaphus* extract, 0.2 ml. *Cypridina* luciferin, 2 ml. of 0.2 M Tris (*p*H 8.0). *Cypridina* spectrum (- - -): 0.3 ml. of *Cypridina* luciferase (1.73×10^{-6} mg/ml., in water), 0.2 ml. *Cypridina* luciferin, 2.7 ml. of 0.2 M Tris (*p*H 8.0). The monochromator was calibrated with a mercury light source but the curves are not corrected for either photomultiplier (RCA 1P21) sensitivity or luminescence decay.

Results with *Diaphus* extracts have been confirmed with extracts of the dermal photophores and infracaudal organ of another myctophid, *Stenobrachius leucopsarus*, collected off the coast of southern California. Cell-free extracts of the ventral photophores of the hatchet fish, *Polyipnus spinosus*, from Suruga Bay, and *Sternoptyx diaphana*, from southern California, also cross-reacted with the *Cypridina* system and showed properties similar to the myctophid system. We conclude that in *D. elucens*, and the other species studied, the biochemical mechanism for luminescence involves a luciferin and luciferase similar to the luciferin and luciferase of *Cypridina*.

We thank Dr B. G. Nafpaktitis and Mr W. T. O'Day for collecting *S. leucopsarus* and *S. diaphana* and Dr T. Abe for identifying *D. elucens*. The study was supported by grants from NSF and the Japan Society for the Promotion of Science under the US–Japan Cooperative Science Program.

FREDERICK I. TSUJI

Department of Biophysics and Microbiology,
University of Pittsburgh, and
Veterans Hospital,
Pittsburgh, Pennsylvania

YATA HANEDA

Yokosuka City Museum,
Yokosuka

Received June 7; revised July 13, 1971.

[1] Nicol, J. A. C., *J. Mar. Biol. Assoc. UK*, **39**, 27 (1960).
[2] Anctil, M., and Gruchy, C. G., *J. Fish. Res. Board Canada*, **27**, 826 (1970).
[3] Bolin, R. L., *Stanford Ichthyol. Bull.*, **1**, 89 (1939).
[4] Tsuji, F. I., Lynch, R. V., III, and Haneda, Y., *Biol. Bull.*, **139**, 386 (1970).
[5] Tsuji, F. I., and Sowinski, R., *J. Cell. Comp. Physiol.*, **58**, 125 (1961).
[6] Chase, A. M., in *Methods of Biochemical Analysis*, III (edit. by Glick, D.), 61 (Interscience Publishers, New York, 1960).
[7] Tsuji, F. I., Davis, D. L., and Donald, D. H., *J. Immunol.*, **102**, 519 (1969).
[8] Tsuji, F. I., Davis, D. L., and Sowinski, R., *J. Immunol.*, **84**, 615 (1960).

BOOK REVIEWS

Weakness at Reading

Reading and its Difficulties: a Psychological Study. By M. D. Vernon. Pp. viii+211. (Cambridge University: London, September 1971.) £3.20; $10.50.

THIS book is a sequel to Professor Vernon's *Backwardness in Reading,* published in 1957, and it deals for the most part with research which has been carried out since that date. A wide variety of experiments are cited: on visual perception, on auditory and linguistic development in children, on the relation of reading to intelligence, and on the social and motivational factors which are associated with high and low scores on reading tests. Finally, Professor Vernon examines some of the evidence relating to specific dyslexia (a disability, or group of disabilities, in which weakness at reading is accompanied by directional confusion, difficulty in spatial and temporal sequencing and very strange spelling mistakes).

As usual, Professor Vernon's standards of thoroughness and scholarship are admirable. The evidence on perception of form and print is particularly well presented; and above all I should like to pay tribute to the chapter on dyslexia, which I wish could be made compulsory reading for everybody concerned with educational planning.

I have one chief criticism. In parts of the book, particularly in the chapters on the relevance to reading of intelligence and of social and motivational factors, Professor Vernon seems to me to have been too charitable to research of dubious merit. Occasionally, it is true, she refers to inadequacies of experimental design or suggests that an author's interpretation of his findings may be questionable. It may well be, however, that educational research, if it is to prosper, requires not mere improvement here and there but radical re-thinking. Countless man-hours have been spent on "measuring" such allegedly useful entities as "IQ" and "reading age", on making judgments about parental support or children's degree of application (sometimes, incidentally, value-laden and arrogant ones, for example, "On the basis of interviews . . . they divided the parents into four classes: demanding, over-anxious, unconcerned and normal", page 107), and on correlating and in some cases factor analysing the resultant scores. Yet an IQ figure is a summary of a person's performance on a wide variety of tasks, the component elements of which are unknown; correlation coefficients are liable to be treacherous even for the sophisticated (because, having obtained them, we may still not know what to do with them), and surveys of what has happened in a particular educational milieu must surely be of less interest to science than generalizations about what can be made to happen by systematic manipulation of the environment of individual children. I wish, therefore, that Professor Vernon had done more to encourage research workers in the educational field to move in this latter direction rather than content themselves with large-scale surveys of the traditional kind.

This, however, is basically a criticism of current educational research, not of Professor Vernon, who in fact has done an excellent job with the material at her disposal.

T. R. MILES

Medical Illustration

History of Medical Illustration from Antiquity to AD 1600. By Robert Herrlinger. Pp. 178. (Pitman: London, 1971.) £7.

THIS book was designed as the first volume of a two volume series to cover medical illustration from the earliest times to the present day. Regrettably because of the premature death of the author, only the first volume was completed and this covers the period from antiquity to AD 1600. The author, a distinguished medical historian, also received recognition as an art historian and as a result his illustrations are particularly notable for their artistry as well as their appositeness.

There is a masterly foreword by Dr Poynter, director of the Wellcome Institute of the History of Medicine. The book is profusely illustrated with nearly four hundred illustrations, many of which are beautifully reproduced in colour. Apart from the occasional American spelling the translation is good and lively. The text presents a scholarly interpretation and communication of historical research and there is a proper enquiry of the aims of medical illustration at particular periods. Herrlinger demonstrates by text and illustration that schematization is more typical of medical illustrations of the middle ages than naturalism, but everywhere the text shows the erudition and clarity of mind of the author. He regards the early cauterization pictures as examples of the use of indication lines and later points out that couches and beds supporting invalids are symbols of illness rather than actual representations. In like manner, he illuminates the symbolism behind the apparent crudity of many medical illustrations and reaches the conclusion that antique medical illustration included schematic, semi-schematic and naturalistic forms, in fact, all the categories of modern medical illustration. The early references to "squatting figures" are carried throughout the text and illustrations to make a continuous and recognizable record. Herrlinger explains this curious posture found in many early texts as derived from the need to make thighs and genitals accessible when the corpse is laid on the table for dissection and therefore feels it is more apt to use the term "mensa figures". There are a few spelling mistakes, for example "skillful" for skilful on page 49, but this is unusual and the text is on the whole beautifully printed.

This is a bibliophile's book not only for the quality of the printing and the binding but also because of the many references to title pages, frontispieces and other bibliophilic details to delight the heart of all who love books. It is somewhat disappointing therefore to see that the plates are not always set as conveniently in relation to the text as one would like. With such profuse illustration this can be difficult, but there seems little reason for setting plate 3, referred to on pages 15 and 16, at page 35, and plate 2 is referred to on page 41 and is set at page 18. Again plate 4 is referred to on page 19 and is set at page 36.

There is a list of locations of the manuscripts referred to in the text and an index devoted more to illustrators than illustrations. Herrlinger pays particular attention to Vesaelius; nearly thirty pages detail the development and influence of this prince of medical illustrators. Sixteenth century illustrations of surgery and instruments are treated less fully although there is a section on sixteenth century title pages.

These are relatively minor objections, however, to a fascinating history, although I doubt that many medical students would be prepared to spend this amount of money even on a beautifully produced, authoritative and profusely illustrated work of medical history and illustration. Certainly every medical library should have a copy.

EDWARD HITCHCOCK

Training and Testing

Animal Psychophysics: The Design and Conduct of Sensory Experiments. Edited by William C. Stebbins. Pp. xii+433. (Appleton–Century–Crofts: New York, December 1970.) $18.75.

THIS book is the outcome of a symposium held at the University of Michigan in 1969, during which the participants discussed their work on the behavioural analysis of sensory function in animals. The book contains eighteen articles, and the emphasis throughout is on the methods by which the animals were trained and tested. Such coherence as the book possesses thus comes from the techniques shared in common by the participants, and not from any of the problems that they are studying by means of these techniques. The book covers a wide range of sensory experiments on animals, including, for example, the detection of ultrasound by bats, of X-rays by rats and monkeys, and of sound by opossums and hedgehogs. In all cases, there are descriptions of how the stimuli were presented and the construction of the apparatus, and details of any idiosyncrasies that make that particular species difficult to work with.

The problems of obtaining accurate behavioural data on the sensory capacities of animals are important; among other things this is the only way in which the significance of physiological experiments on sensation can be assessed. The articles in this book show how well these problems have been solved in a variety of cases, and that it is possible to obtain psychophysical data from animals which are as accurate as those obtained from man. As a whole, however, the book has little unity. The various techniques of animal psychophysics that have been used do not seem to have very much in common with each other, and few general principles emerge. As in other fields of animal behaviour, each species presents different problems which need different solutions, and reading a paper on, for example, how to train and test monkeys helps only a little in designing an experiment for fish. A book this length can also include only an arbitrary and limited selection of the work in this field. The final impression is thus of a fairly random selection of contributions, often interesting in themselves, but only tenuously interrelated.

W. R. A. MUNTZ

Problems Solved

Applied Diffraction Theory. By F. H. Northover. (Modern Analytic and Computational Methods in Science and Mathematics: a Group of Monographs and Advanced Textbooks, No. 28.) Pp. 632. (Elsevier: New York, 1971.) £20.

THIS book provides a mathematical analysis of the solutions to problems involving time harmonic scalar waves and time harmonic electromagnetic waves. The author's selection of obstacles is restricted and the application of his mathematical results to problems in physics and engineering is not always emphasized. The choice of material is clearly personal and he does not attempt to review the literature relating to diffraction theory. He has, however, succeeded in providing the full mathematical analysis of problems that have interested him for more than twenty years. The mathematical topics are sophisticated and include separation of variables, eigenfunction expansions, special functions, contour integration, asymptotic methods and integral equations.

The early chapters are concerned principally with the propagation of electromagnetic waves around a perfectly conducting sphere embedded in a dielectric medium (a model for very high frequency propagation of radio waves around the Earth surrounded by a nonhomogeneous atmosphere). They reflect the author's interests before 1955. I was astonished and dismayed to read on page 114: "Since 1954 the writer's interest has moved from the subject of tropospheric propagation of radio waves to propagation through ionized gases and laboratory-type microwave diffraction problems. Therefore no attempt is made here to describe work in this field subsequent to 1955."

The next problem to be considered is diffraction of electromagnetic waves by a perfectly conducting cone of finite dimensions. The author published a solution in 1962 and a revised solution in 1965. On reviewing these papers, he found that certain aspects of his earlier solutions were open to criticism. In this book he presents a third investigation, paying special attention to the edge conditions, which were ignored in his earlier papers.

The third and last major topic concerns microwave lenses and the Fabry–Perot microwave interferometer, and is based on the Kirchhoff–Huygens diffraction theory. Fredholm integral equations are used extensively in the solution of some particular problems for interferometers consisting of two curved plates in the form of equal spherical caps.

The introduction to the ideas of diffraction theory in the first chapter is unsound. Insufficient attention is paid to the radiation condition at infinity. The reasoning behind the choice of the velocity potential of the diffracted wave in section 6.1 (the sphere diffraction problem—the acoustic case) is erroneous. There are statements of some possible boundary conditions, but no discussion of these boundary conditions and no mention of impedance boundary conditions. The necessity of imposing edge conditions in certain circumstances receives no attention, even though this is central to the investigation of the finite cone problem in the eighth and ninth chapters.

Also in the first chapter are exact solutions of some problems involving spherical obstacles. The author obtains some complete solutions using the separation of variables. His solutions by means of the infinite Legendre integral transform method are incomplete and purely formal. It is not clear why the integral transform method is worthy of consideration: the integral representing the solution of even the simplest problem looks intractable and attracts no comment. There is no discussion of the relative merits of the two methods of solution, although an oblique remark appears on page 622.

The third chapter, an account of the WKB approximation, would have been more widely usable (as an introduction) with some worked examples and exercises. The analysis is applied to the Airy equation, but the reader is expected to work through the previous twenty pages of technically difficult mathematics to appreciate the results stated for this particular case. There are three references, the most recent dated 1962. A slightly modified version of the chapter has been published by the author in the *Journal of Mathematical Physics* (**10**, 715; 1969).

The tenth chapter is devoted to some of the more important properties of linear integral equations. Some knowledge of standard Fredholm theory is presupposed. One reference (to Lovitt's book, first published in 1924) is provided in the sixty-seven pages of analysis written in theorem-proof style. In the author's subsequent use of integral equations (in connexion with the microwave problems) there is only one reference to this chapter. A shortened version of the chapter has appeared in the *Journal of Mathematical Analysis and Applications* (**29**, 305; 1970).

Much of the detailed analysis is in an appendix (of 142 pages). The mathematical supplement at the end of the book is in two parts, the first devoted to the method of steepest descents and the second devoted to the Watson transformation. This is a useful, well written section. The first part has been published in the *Journal of Mathematical Analysis and Applications* (**29**, 216; 1970). There are several references in the second part; however, in some cases only authors' names are given.

In my copy, there is a serious omission of the details of references 67–96, and there are two obvious mistakes (on page 18 $r^{-1}\ e^{ikr}$ appears instead of $r^{-1}\ e^{-ikr}$ and in section 6.1 of the sixteenth chapter Legendre appears instead of Laguerre).

To summarize, *Applied Diffraction Theory* is a detailed account of the mathematical solutions of a small selection of problems. It will interest a highly specialized readership.

D. PRYS THOMAS

CORRESPONDENCE

Skuas

SIR,—B. R. Johnston's "Skua Numbers and Conservation Problems at Cape Hallett, Antarctica" (*Nature*, **231**, 468; 1971) has been read with interest and understanding. However, the report requires further elaboration.

With reference to paragraph 3, it should be said that the resident penguins were moved from the area selected for establishing Hallett Station into other parts of the rookery. They were not killed or shipped from Cape Hallett as the verb "were removed" might suggest. This 1956 operation, supervised by Carl Eklund, USNC IGY, was carried out as humanely as possible, without losses, and in light of information then available on penguin behaviour.

With reference to paragraph 5, the remark that there was no significant loss of monel-metal bands is unclear. The statement on mean annual loss of breeding skuas, and on the fifty breeding birds lost between 1956 and 1967, requires substantiating data to indicate how these figures were derived. Similarly, figures for chicks fledged/females breeding may be based on end-of-the-season count and may result in greater actual breeding success than really exists.

For the benefit of those unfamiliar with skuas, it should be stated that the species is highly adaptive and quickly takes to feeding on food and other wastes available at all Antarctic camps; and that its willingness to take anything that can be swallowed has contributed to its mortality.

With reference to the last sentence, next to the last paragraph, Cape Hallett is not a Specially Protected Area. A very small part of Seabee Hook inland toward the cliffs has been designated as a Specially Protected Area for terrestrial botanical reasons.

In 1970–71, USARP biologists at Hallett Station began the restitution of the natural area; the work is continuing and will result in returning a large part of the land occupied by Hallett Station to the original inhabitants. The 1971–72 scientific work at Hallett Station includes a skua-banding programme by Dr John R. Baker, Iowa State University, in cooperation with Mr C. J. R. Robertson, Department of Internal Affairs, Wellington, New Zealand, for data which may help to establish the true status of the skua population at Cape Hallett, Antarctica.

Yours faithfully,

GEORGE A. LLANO

Polar Biology Program,
US Antarctic Research Program,
National Science Foundation,
Washington DC 20550

British Diary

Monday, November 1

Modern Food and Food Additives (6.30 p.m.) Dr D. W. Kent-Jones, Society of Chemical Industry, in association with the Industrial Division of the Chemical Society, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

Some Aspects of Drying Oils Technology (7 p.m.) Mr G. Hutchinson, Oil and Colour Chemists' Association, at the Queen's Hotel, George Street, Hull.

Tomorrow's World in Telecommunications (7 p.m.) Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

Acrylic Resins (4 p.m.) Mr A. R. H. Tawn, Oil and Colour Chemists' Association, Thames Valley—Student Group, in the Main Lecture Theatre, Slough College, Slough.

Tuesday, November 2

Automatic Circuit Testing (7.30 p.m.) Mr D. Palmer, Institute of Measurement and Control, at the Peahen Hotel, St Albans.

Electrons in Solids (5.15 p.m.) Professor L. Pincherle, University of London, at Bedford College, Regent's Park, London NW1.

Migration and Interaction of Triplet Excitons (5.15 p.m.) Professor H. C. Wolf, Faraday Society, at the School of Chemistry, The University of Newcastle upon Tyne. (Bourke Lecture.)

The Excavations at Kalibangan (5.45 p.m.) Mr B. K. Thapar, University of London, at the Institute of Archaeology, 31/34 Gordon Square, London WC1.

Wednesday, November 3

Food Additives and Contaminants (3 p.m.) Society for Analytical Chemistry, at the Polytechnic of the South Bank, Borough Road, London SE1.

Henry Armstrong and School Science, 1880–1930 (1 p.m.) Dr W. H. Brock, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Migration and Interaction of Triplet Excitons (4 p.m.) Professor H. C. Wolf, Faraday Society, in the Department of Chemistry, The University of Manchester.

The Effectiveness of Modern Visual Communication Systems (6 p.m.) Mr B. Stapley, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

The Ionosphere and Radio Engineering (7 p.m.) Mr G. Millington, Institution of Electrical Engineers, at the University of Essex, Wivenhoe Park, Colchester, Essex.

Using Existing Resources Better—Planning for Future Growth (11 a.m. symposium) Institution of Chemical Engineers, jointly with the Society of Chemical Industry, at the University of Aston in Birmingham.

Thursday, November 4

Fermentation Pilot Plant Organization and Instrumentation (10.30 a.m., panel discussion) Society of Chemical Industry, Microbiology Group, at 14 Belgrave Square, London SW1.

Hazard Analysis — a Quantitative Approach to Safety (6.30 p.m.) Mr T. A. Kletz, Oil and Colour Chemists' Association, at the Royal Turks Head Hotel, Gret Street, Newcastle upon Tyne.

Migration and Interaction of Triplet Excitons (5 p.m.) Professor H. C. Wolf, Faraday Society, in the Department of Chemistry, The University of Sheffield.

Pressure Transmitter Design Parameters (7.30 p.m.) Mr C. H. Goode, Institute of Measurement and Control, at the Hotel Majestic, Park Place, Cheltenham.

Recent Developments in the Preservation of Foods (6.30 p.m.) Mr S. D. Holdsworth, Society of Chemical Industry, Food Group, at the School of Chemistry, University of Bristol.

Statistical Interpretations (7 p.m.) Mr M. Keegan, Society of Cosmetic Chemists of Great Britain, at the Royal Society of Arts, 6–8 John Adam Street, London WC2.

The Laws of Disorder. Part 1: Entropy; Part 2: The Second Law of Thermodynamics (1 p.m., ICI science films) Royal Institution, at 21 Albemarle Street, London W1.

The Pulsars (5.30 p.m.) Professor F. G. Smith, Institution of Electrical Engineers, Savoy Place, London WC2. (Appleton Lecture.)

Friday, November 5

Computer Controlled Frequency Response Measurements (5.30 p.m.) Mr A. J. Ley and Mr A. J. Martin, Institution of Electrical Engineers, at Savoy Place, London WC2.

Industrial Progress Associated with Analytical Research—Some Personal Experiences (5 p.m.) Mr A. F. Williams, Society for Analytical Chemistry, at the University of Strathclyde, Glasgow.

Making Sense of Music (9 p.m.) Professor H. C. Longuet-Higgins, FRS, Royal Institution, at 21 Albemarle Street, London W1.

Radiolysis of Nucleic Acids and Related Compounds (1 p.m.) Dr G. Scholes, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Saturday, November 6

Inca Architecture of Highland Peru (3.30 p.m.) Miss Anne Kendal, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Reports and Publications

not included in the monthly Books Supplement

Great Britain and Ireland

Science Research Council. The Work of the Rutherford Laboratory in 1970. Edited by J. R. Smith and F. M. Telling. Pp. 206. (Chilton, Didcot, Berks: Rutherford High Energy Laboratory, 1971.) [146

Papers and Proceedings of a Conference on Research into the Value of Time, held on 7th–8th May 1970, St. Christopher House, Southwark Street, London, SE1. Edited by N. W. Mansfield. (Time Research Note No. 16.) Pp. vi+131. (London: Highway Economics Unit, Department of the Environment, 1971.) *gratis* [146

PEP Broadsheet 527: Opting out of the NHS—a Review of the Private Sector of Medical Care. By Michael Lee. Pp. iv+28. (London: PEP, 1971.) 50p. [146

Productivity Measurement—a Symposium for the Seventies. Contributors: R. Allard, J. E. Keal, H. W. Mount, P. J. Samuel, G. R. Smith and A. R. Swannack. Pp. 50. (London: Institute of Personnel Management, 1971.) £1. [146

Xenobiotica, Vol. 1, No. 1 (January 1971). Published bi-monthly. Annual Subscription price (payable in advance) £15 postage paid. Subscribers in the USA., Canada and Mexico (air-freight) £16; $39.20 postage paid. Per part £3; $7.35 plus postage. Pp. 1–104. (London: Taylor and Francis, Ltd., 1971.) [146

J. J. Thompson Physical Laboratory, University of Reading. Research Report 1970–71. Pp. iv+61. (Reading: J. J. Thompson Physical Laboratory, 1971.) [146

Prospects in Health. Pp. 24. (London: Office of Health Economics 1971.) 15p. [176

Agricultural Research Council. Letcombe Laboratory—Annual Report 1970. Pp. ix+42. (Wantage: Letcombe Laboratory, 1971.) 50p. [186

A Guide to the Performance of the Standardized Rubella Hemagglutination-Inhibition Test. (Based on the publication of October 1970 by US Department of Health, Education and Welfare, Public Health Service, Health Services and Mental Health Administration, Center for Disease Control, Atlanta, Georgia, USA.) Pp. 43+9. Irvine, Ayrshire: Flow Laboratories, Ltd., Victoria Park, Heatherhouse Road, 1971.) 60p. [186

Potato Marketing Board: Sutton Bridge Experimental Station. Report No. 6: Storage Design and Equipment for Environmental Control in Potato Stores. Part 1: Storage Buildings. By O. J. H. Statham. Pp. ii+30. (London: Potato Marketing Board, 1971.) [216

Competition in Television. (An Address given by Huw Wheldon, Managing Director, BBC Television, at a Joint Meeting of the Faculty of Royal Designers for Industry and the Royal Society of Arts, 26 April 1971.) Pp. 15. (London: BBC, 1971.) [216

The Royal Observatory, Edinburgh. Publications—Vol. 7, No. 3: Photographic Photometry with Calcite Filter Calibration. (a) Inherent Error in the Method. By Peter W. J. L. Brand. (b) Its Use in the Near Infrared. By M. T. Brück. Pp. 35–45. Publications—Vol. 7, No. 4: Multicolour Photometry of the Stars in NGC 2264. By K. Nandy. Pp. 47–62. (Edinburgh: The Royal Observatory, 1971.) [216

Progress in Reaction Kinetics, Vol. 6, Part 1. Pp. 1–74. £1.60; $4.25. Progress in Reaction Kinetics, Vol. 6, Part 2. Pp. 75–141. £1.50; $4. (Oxford and New York: Pergamon Press, 1971.) [226

Daresbury Nuclear Physics Laboratory. Annual Report 1970. Pp. 134. (Daresbury, near Warrington: Science Research Council, Daresbury Nuclear Physics Laboratory, 1971.) [226

Guide to the Oxford Botanic Gardens. Pp. 42. (Oxford: Clarendon Press; London: Oxford University Press, 1971.) 40p net. [226

Field Studies Council. The Effects of Industry on the Environment. (Proceedings of a Symposium held at the Orielton Field Centre, Pembroke, South Wales, as part of a Contribution for European Conservation Year.) Pp. 58. (London: Field Studies Council, 9 Devereux Court, WC2, 1970.) [226

Courtaulds. Report and Accounts, 1970/1971. Pp. 25. (London: Courtaulds, Ltd., 1971.) [226

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 261, No. 839 (17 June 1971): A Discussion on Subcellular and Macromolecular Aspects of Synaptic Transmission. Organized by H. K. F. Blaschko and A. D. Smith. Pp. 273–437+plates 47–90. £7.75; $20. (London: The Royal Society, 1971.) [236

A Bulletin of Current Documentation, Vol. 1, No. 1, June 1971. Pp. 1–32. Published June, December and March. Annual subscription 75p for UK; £1; $2.50 elsewhere. (London: Association of Commonwealth Universities, 1971.) [236

Cement and Concrete Association. Report for the year 1970. Pp. 55. (London: Cement and Concrete Association, 1971.) [246

Other Countries

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 29, Fasc. 5: Transformations Conformes er Quasi-Conformes des Variétés Riemanniennes Compactes (Démonstration de la Conjoncture de A. Lichnerowicz). Par Jacqueline Lelong-Ferrand. Pp. 44. (Bruxelles: Académia Royale de Belgique, 1971.) 100 francs. [116

The Use of Modules in College Biology Teaching. Edited by Joan G. Creager and Darrel L. Murray. Pp. viii+173. (Washington, DC: The Commission on Undergraduate Education in the Biological Sciences, 3900 Wisconsin Avenue, N.W., 1971.) *gratis*. [116

Académie Royale de Belgique. Annuaire pour 1971. Pp. 346+213. (Bruxelles: Académie Royale de Belgique, 1971.) [116

International Union for Conservation of Nature and Natural Resources. IUCN Publications, New Series, No. 20: IUCN Eleventh Technical Meeting—Papers and Proceedings, New Delhi, India, 25–28 November 1969. Vol. IV, Fifth Session, Commission on Education—Education Workshop. Environmental Conservation Education Among Populations of Rural and Woodland Areas. Pp. 156. (Morges, Switzerland: International Union for Conservation of Nature and Natural Resources, 1971.) [146

Académie Royale de Belgique. Classe des Sciences Mémoires, Tome 39, Fasc. 4: Chimie Organostannique—Structure, Mécanisme, Stéréochimie. Par J. Nasielski. Pp. 59. (Bruxelles: Académie Royale de Belgique, 1971.) 160 francs. [146

Commonwealth of Australia: Department of Supply. Defence Standards Laboratories Annual Report. 1969–1970. Pp. 62. (Maribyrnong, Victoria: Chief Superintendent, Defence Standards Laboratories, 1971.) [146

Les Sciences Occultes d'Après les Documents Littéraires Italiens du XVI^e^ Siècle. Par Viviana Paques. (Mémoires de l'Institut d'Ethnologie, No. VI.) Pp. 220. Le Bateau de Berck. Par F. Beaudouin. (Mémoires de l'Institut d'Ethnologie, V.) Pp. 210. (Paris: Université de Paris, Institut d'Ethnologie Musée de l'Homme, 1970 et 1971.) [146

Australia: CSIRO. Annual Report of the Division of Applied Chemistry 1969/1970. Pp. 41. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1971.) [166

World Health Organization. Technical Report Series, No. 463: WHO Expert Committee on Biological Standardization—Twenty-third Report. Pp. 94. (Geneva: WHO; London: HMSO, 1971.) 5 Swiss francs; 50p.; $1.75. [166

Proceedings of the North American Palaeontological Convention, Field Museum of Natural History, Chicago, September 5–7, 1969. Part G: Ultra Microplankton. 705–1010. Part H: Evolution of Higher Categories. Pp. 1011–1152. Part I: Extraordinary Fossils. Pp. 1153–1272. Part J: Reef Organisms Through Time. Pp. 1273–1482. Part K: Phosphate in Fossils. Pp. 1483–1562. Part L: Cretaceous Biogeography. Pp. 1563–1674. (Lawrence, Kansas: Allen Press, Inc. 1971.) [166

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 71–21: Massive Ice and Icy Sediments Throughout the Tuktoyaktuk Peninsula, Richards Island, and Nearby Areas, District of Mackenzie. By V. N. Rampton and J. Ross Mackay. Pp. v+16. Paper 70–45: Geology of Ennadai Lake Map-Area, District of Keewatin. By K. E. Eade. Pp. 19. $1.50. Paper 70–53: Geology, History and Potential of Vancouver Island Coal Deposits. By J. E. Muller and M. E. Atchison. Pp. viii+50. $2. Paper 70–60: Some Devonian Charophytes from Western Canada. By H. M. A. Rice. Pp. v+23. $1.50. Paper 70–67: Quaternary Geology Road Logs-Banff Area, Alberta. By Nathaniel W. Rutter. Pp. v+25. $0.75. (Ottawa: Information Canada, 1971.) [176

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.
The direct postal price per subscription is:-
12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A. & Canada
Nature (Friday)	**£14**	**$48**
Nature+ Nature Physical Science	**£24**	**$83**
Nature+ Nature New Biology	**£24**	**$83**
All three editions	**£29.50**	**$108**
Annual Index	**£1**	**$3**

* Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF
Telex: 262024
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355. Telex: 64280
Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 29242
American display advertisements
NATURE SCIENTIFIC PUBLICATIONS INC.
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
All other advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED
Registered as a newspaper at the Post Office
Copyright © Macmillan Journals Limited, October 29 1971